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 cytokins 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 cortexes 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 supresses 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 β_2 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 β_1 and β_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 a*l., 2002). Its expression and serotonin uptake are positively regulated by fibroblast growth factor (Kubota e*t al*., 2001). By binding to astrocytic $5HT_1$ receptors , serotonin stimulates production of neurotrophic S100β which is also produced after exposition to SSRI fluoxetine (Wilson *et al*., 1998, Donato, 2001, Manev *et al.*, 2001). Plasma level of S100β 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 phosphoinositide and cAMP accumulation, there is a potentiation of β-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 simillar 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 β_2 adrenoreceptors by clenbuterol induced the synthesis of nerve growth factor (NGF), fibroblast growth factor (FGF) and transforming growth factor β1 (TGF β1) 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 β-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 a*l., 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 α_s, G $\alpha i_{1,2}$) and phospholipase C (G $\alpha_{q/11}$), 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 $\alpha_{q/11}$ and G α_s 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-HT_1 and 5-HT_2 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 G $\alpha_{q/11}$ subunit in C6 glioma cells, whereas after chronic exposition an increased G $\alpha_{q/11}$ level was determined. Acute fluoxetine administration causing a reduced membrane G $\alpha_{q/11}$ amount was linked to subunit translocation into cytoplasm where it was proved (Kovářů *et al*., 1997, 1998). Reduced membrane G $\alpha_{q/11}$ subunit levels caused a lower degree of phospholipase C signalling with subsequent decreased substrate 1,4,5 inositoltriphosphate formation. Antidepressants from different classes are able to induce G α 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 fluoxetin 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 G α 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 (Gα q/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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REFERENCES

ABERG MA , ABERG ND, HEDBACKER H, OSCARSSON J,ERIKSSON PS: Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci **20** (8): 2896–2903,2000.

ALFONSO J, POLLEVICK GD, VAN DER HART MG, FLUEGGE G, FUCHS E, FRASCH AC: Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. Eur J Neurosci **19**(3): 659-666, 2004.

ALT A, MCFADYEN IJ, FAN CD, WOODS JH, TRAYNOR JR: Stimulation of guanosine-5'-o-(3-[35S]thio)triphosphate binding in digitonin-permeabilized C6 rat glioma cells: evidence for an organized association of mu-opioid receptors and G protein. J Pharmacol Exp Ther **298**(1): 116-121, 2001.

ARAQUE A, PARPURA V, SANZGIRI RP, HAYDON PG: Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci **22**(5): 208–15,1999.

AROLT V, PETERS M, ERFURTH A, WIESMANN M, MISSER U, RUDOLF S, KIRCHNER H, ROTHERMUNDT M: S100B and response to treatment in major depression: a pilot study Eur Neuropsychopharmacol **13**(4): 235-9, 2003.

AVISSAR S, SCHREIBER G: Toward molecular diagnostics of mood disorders in psychiatry. Trends Mol Med **8**(6): 294-300, 2002.

AVISSAR S, SCHREIBER G: The involvement of G proteins and regulators of receptor-G protein coupling in pathophysiology, diagnosis and treatment of mood disorders. Clin Chim Acta **336** (1-2**)**, 37-47, 2006.

BAL N, FIGUERAS G, VILARO MT, SUNOL C, ARTIGAS F: Antidepressant drugs inhibit a glial 5-hydroxytryptamine transporter in rat brain. Eur J Neurosci. **9**(8) :1728–38, 1997.

BARNES NM, SHARP T: A rewiew of central 5-HT receptors and their function. Neuropharmacology **38**(8): 1083-152, 1999.

BENMANSOUR S, OWENS WA, CECCHI M, MORILAK DA, FRAZER A: Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. J Neurosci **22**(15): 6766–72, 2002.

BOWLEY MP, DREVETS WC, ÖNGÜR D, PRICE JL: Low glial numbers in the amygdala in major depressive disorder. Biol Psych **52**(5): 404-12,2002.

BROWN ES, RUSH AJ, MCEWEN BS: Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology **21**(4):474-84, 1999.

BROWN DR: Neurons Depend on Astrocytes in a Coculture System for Protection from Glutamate Toxicity. Mol Cell Neurosci. **13**(5):379-89, 1999.

COTRINA ML, LIN JH, LOPEZ-GARCIA JC, NAUS M, NEDERGAARD M: ATP-mediated glia signaling, J Neurosci **20**(8): 2835–44,2000.

COVIC T, TYSON G, SPENCER D, HOWE G: Depression in rheumatoid arthritis patients: demographic, clinical and psychological predictors. J Psychosom Res. **60** (5): 469-76, 2006

CHEN J, RASENICK M: Chronic treatment of C6 glioma cells with antidepressant drugs increases functional coupling between a G protein (Gs) and adenylyl cyclase.J Neurochem. **64**(2),724-32,1995.

CZECH B, WELT T, FISCHER AK, ERHARDT A, SCHMITT W, MULLER MB, TOSCHI N, FUCHS E, KECK ME: Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. Biol Psychiatry **52**(11): 1047–56, 2002.

DANBOLT NC: Glutamate uptake. Prog Neurobiol. **65**(1): 1–105, 2001.
DE KEYSER J, ZEINSTRA E, WILCZAK N: Astrocytic β_2 adrenoreceptors receptors and multiple sclerosis. Neurobiol. Dis, **15** (2):331-339, 2004.
DESCHEPPER CF: Peptide receptors on astrocytes. Front Neuroendocrinol. **19**(1):20-46, 1998.
DIAMOND M, KELLY JP, CONNOR TJ: Antidepressants suppress production of the Th_1 cytokine interferon-γ, independent of monoamine transporter blockade. Eur Neuropsychopharmacol **16**(7): 481-90, 2006
DOETSCH F, HEN R: Young and excitable: the function of new neurons in the adult mammalian brain. Curr Opin Neurobiol **15**(1): 121-8, 2005.
DONATI RJ, RASENICK MM: Chronic antidepressant treatment prevents accumulation of gsalpha in cholesterol-rich, cytoskeletal-associated, plasma membrane domains (lipid rafts). Neuropsychopharmacology 3**0(7)**: 1238-45, 2005.
DONATO R: S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol **33**(7):637-68, 2001.
DUMAN RS: Depression: a case of neuronal life and death? Biol Psychiatry **56**(3): 140-5, 2004.
DUMAN RS, MONTEGGIA LM: A neurotrophic model for the stress-related mood disorders. Biol Psychiatry. **59**(12): 1116-27, 2006.
DRUKARCH B, SCHEPENS E, STOOF JC, LANGEVELD CH, VAN MUISWINKEL FL: Astrocyte-enhanced neuronal survival is mediated by scavenging of extracellular reactive oxygen species. Free Radic Biol Med. **25**(2): 217–20, 1998.
FATEMI SH, LAURENCE JA, ARAGHI-NIKNAM M, STARY JM, SCHULZ SC, LEE S, GOTTESMAN II: Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophr Res. **69**(2-3): 317-23, 2004.
FIŠEROVÁ A, KOVÁŘŮ H, HAJDUOVA Z, MAREŠ V, STAREC M, KŘEN V, FLIEGER M, POSPÍŠIL M: Neuroimmunomodulation of natural killer (NK) cells by alkaloid derivates. Phys Res **46**(2**)**: 119-25, 1997.
FIŠEROVÁ A, STAREC M, KULDOVÁ M, KOVÁŘŮ H, PÁV M, VANUCCI L, POSPÍŠIL M: Effects of D2-dopamine and alpha-adrenoceptor antagonists in stress induced changes on immune responsiveness of mice. J Neuroimmunol. **130**(1-2): 55-65, 2002.
FULLER RW,WONG DT: Serotonin uptake and serotonin uptake inhibition. Ann N Y Acad Sci. **600**: 68–78, 1990.
GONZALES-MAESO J, RODRIGUES-PUERTAS R, MEANA JJ, GARCIA-SEVILLA JA, GUIMON J: Neurotransmitter receptor-mediated activation of G-proteins in brains of suicide victims with mood disorders. Mol Psychiatry **7**(7): 755-67, 2002.
GOULD TD, MANJI HK: Signaling networks in the pathophysiology and treatment of mood disorders. J Psychosom Res. **53**(2): 687-97, 2002.
GUBBA EM, FAWCETT JW, HERBERT J: The effects of corticosterone and dehydroepiandrosterone on neurotrophic factor mRNA expression in primary hippocampal and astrocyte cultures. Brain Res Mol Brain Res. **127**(1-2): 48-59,2004.
GUILLEMIN GJ, KERR SJ, SMYTHE GA, SMITH DG, KAPOOR V, ARMATI PJ, CROITORU J, BREW BJ: The kynurenine pathway metabolism in human astrocytes: paradox for neuroprotection. J Neurochem **78**(4): 842-53, 2001.
GUILLEMIN GJ, SMYTHE G, TAKIKAWA O, BREW BJ: Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes and neurons. Glia **49**(1**)**:15-23, 2005.

HADDAD JJ, SAADE NE, SAFIEH-GARABEDIAN B: Cytokines and neuro–immune–endocrine interactions: a role for the hypothalamic–pituitary–adrenal revolving axis. J Neuroimmunol **133**(1-2):119, 2002.
HAGG T: Molecular regulation of adult CNS neurogenesis: an integrated review. Trends Neurosci. **28**(11): 589-95, 2005.
HANSSON E, SIMONSSON P, ALLING C: Interactions between cyclic AMP and inositol phosphate transduction systems in astrocytes in primary culture. Neuropharmacology **29**(6):591–8,1990.
HAUWEL M, FURON E, CANOVA C, GRIFFITHS M, NEAL J, GASQUE P: Innate (inherent) control of brain infection, brain inflammation and brain repair: the role of microglia, astrocytes, "protective" glial stem cells and stromal ependymal cells. Brain Res Brain Res Rev **48**(2): 220-33,2005.
HAVRDOVA E: Aggressive multiple sclerosis- is there a role for stem cell transplantation? J Neurol **252** (Suppl 3): iii34-iii37, 2005.
HAYLEY S, POULTER MO, MERALI Z, ANISMAN H: The pathogenesis of clinical depression: stressor and cytokine induced alterations of cytoplasticity. Neuroscience. **135**(3): 659-78, 2005.
HERTZ L, ZIELKE HR: Astrocytic control of glutamatergic activity: astrocytes as stars of the show. Trends Neurosci. **27**(12): 735-43,2004.
HISAOKA K, NISHIDA A, KODA T, MIYATA M, ZENSHO H, MORINOBU S, OHTA M, YAMAWAKI S: Antidepressant drug treatments induce glial cell line-derived neurotrophic factor (GDNF) synthesis and release in rat C6 glioblastoma cells. J Neurochem **79**(1): 25-34, 2001.
HISAOKA K, NISHIDA A, TAKEBAYASHI M, KODA T, YAMAWAKI S, NAKATA Y.: Serotonin increases glial cell line-derived neurotrophic factor release in rat C6 glioblastoma cells. Brain Res. **1002**(1-2): 167-70, 2004.
HORNER HC, PACKAN DR, SAPOLSKY RM: Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. Neuroendocrinology. **52**(1): 57–64,1990.
HÖSLI E, HÖSLI L: Receptors for neurotransmitters on astrocytes in the mammalian central nervous system. Prog Neurobiol **40**(4)**:** 477–506, 1993.
HUGHES EG, MAGUIRE JL, MCMINN MT, SCHOLZ RE, SUTHERLAND ML: Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. Brain Res Mol Brain Res. **124**(2):114-23, 2004.
INAZU M, TAKEDA H, MATSUMIYA T. Functional expression of the norepinephrine transporter in cultured rat astrocytes. J Neurochem. **84**(1): 136-44, 2003.
INAZU M, TAKEDA H, IKOSHI H, SUGISAWA M, UCHIDA Y, MATSUMIYA T: Pharmacological characterization and visualization of the glial serotonin transporter. Neurochem Int. **39**(1):39-49, 2001.
INOUE S, SUSUKIDA M, IKEDA K, MURASE K, HAYASHI K: Dopaminergic transmitter up-regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) synthesis in mouse astrocytes in culture. Biochem Biophys Res Commun. **238**(2): 468–72,1997.
JACOBS BL, PRAAG H, GAGE FH: Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry. **5**(3): 262–9,2000.
JUNKER V, BECKER A, HUHNE R, ZEMBATOV M, RAVATI A, CULMSEE C, KRIEGLSTEIN J: Stimulation of beta-adrenoceptors activates astrocytes and provides neuroprotection. Eur J Pharmacol. **446(1-3)**: 25– 36, 2002.

KELLEY KW, BLUTHE RM, DANTZER R, ZHOU JH, SHEN WH, JOHNSON RW, BROUSSARD SR: Cytokine-induced sickness behavior. Brain Behav Immun. **17** Suppl 1: S112-8, 2003.
KEMPERMANN G, WISKOTT L, GAGE FH: Functional significance of adult neurogenesis. Curr Opin Neurobiol. **14**(2): 186-91,2004.
KEMPERMANN G, KRONENBERG G: Depressed new neurons- adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry **54**(5): 499–503,2003.
KOVÁŘŮ H, FIŠEROVÁ A, LISÁ V, ŠPANOVÁ A, KOVÁŘŮ F, VELEK J: Fluoxetine induced changes in C6 glioma cells and NK lymphocytes. In: Biologické základy psychických poruch. (Eds J.Sikora, Z.Fišar et al.), Galén Publ.Co, Praha, 108-112, 1997.
KOVÁŘŮ H, FIŠEROVÁ A, ŠPANOVÁ A, LISÁ V, FIŠAR Z, VELEK J: Antidepressants as neuroimmunomodulators at postreceptor level. J Neuroimmunol **90**: 41, 1998.
KOVÁŘŮ H, LISÁ V, FIŠAR Z, MALBOHAN I: Comparative analyses of antidepressant induced changes in G alpha subunit profiles. NeuroImmunomodulation **6**: 460,1999.
KOVÁŘŮ H, FIŠEROVÁ A, KOVÁŘŮ F, PACLT I, LISÁ V: Antidepressant or immunomodulator induced regulation of cell signalling in brain and immune system. Eur Neuropsychopharmacol **10** Suppl 3: S269- S270, 2000.
KOVÁŘŮ H, FIŠEROVÁ A, KOVÁŘŮ F, POSPÍŠIL M, LISÁ V: Modulation of heterotrimeric GTP-binding proteins in immune system and brain. Czech J Anim Sci, 46:62-67,2001.
KOVÁŘŮ H, KOVÁŘŮ F: Basis of neuroimmunomodulation. In: Základy neuroimmunomodulace H.Kovářů, F.Kovářů, Galén, Praha: 203-208, 2005.
KUBOTA N, KIUCHI Y, NEMOTO M, OYAMADA H, OHNO M, FUNAHASHI H, SHIODA S, OGUCHI K: Regulation of serotonin transporter gene expression in human glial cells by growth factors. Eur J Pharmacol. **417**(1-2):69-76, 2001.
LAMING PR, KIMELBERG H, ROBINSON S, SALM A, HAWRYLAK N, MULLER C, ROOTS B, NG K: Neuronal–glial interactions and behaviour. Neurosci Biobehav Rev **24**(3): 295-340,2000.
LEE HJ, CHA JH, HAM BJ, HAN CS, KIM YK, LEE SH, RYU SH, KANG RH, CHOI MJ, LEE MS: Association between a G-protein beta3 subunit gene polymorphism and the symptomatology and treatment responses of major depressive disorders. Pharmacogenomics J. **4**(1): 29-33, 2004.
LEONARD BE: Inflammation and Depression: Is there a causal Connection with Dementia? Psychiatrie **10**(3):172-178, 2006 (in czech).
LUCASSEN PJ, HEINE VM, MULLER MB, VAN BER BEEK VM, WIEGANT VM, DE KLOET ER, JOELS M, FUCHS E, SWAAB DF, CZEH B: Stress, depression an hippocampal apoptosis. CNS Neurol Disord Drug Targets. **5**(5):531-46, 2006.
MANEV R, UZ T, MANEV H: Fluoxetine increases the content of neurotrophic protein S100β in the rat hippocampus. Eur J Pharmacol **420**(2-3): R1-2, 2001.
MAREŠ V, GIORDANO PA, PELLICCIARI C, SCHERINI E, LISÁ V, BOTTONE G, BOTTIROLI G: Changes in cell cycle and chromatin distribution in C6 glioma cells treated by dibutyryl cyclic AMP. Cell Prolif. **24**(6**)**: 569-77,1991.
MCEWEN BS, MAGARINOS AM, REAGAN LP: Structural plasticity and tianeptine: cellular and molecular targets. Eur Psychiatry. **17**(3): 318-30, 2002.
MERCIER G, LENNON AM, RENOUF B, DESSOROUX A, RAMAUGE M, COURTIN F, PIERRE M: MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. J Mol Neurosci **24**(2): 207-16, 2004.

MIGUEL-HIDALGO JJ, RAJKOWSKA G: Comparison of prefrontal cell pathology between depression and alcohol dependence. J Psych Res. **37**(5): 411–420, 2003.
MIYAZAKI I, ASANUMA M, DIAZ-CORRALES FJ, MIYOSHI K, OGAWA N. Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. Brain Res **1029**(1): 120-3, 2004.
MIZUTA I, OHTA M, OHTA K, NISHIMURA M, MIZUTA E, HAYASHI K, KUNO S: Selegiline and desmethylselegiline stimulate NGF, BDNF, and GDNF synthesis in cultured mouse astrocytes. Biochem Biophys Res Commun. **279**(3): 751-5, 2000.
MOJCA-JURIC DM, MIKLIC S, CARMAN-KRZAN M: Monoaminergic neuronal activity up-regulates BDNF synthesis cultured neonatal rat astrocytes. Brain Res. 1108(1): 54-62, 2006.
MÜLLER HV, JUNGHANS U, KAPPLER J: Astroglial neurotrophic and neurite-promoting factors. Pharmacol Ther. **65**(1):1-18, 1995.
NAKAGAWA T, SCHWARTZ JP: Expression of neurotrophic factors and cytokines and their receptors on astrocytes in vivo. Adv Cell Mol Biol **31** (Pt 2): 561-574, 2004.
NAKYAMA T, SOMOGI-SOGA T, INOUE N. Astrocyte-derived factors instruct differentiation of embryonic stem cells into neurons. Neurosci Res **46**(2):241-9, 2003.
NESTLER EJ, BARROT M, DILEONE RJ, EISCH AJ, GOLD SJ, MONTEGGIA LM: Neurobiology of depression. Neuron. **34**(1): 13-25, 2002.
NEUMEISTER A.: Tryptophan depletion, serotonin and depression: where do we stand? Psychopharmacol Bull. **37**(4): 99-115, 2003.
NICHOLS NR, AGOLLEY D, ZIEBA M, BYE N: Glucocorticoid regulation glial responses during hippocampal neurodegeneration and regeneration. Brain Res Brain Res Rev **48**(2): 287-301, 2005.
NOWAK G, TRULLAS R, LAYER RT, SKOLNICK P, PAUL IA : Adaptive changes in the *N*-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid. J Pharmacol Exp Ther. **265**(3): 1380–6, 1993.
OHTA K, KUNO S, MIZUTA I, FUJINAMI A, MATSUI H, OHTA M: Effects of dopamine agonists bromocriptine, pergolide, cabergoline, and SKF-38393 on GDNF, NGF, and BDNF synthesis in cultured mouse astrocytes. Life Sci **73**(5): 617-26, 2003.
O'KUSKY J, COLONNIER M: A laminar analysis of the number of neurons, glia, and synapses in the adult cortex (area 17) of adult macaque monkeys. J Comp Neurol **210**(3)**:** 278–90, 1982.
ÖNGÜR D, DREVETS WC, PRICE JL: Glial reduction in the subgenual prefrontal cortex in mood disorders. Proc Natl Acad Sci U.S.A. **95**(22): 13290–5,1998.
PACHECO MA, STOCKMEIER C, MELTZER HY, OVERHOLSER JC, DILLEY GE, JOPE RS. Alterations in phosphoinositide signalling and G-protein levels in depressed suicide brain. Brain Res **723**(1-2**)**: 37–45, 1996.
PARPURA V, HAYDON PG: Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. Proc Natl Acad Sci U S A. **97**(15): 8629-34,2000.
PAWLAK J, BRITO V, KŰPPERS E, BEYER C: Regulation of glutamate transporter GLAST and GLT-1 expression in astrocytes by estrogen. Brain Res Mol Brain Res. **138**(1): 1-7, 2005.
PORTER JT, MCCARTHY KD: Atrocytic neurotransmitter receptors in situ and in vivo. Progress in Neurobiology **51**(4): 439-55,1997.
RAJKOWSKA G: Depression: What We Can Learn from Postmortem Studies. Neuroscientist **9**(4): 273–84, 2003.

REYNAERT C, JANNE P, BOSLY A, STAQUET P, ZDANOWICZ N, VAUSE M, CHATELAIN B, LEJEUNE D. From health locus of control to immune control: internal locus of control has a buffering effect on natural killer cell activity decrease in major depression. Acta Psychiat Scand **92**(4): 294-300, 1995.
RIDET JL, MALHOTRA SK, PRIVAT A, GAGE FH: Reactive astrocytes: cellular and molecular cues to biological function.Trends Neurosci **20**(12): 570-7, 1997.
SAPENA R, MORIN D, ZINI R, MORIN C, TILLEMENT JP: Desipramine treatment differently down-regulates beta-adrenoceptors of freshly isolated neurons and astrocytes. Eur J Pharmacol. **300**(1-2): 159-62,1996.
SCHILDKRAUT JJ, MOONEY JJ: Toward a rapidly acting antidepressant: the normetanephrine and extraneuronal monoamine transporter (uptake 2) hypothesis. Am J Psychiatry **161**(5): 909-11, 2004.
SEKOT M, GURLICH R, MARUNA P, PÁV M, UHLÍKOVÁ P: Evaulation of anxiety and depression of patients with malignant gastrointestinal cancer. Čes. Slov. Psychiatrie **5**: 252-257, 2005 (in czech) .
SERI B, GARCIA-VERDUNO JM, MCEWEN BS, ALVAREZ-BUYLLA A: Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci **21**(18):7153-60, 2001.
SHARMA G, VIJAYARAGHAVAN S: Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. Proc Natl Acad Sci U S A. **98**(7):3631-2, 2001.
SHELINE ZI, MITTLER BL, MINTUN MA: The hippocampus and depression. Eur Psychiatry. **17(3)**: 300-305, 2002.
SHELINE ZI: Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry **54**(3): 338-52, 2003.
SHIEPERS O JG, WICHERS MC, MAES M: Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry. **29**(4): 637-8, 2005.
SLEZAK M, PFRIEGER FW: New roles for astrocytes: Regulation of CNS synaptogenesis. Trends Neurosci **26**(10): 531-5, 2003.
SOFRONIEW MW. Reactive astrocytes in neuronal repair and protection. Neuroscientist. **11**(5): 400-7, 2005.
SPIEGEL AM: Defects in G protein coupled signal transduction in human disease. Ann Rev Physiol. **58**: 143-70, 1996.
STEFFENS DC, BYRUM CHE, MCQUOID DR, GREENBERG DL, PAYNE ME, BLITCHINGTON TF, MACFALL JR, KRISHNAN KRR: Hippocampal Volume in Geriatric Depression. Biol Psychiatry **48**(4): 301–309, 2000.
STONE EA, ARIANO MA: Are glial cells targets of the central noradrenergic system: a review of the evidence. Brain Res Brain Res Rev. **14**(4)**:** 297–309, 1989.
STRUBING C, ROHWEDEL J, AHNERT-HILGER G, WIEDENMANN B, HESCHELER J, WOBUS AM: Development of G protein-mediated Ca2+ channel regulation in mouse embryonic stem cell- derived neurons. Eur J Neurosci. **9**(4): 824-32, 1997.
SYKOVÁ E: Glia and volume transmission during physiological and pathological states. J Neural Transm. **112**(1):137-47,2005.
ŠPANOVÁ A, KOVÁŘŮ H, LISÁ V, LUKÁŠOVÁ E, RITTICH B: Estimation of apoptosis in C6 glioma cells treated with antidepressants. Physiol Res **46**(2): 161-164, 1997.
TACUMA K, BABA A, MATSUDA T: Astrocytic apoptosis: implications for neuroprotection. Prog Neurobiol **72**(2): 111-127, 2004.

TAFET GE, BERNARDINI R: Psychoneuroendocrinological links between chronic stress and depression. Prog Neuropsychopharmacol Biol Psychiatry **27**(6): 893– 903, 2003.
VEREKER E, O´DONNELL E, LYNCH A, KELLY A, NOLAN Y, LYNCH MA: Evidence that interleukin -1β and reactive oxygen species production play a pivotal role in stress induced impairment of LTP in the rat dentate gyrus. Eur J Neurosci **14**(11): 1809-19, 2001.
VERKHRATSKY A, STEINHÄUSER CH: Ion channels in glial cells. Brain Res Rev **32**(2-3): 380-412, 2000.
VILLOSLADA P, GENAIN P: Role of nerve growth factor and other trophic factors in brain inflammation. Prog Brain Res, **146**: 403-414, 2004.
VITHINGHAM M, HEIM C, NEWPORT J, MILLER AH, ANDERSON E, BRONEN R, BRUMMER M, STAIB L, VERMETTEN E, CHARNEY DS, NEMEROFF CB, BREMNER JD: Childhood trauma associated with smaller hippocampal volume in women with major depression. Amer J Psychiatr. **159**: 2072-80, 2002.
WILSON CC, FABER KM, HARING JH: Serotonin regulates synaptic connections in the dentate molecular layer of adult rats via 5-HT_{1a} receptors: evidence for a glial mechanism. Brain Res **782**(1-2): 235-239, 1998.
YOUNG LT, LI PP, KISH SJ, SIU KP, KAMBLE A, HORNYKIEWICZ O, WARSH JJ: Cerebral cortex Gs alpha protein levels and forskolin-stimulated cyclic AMP formation are increased in bipolar affective disorder. J Neurochem **61**(3): 890-8,1993.
ZELENAIA O, SCHLAG D, GOCHENAUER O, GANEL R, SONG W,BEELSEY JS, GRINSPAN JB, ROTHSTEIN JD, ROBINSON MB: Epidermal growth factor receptor agonists increase expression of glutamate transporter GLT-1 in astrocytes through pathways dependent on phosphatidylinositol 3-kinase and transcription factor NF-kB. Mol Pharmacol **57**(4): 667-78, 2000.

Scheme 1.

Astrocyte receptors and membrane transporters.

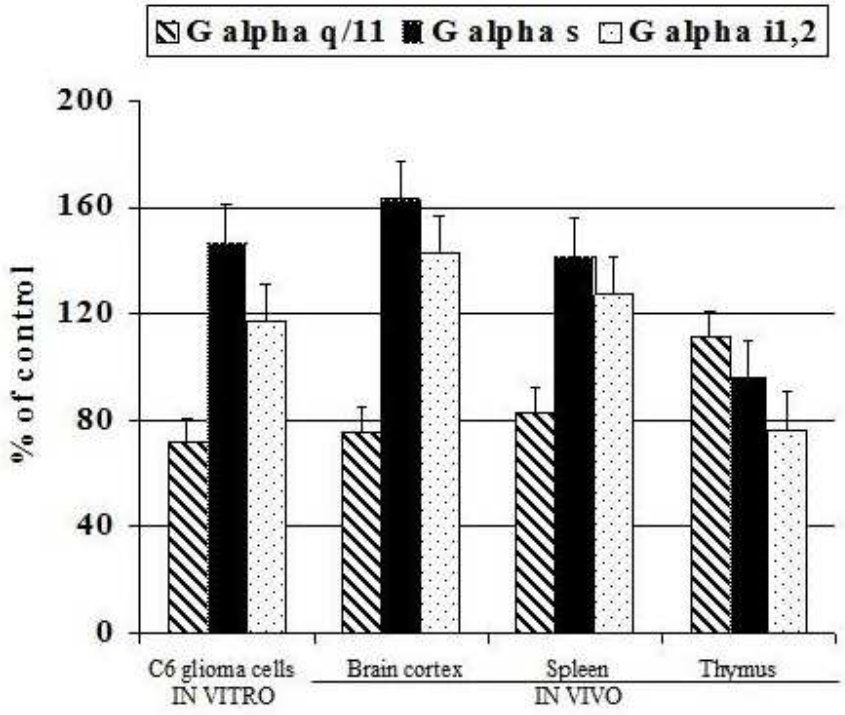
Figure 1.

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

Adrenergic $\beta_{2,}$ $\beta_{1,}$ α_1,$\alpha_{2A/D}$	Junker *et al*, 2002, Hösli and Hösli, 1993, Porter *et al*, 1997
Serotonergic $5HT_{1A,}$ 5-HT_{2A}, 5HT7	Wilson et al., 1998, Barnes and Sharp 1999, Manev *et al.*, 2001, Hannson *et al*., 1990
$GABA_A$, $GABA_{B1a}$ $GABA_{B1b}$, $GABA_{B2}$	Charles 2003, Porter *et al*, 1997
Glutamate AMPA/kainate, NMDA, mGLURs1, mGLURs5	Porter *et al*, 1997, Hertz and Zielke 2004
Purinergic P1, $P2X_1$, $P2X_1$, $P2Y_2$, $P2X_3$, $P2X_4$, $P2X_6$, $P2X_7$, $P2Y_2$, $P2Y_4$	Müller *et al.*, 1995, Washburn and Neary 2006
Acetylcholine nikotinic receptors	Sharma and Vijayaraghavan 2001
Dopamine D_1 , D_3, D_4 , D_5	Ohta *et al*., 2003, Myiazaki *et al*., 2004
Serotonin transporter SERT	Fuller and Wong 1990, Bal *et al*, 1997, Inazu *et al*., 2001
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 ńeurotrophic factors (NGF, BDNF, IGF, FGF, VEGR, EGFR, NT-3)	Müller *et al.*, 1995, Hughes *et al*., 2004, 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


G alpha q/11
G alpha s
G alpha i1,2
% of control
200
160
120
80
40
0
C6 glioma cells
IN VITRO
Brain cortex
Spleen
Thymus
IN VIVO