Neuroendocrinology Letters Volume 30 No. 5 2009

ORIGINAL ARTICLE

Acute and chronic effects of antidepressants on the G-protein alpha subunit profiles *in vitro* and *in vivo*

Marek Pav [1], Hana Kovaru [1], Frantisek Kovaru [2,3], Vera Lisa [4], Petra Ondrackova-Zelnickova [2], Anna Fiserova [5]

[1] Charles University, 1st Faculty of Medicine in Prague, Prague, Czech Republic
[2] University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3] Institute of Animal Physiology and Genetics, Academy of Sciences CR, Brno, Czech Republic
[4] Institute of Physiology, Academy of Sciences CR, Prague, Czech Republic
[5] Institute of Microbiology, Academy of Sciences CR, Prague, Czech Republic

Correspondence to: Prof. RNDr. Hana Kovářů, DrSc.
Charles University in Prague, 1st Faculty of Medicine
Ke Karlovu 11, 120 00 Prague 2, Czech Republic
PHONE: +420 224965382; FAX: +420 224923077;
E-MAIL: hkova@lf1.cuni.cz

Submitted: 2009-05-04 Accepted: 2009-06-09 Published online: 2009-11-10

Key words: **C6 glioma cells; C6 astrocytoma cell line; cAMP; IP3; antidepressant; depressive disorder; glia; trimeric GTP binding protein; G-protein**

Neuroendocrinol Lett 2009; **30**(5):592–598 **PMID:** 20035268 NEL300509A18

Abstract

OBJECTIVES: Neurochemical studies on the etiopathogenesis of depression are also focusing on the transduction system beyond receptors. Trimeric G-proteins play a crucial role in the transmembrane signalling, signal amplification and intracellular processing. Abnormalities of G-protein levels are observed in subjects with depression, G-protein modulation is considered to play a role in the antidepressant mode of action.

METHODS: We studied acute or chronic administration of antidepressants from different pharmacological groups. We used immunochemical estimation (ELISA) of the main types of G-protein alpha subunits from isolated membranes of C6 glioma cells and rat brain tissue.

RESULTS: Significant elevation of G alpha q/11 subunits after chronic administration of sertraline and significant reduction of G alpha s subunit levels following both acute and chronic administrations of sertraline were found. In contrast, no significant effects on G alpha subunit levels following acute desipramine and moclobemide administration were observed in vitro. Chronic moclobemide effect in vivo is causing significant elevation of Galpha s and Galpha i1,2 subunit levels.

CONCLUSIONS: Results show involvement of antidepressant drugs in the C6 glioma signal transduction cascades modulation in dependence on the antidepressant class. Significant influence in the cAMP system modulation is observed after administration both SSRI and MAOA inhibitors. Astrocytoma cells – C6 glioma cells also can offer a model system of the glia where modulation of cell signalization cascades can influence cell functioning and production of neurotrophic factor molecules relevant to the antidepressant tratment and depression etiopathogenesis.

Abbrevations:

AC	– adenylyl cyclase
cAMP	– cyclic adenosine monophoshate
BDNF	– brain derived neurotrophic factor
CREB	– cAMP response element binding protein
G-protein	– trimeric GTP binding protein
GDNF	– glia derived neurotrophic factor
GTP	– guanosine triphosphate
IP3	– 1,4,5 inositol triphosphate
MAOA	– monoaminooxidase A
MAPK	– MAP kinase
NA	– noradrenaline
5-HT	– 5-hydroxytryptamine, serotonin
PKA	– protein kinase A
PKC	– protein kinase C
PLC	– phopholipase C
RIMA	– reversible inhibitor of monoaminooxidase A
SSRI	– selective serotonin reuptake inhibitor
TCA	– tricyclic antidepressant

INTRODUCTION

Although antidepressant medication has been used to treat affective disorders for long time, our understanding of its action is still incomplete. Nonetheless, to act, antidepressants are likely to have one or more molecular targets, some of these targets are monoamine uptake sites but it is difficult to reconcile the clinical requirement for chronic drug or electroconvulsive shock treatment with uptake inhibition which is contemporaneous with acute drug exposure (Donati *et al.* 2008). Chronic antidepressant administration results in the modulation of the cellular signaling components, in the signal transduction from membrane into series of downstream cell responses, the key role are playing trimeric GTP binding proteins (Spiegel, 1996, Avissar and Schreiber 2006).

Signal transduction by GTP-binding proteins (G proteins) are most widely recognized mechanisms of information signal transducttion induced by first messengers – neurotransmitters, hormones, chemokines, etc. – ligands of receptor (seven transmembrane spanning type) coupled to G protein across membrane to effector and signal is processed via signalling pathways within the cell (Spiegel 1996; Wettschureck and Offermanns 2005). These receptors are coupled to limited repertoire of G proteins and interact with few effector molecules that include adenylylcyclases, phosphodiesterases, phospholipase C and various ion channels, etc. Trimeric G proteins are composed of 3 subunits : functionally highly important is G alpha subunit with intrinsic GTPase activity, cooperating with beta/gamma subunits. Alpha subunits influence various effectors: G s stimulates and G i inhibits adenylylcyclase, G q operate via phospholipase C, and G(o) is related to ion channels. In many events effects of G proteins are combined (Milligan 1988). There are feedback loops adjusting levels of activity in separate signalling pathways, as well as significant cross-talk between separate pathways at different levels *(*Spiegel 1996; Wettschureck and Offermanns 2005).

Many data suggest that long-term antidepressant treatment is facilitating signalization cascade initiated by the G alpha s subunit, activation of adenylyl cyclase, increased cAMP formation, activation of protein kinase A, phosphorylation of transcription factor CREB, and facilitation of CREB mediated transcription with subsequent enhanced production of neurotrophins, including BDNF (Chen and Rasenick 1995;. Nair and Vaidya 2006). Resulting changes of neuroplastic changes and increased neuroregeneration as an effect of the increased availability of neurotrophic factors, are considered to mediate at least a part of the antidepressant effect (Nestler *et al.* 2002; Duman 2004; Tardito el al. 2006; Maes 2008). In the activation of CREB by antidepressants, besides cAMP pathway, cascade initiated by the G alpha q/11 subunits also participates, with final activation of PKC and Ca^{2+}/calmodulin-dependent kinases signalling to cell nucleus (Shaywitz and Greenberg 1999; Tiraboshi *et al.* 2004).

Antidepressants are modulating cell signalization not only in the neuronal cells but also in the non-neuronal (glial) ones, and in leukocytes and thrombocytes, this refers to the systemic character of the depression disorder and dysregulation in different homeostatic systems (Maes 2001; 2008; Kovářů and Kovářů 2005; Avissar and Schreiber, 2006, Kitzlerová and Anders 2007; Páv *et al.* 2008).

The C6 glioma cell line is widely used to study postsynaptic antidepressant effect due to expressing substantial levels of beta-adrenoreceptors tightly coupled with adenylyl cyclase There is also no change in the content of G-protein subunits or mRNA levels in the rat cortex after TCA antidepressant treatment (Emamghoreishi *et al.* 1996, Chen a Rasenick 1995). On the hand, fluoxetine (SSRI) induced mRNA changes of G alpha subunits were estimated in rat brain (Lesch *et al.* 1992b). The C6 glioma cells are also used to study antidepressant effects on Galpha subunit levels and production of growth factors or cytokines (Toki *et al.* 1999; Jenab and Quinone-Jenab 2002, Hisaoka *et al.* 2001; 2008).

Altered levels or function of signalling proteins, especially the alpha subunits of G-proteins, as well as changed mRNA levels were found in post-mortem brain tissue of patients suffering from the bipolar disorder (Young *et al.* 1993; Manji *et al.* 1995). Another data demonstrate diminished both G-protein influenced cAMP synthesis and G-protein induced activation of the phosphatidylinositol system signal transduction in post-mortem brain cortex regions of suicide victims suffering from major depression (Menkes *et al.* 1983; Pacheco *et al.* 1996). Depressed patients had markedly hypofunctional Galpha s and G i1,2 granulocyte G-proteins, these were suggested as depression „state markers", predictors of antidepressant therapeutic response

(Avissar *et al.* 1998; Gurguis *et al.* 1999; Avissar and Schreiber 2006).

In this study we examine acute and chronic effects of antidepressants from different classes (TCA, SSRI, RIMA) on the rat C6 glioma G alpha subunit levels *in vitro* and *in vivo* using rat model. Despite a number of studies performed with antidepressants from the tricyclic class, there is a limited knowledge assessing the effect of newer antidepressants, such as SSRIs or RIMA on the Galpha subunit profiles of main G protein types. There is also a lack of data accessing dynamics of the G alpha level change after sertraline and moclobemide administration in comparison with tricyclic antidepressant response.

MATERIAL AND METHODS

Animals. We used inbred Wistar strain of male rats (Charles River Co.) with initial weight 180–200 g fed with standard ST1 diet and water ad libitum. Antidepressant was administered orally each day (5mg/kg – citalopram, and 25mg/kg – moclobemide) for 3 weeks. Groups of 6 animals were used, the control group consisted of 6 animals kept under same conditions. All procedures were performed in accordance with the European convention for care and use of laboratory animals, and with the Czech law (246/1992 Coll. and later regulations) and. according to recommendation of FELASA and European community.

All chemicals were purchased from SIGMA Co., if not indicated otherwise.

Preparation of crude membrane fraction. Cells or fragments of brain tissue were homogenised in 50 mM Tris-HCl pH 7.4, containing proteolytic inhibitors – 0.1 mM benzamidine, 0.3mM PMSF (phenyl-methylsulphonyl fluoride, 1mM DTT (dithiothreitol) and 1 mM EDTA and centrifuged at 1000xg for 10 min at 4 °C. Resulting supernatant was then centrifuged at 40,000xg for 20 min at 4 ° C and membrane protein was extracted for 1 hr at 4 ° C in 20 mM Tris-HCl pH 8.0 containing 25mM NaCl, 1mM EDTA and 1% sodium cholate. Extract was centrifuged at 100,000xg for 1hr at 4 ° C and supernatants were stored at –80 °C.

C6 glioma cell line (ATCC CLM, Rockville, MD) was cultured in MEM medium (pH 7.3), supplemented with 5% bovine fetal bovine serum in a humified atmosphere of 95% of air and 5% of CO_2 at 37°C under standard condtions for 3 days after splitting, for other details (Mareš *et al.* 1991). Confluent cultures were exposed to the antidepressant (final 1μM concentration for all tested drugs) for 24 hours (acute model) or five days (chronic model); the antidepressant was supplemented to medium each day. After exposition to the antidepressant, the cells were washed with PBS, then harvested by scraping with rubber policeman in PBS containing proteolytic inhibitors as above mentioned. Cells were then three times washed and collected by centrifugation at 1 000x g, and cholate membrane extracts were prepared with anti-proteolytic. solution and stored at –80C.. We used physiologically optimal final 1μM concentration of antidepressant treatment in vitro. For other details (Kovářů *et al.* 2001).

Alpha subunits of G proteins. We used our rabbit monospecific antibodies against C-terminal dekapeptides of alpha chains – Gs, Gi1,2 and Gq/11 with amino acid according sequences (Milligan 1988, Kovářů *et al.* 1998, 2001). In control tests, no cross-reaction of antisera against other C – terminal dekapeptides of alpha chains was proved.

ELISA immunoassay The levels of G alpha subunits were estimated in cholate membrane extracts (5–10 μg protein/well) by ELISA microplate competitive inhibition immunoassay, compared with Western immunoblotting, for other details see (Fišerová *et al.* 1997; Kovářů *et al.* 1998;2001). ELISA immunoassay of competitive inhibiton was performed (Tijjsen, 1993; Ransnas and Insel, 1989; Lesch and Manji 1992a; Tijjsen, 1993) with followed modifications. We used 96 – microtitration plates of U shape well for noncovalent peptide binding. We used high binding microplates (Costar) for Galpha q/11 and G alpha s level estimation and Maxisorp microtitration plates (NUNC) for Gα i1,2 subunit. Shortly, first antibody was monospecific rabbit antibody against.G alpha subunit tested.

Immunochemical staining with alkaline phosphatase conjugated with goat antirabbit IgG and p-nitrophenyl phosphate as substrate was used (Tijssen 1993, Kovářů *et al.* 1997b). Quantity of p-nitrophenole formation was measured by ELISA reader at 405 nm. Other steps during ELISA procedure (washing, etc) were used according traditional protocol (Tijjsen 1993).

Statistical analysis.

All results are expressed as the arithmetical mean +/– S.E.M. The differences between experimental samples were evaluated by Student's t- test for unpaired values.

P values smaller than 0.05 were considered significant ($p < 0.05$, marked with(*) and $p < 0.01$ (**)..

RESULTS

Used antidepressants are belonging to the three different pharmacological groups: TCAs (desipramine, imipramine), SSRIs (sertraline, citalopram) and RIMA (moclobemide). TCAs non-specifically block reuptake of noradrenaline and serotonin, as well as histamine and muscarine receptors. SSRIs are specific serotonin reuptake inhibitors, acting on blockade of serotonin transporter SERT, moclobemide is a reversible inhibitor of monoaminooxidase A (RIMA), blocking deamination of serotonin, adrenaline, noradrenaline, melatonine, and dopamine.

Fig. 1. summarizes acute effects of administration of the antidepressants on the C6 glioma cells culture. Results show statistically significant G alpha q/11 sub-

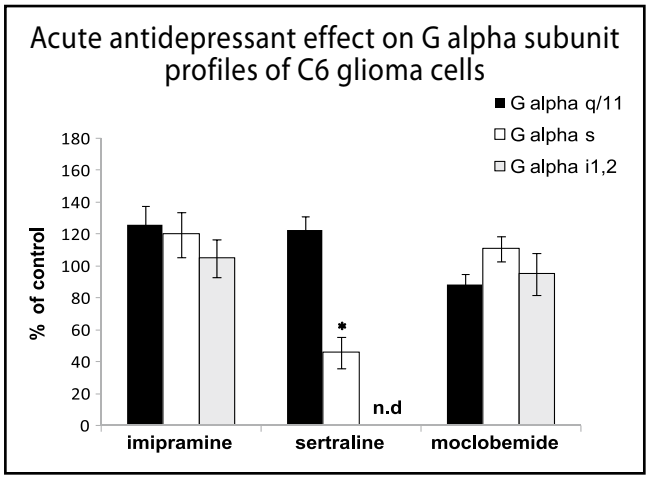

Fig. 1: Results are arithmetical means of 6-8 measurements in tetraplets, +/- S.E.M. Indicated values are significant P<0.05 (*), P<0.01 (**).n.d. not determined

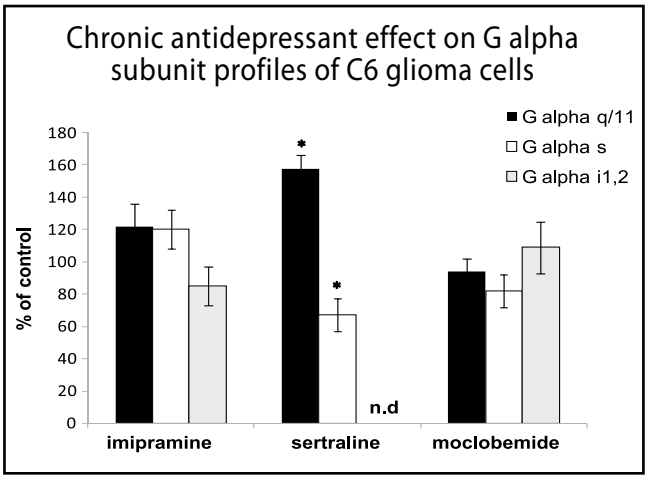

Fig. 2: *Results are arithmetical means of 6-8 measurements in tetraplets, +/- S.E.M..Iindicated values are significant P<0.05 (*), P<0.01 (**).n.d.not determined*

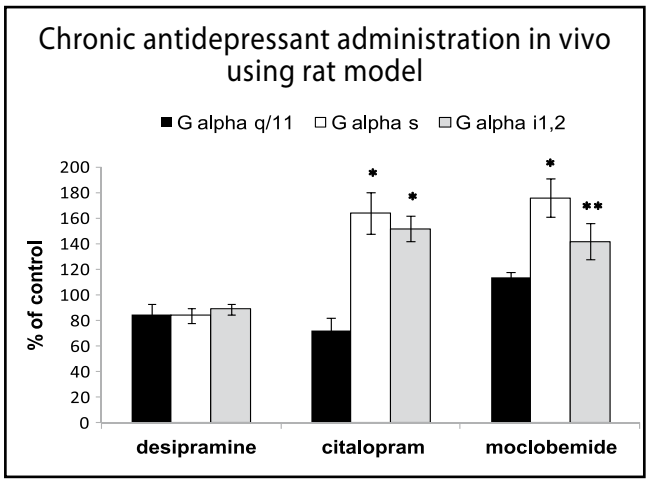

Fig.3: *Results are arithmetical means +/- S.E.M. Indicated values are significant P<0.05 (*), P<0.01 (**).*

units elevation concurrent with significant decrease of G alpha s as an effect of acute sertraline (SSRI) administration. In contrast, imipramine (TCA) and moclobemide (RIMA) have no effect on the G alpha subunits levels. Other G alpha subunits were influenced insignificantly.

We know that antidepressants show therapeutic effects within weeks of administration, chronic effect is crucial to determine changes taking place *in vivo*. Therefore, the 5-day-lasting exposition to the examined drug *in vitro* was performed which is corresponding to the 21-day-lasting administration *in vivo* when usually clinical response to the antidepressant treatment is observed. In Fig 2, chronic (5 days) antidepressant effect shows statistically significant G alpha q/11 subunits elevation as an effect of sertraline (SSRI) administration, with G alpha s subunit level reduction. Chronic response of the C6 glioma cell line is thus similar to the acute exposition. After chronic administration, imipramine (TCA) and moclobemide (RIMA) demonstrate no significant elevation of G alpha subunit levels, therefore a certain similarity of G alpha subunit profile in comparison with the acute effect (Fig.1) can be evident.

To determine changes occurring *in vivo,* and to allow comparison with *in vitro* data, we administered antidepressants from different classes to rats for 3 weeks (Fig.3). TCA desipramine induces no significant response in G alpha subunit levels in the rat brain membranes. Chronic citalopram (SSRI) effect is elevation of G alpha s and G alpha i1,2 subunit levels. Moclobemide (RIMA) is also inducing significant increase of G alpha s and G alpha i1,2 subunits.

DISCUSSION

Our results demonstrate prominent influence of sertraline (SSRI) on the G alpha q/11 system with elevation of G alpha q/11 subunit levels after both acute and chronic exposures *in vitro* (Fig. 1,2).. Besides this sertraline shows the effect on the G alpha s subunit levels during acute and chronic administrations, significantly reducing its levels. These findings cannot be compared with other results, there are no data concerning influence of sertraline to the

G alpha subunits. No significant effects on the monoamine receptor or adenylyl cyclase activity during chronic administration were found, subacute administration of sertraline *in vivo* results in the down-regulation of beta-adrenoreceptors, protein kinase A activity increase in the rat frontal cortex was also observed (Koe and Lebel 1995; Tadokoro *et al.* 1997; 1998).

In vitro results correspond with chronic exposure response of C6 glioma cells of another member of SSRI

group – zfluoxetine during chronic administration, previous results of our group show biphasic response to fluoxetine administration with initial decrease of G alpha q/11 and elevation after chronic administration, with no effect on G alpha s levels (Kovářů *et al.* 2000; 2001).

We also demonstrated dynamic changes in levels of both G alpha q/11 and G alpha s subunits of peripheral blood granulocytes of patients with unipolar depression during fluoxetine administration on days 3 – 28 (Kovářů *et al.* 2000; Kovářů and Kovářů 2005). Due to a limited amount of data in the literature, it is necessary to discuss data obtained in different models, a support for comparability of different models stems e.g. from previous results where citalopram shows a similar profile of response in *in vitro* data and *in vivo* model (Kovářů and Kovářů 2005; Páv *et al.* 2008).

Effects of G alpha q/11 subunit induce PLC cascade activation, and increase of inositol 1,4,5-triphosphate production (IP3), and vice versa in our estimations correlated both decreased G alpha q/11 subunit lower IP3 formation (Kovářů *et al.* 1998;.Kovářů et al.2000). PLC pathway and IP3 stimulate release of Ca^{2+} from intracellular stores and Ca^{2+}- dependent kinases CaMK I and IV, and p38-MAPK are subsequently activated, etc (Shaywitz and Greenberg 1999; Hisaoka *et al.* 2008*).* Ca^{2+} -dependent kinase activation in the antidepressant induced CREB phosphorylation via G alpha q/11 cascade activation was observed (Tiraboshi *et al.* 2004). Importance of G alpha q/11 initiated cascade is supported by showing antidepressant effect of elevation protein kinase C levels (Sun and Alkon 2005). Other consequences of intracellular signalling by antidepressants are not excluded.,e.g. we found apoptic events in fluoxetine affected C6 glioma cells (in little population) in contrast to no change in cells treated with tricyclic antidepressants (Španová *et al.* 1997).

G alpha q/11 also regulates MAP kinase cascade, probably via transactivation mechanisms, participation of beta-gamma subunit complex is also suggested (Hawes *et al.* 1995; Peavy *et al.* 2001). When we estimated fluoxetine induced effects on G alpha and G beta subunits, also G beta subunit in C6 glioma cells was increased (Kovářů *et al.* 2001). Serotonin 5HT-2 receptors, implicated in the depression etiopathogenesis, or the actions of some antidepressants, increase phosphoinositide signalization. (Tyeryar and Undie 2007). This direct effect upon G alpha subunit levels can not be excluded and thus interfere with serotonin receptor activation. The effect of sertaline on the G-protein subunit levels of C6 glioma cells therefore corresponds to the above discussed results mainly in the G alpha q/11 cascade modulation.

Moclobemide in our experiment during short-term exposition shows no influence on the G alpha q/11 subunit levels in vitro (Fig 1,2). But long-term administration in vivo using rat model leads to the significant elevation of the G alpha s and G alpha i1,2 subunit levels in brain tissue (Fig 3).. These findings, again, cannot be compared with other results because of lacking data in the literature. There is an observation, demonstrating that long-term, but not acute, moclobemide treatment significantly increased cAMP binding to the PKA in the rat brain cortex (Mori *et al.* 1998). The difference between moclobemide induced changes in our in vitro and in vivo models emphasizes an important component of tissue activity modulation in the action of moclobemide (most probably MAOA activity) with limited direct modulation of postreceptor G-protein signalling machinery in C6 glioma cells. Moclobemide thus appears to modulate signalization in the G alpha s/i1,2 pathways in vivo, interfering with elevation of synaptic neurotransmitter levels and modulation of more receptor function, long–term exposition of moclobemide results in the beta-adrenoreceptor desensitization (Klimek *et al.* 1991).

Comparative analyses of TCA antidepressants imipramine and desipramine *in vitro* and *in vivo* proved no significant changes in G alpha subunit profiles. Results show different mode of TCA action in receptor-G protein-effector transmembrane signalling cascade. Since 1983 it has been known that TCA antidepressants facilitate G-protein activation of adenylyl cyclase without altering G-protein content (Menkes *et al.* 1983; Chen and Rasenick 1995). Despite receptor down-regulation, clinically effective antidepressant treatment increases adenylyl cyclase activity independently of the receptor system (5-HT1A, beta-adrenoreceptors), this so-called uncoupling is considered to be one of the crucial steps in the chronic TCA antidepressant effect (Chen and Rasenick 1995,). These findings are in agreement with older data showing release of activated G alpha s from membrane to the cytoplasm (Ransnas and Insel 1989, Rasenick *et al.* 1995). Chronic antidepressant treatment of C6 glioma cells prevents G alpha s subunit accumulation in cytoskeletal-associated cholesterol and sphingolipid-rich detergent-resistant plasma membrane domains (membrane rafts), causing its redistribution to the cytoplasm (Toki *et al.* 1999; Donati *et al.* 2003; Donati and Rasenick 2005).

Besides the use as a postsynaptic compartment model, C6 glioma cell line can be also considered as a model of astrocytic cell. There are findings showing that glial cell dysfunction may contribute to the pathogenesis of depression and participate in antidepressant action (Manev *et al.* 2003; Lee *et al.* 2007; Rajkowska and Miguel-Hidalgo 2007). Antidepressant induced changes in the cAMP system in astrocytes exert prominent influence upon cellular shape, reorganization of membrane compounds and expression of membrane receptors and transporters (Perea and Araque 2005). Stimulation of cAMP pathway participates in the phosphorylation and activation of nuclear factors, such as CREB with subsequent production of neurotrophic factors relevant to depression including BDNF and GDNF

(Hisaoka *et al.* 2001; 2008). In the production of neurotrophins, such a NGF or BDNF by glia is also involved Gαq/11 initiated cascade as described above (Miklič *et al.* 2004; Hisaoka *et al.* 2001; 2008). This may constitute another mode of antidepressant effect which can interfere with inhibition of uptake and/or direct modulation of postsynaptic signalling cascades in neuronal cells, see review (Páv *et al.* 2008).

CONCLUSION

In conclusion, the above discussed results show modulation of C6 glioma cell G alpha subunit profiles of main G protein subtypes of C6 glioma cell and the effects of sertraline, citalopram (SSRI) and moclobemide (RIMA), studied also in vivo. These drugs differ in the pharmacological modes of action, and we estimated that their influences upon C6 glioma cell G alpha subunit levels are antidepressant type dependent. In contrast to tricyclic antidepressants which do not significantly influence G alpha subunits levels, newer drug molecules modulate G-protein subunit profiles in a much larger extent. Our comparative study of the antidepressants can contribute to idea of signalling diversity of different classes of antidepressants that act through a postsynaptic transmembrane signalling mechanism toward intracellular targets. Our results thus offer possibility of considering modulation of the astrocytic cell type signalling by antidepressant influence. This mechanism can be taken in account when attempting to elucidate antidepressant effect on the complex brain environment and processes of neuroplasticity. Further intensive research is therefore needed to elucidate more detailed antidepressant mechanism of the signalling networks in the neuronal and glial cell populations.

Acknowledgements. This study was supported by grant of Academy of Sciences of Czech Republic, No. IAA 601680801 and grants of Ministery of Education of Czech Republic, No. 6215712403 and No.MSM 0021620849.

REFERENCES

1 Avissar S, Nechamkin Y, Roitman G, Schreiber G (1998). Dynamics of ECT normalization of low G protein function and immunoreactivity in mononuclear leukocytes of patients with major depression. Am J Psychiatry. **155**: 666–671.
2 Avissar S, Schreiber G (2006). The involvement of G proteins and regulators of receptor-G protein coupling in the pathophysiology, diagnosis and treatment of mood disorders. Clin Chim Acta. **366**: 37–47.
3 Chen J, Rasenick MM (1995). Chronic treatment of C6 glioma cells with antidepressant drugs increases functional coupling between G protein (Gs) and adenylyl cyclase. J Neurochem. **64**: 724–732.
4 Donati JR, Rasenick MM (2003). G protein signalling and the molecular basis of antidepressant action. Life Sci. **73**: 1–17.
5 Donati RJ, Rasenick MM (2005). Chronic antidepressant treatment prevents accumulation of Gs alpha in cholesterol-rich, cytoskeletal-associated, plasma membrane domains (lipid rafts). Neuropsychopharmacology. **30**: 1238–1245.
6 Donati RJ, Dwivedi Y, Roberts RC, Conley RR, Pandey GN, Rasenick MM (2008). Postmortem brain tissue of depressed suicides reveals increased Gs alpha localization in lipid raft domains where it is less likely to activate adenylate cyclase. J Neurosci. **28**: 3042–3050.
7 Duman R (2004). Role of neurotrophic factors in the aetiology and treatment of mood disorders. Neuromolecular Med. **5**: 11–25.
8 Emamghoreishi M, Warsh JJ, Sibony D, Li PP (1996). Lack of effect of chronic antidepressant treatment on Gs and Gi alpha-subunit protein and mRNA levels in the rat cerebral cortex. Neuropsychopharmacology. **15**: 281–287.
9 Fišerová A, Kovářů H, Hajduová Z, Mareš V, Starec M, Křen V, Flieger M, Pospíšil M (1997). Neuroimmunomodulation of natural killer (NK) cells by ergot alkaloid derivates. Physiol Res. **46**: 119–125.
10 Hawes BE, van Biesen T, Koch WJ, Luttrell LM, Lefkowitz RJ (1995). Distinct pathways of G(i)- and G(q)-mediated mitogen-activated protein kinase activation. J Biol Chem. **270**: 17148–17153.
11 Hisaoka K, Nishida A, Koda T, Miyata M, Zensho H, Morinobu S, Ohta M, Yamawaki S (2001). Antidepressant drug treatments induce glial cell line-derived neurotrophic factor (GDNF) synthesis and release in rat C6 glioblastoma cells. J Neurochem. **79**: 25–34.
12 Hisaoka K, Maeda N, Tsuchioka M, Takebayashi M (2008). Antidepressants induce acute CREB phosphorylation and CRE-mediated gene expression in glial cells: a possible contribution to GDNF production. Brain Res. **1196**: 53–58.
13 Hubbard KB, Hepler JR (2006). Cell signalling diversity of the Gq alpha family of heterotrimeric G proteins. Cell Signal. **18**: 135–150.
14 Jenab S, Quinone-Jenab V (2002). The effects of interleukin-6, leukaemia inhibitory factor and interfero-gamma on STAT DNA binding and c-fos mRNA in cortical astrocytes and C6 glioma cells. Neuroendocrinol Lett. **23**: 325–328.
15 Kitzlerová E, Anders M (2007). The role of some new factors in the pathophysiology of depression and cardiovascular disease: Overview of recent research. Neuro endocrinol Lett. **28**(6): 832–840.
16 Klimek V, Zak-Knapik J, Maj J (1991). Antidepressants given repeatedly increase the α1-adrenoceptor agonist affinity in the rat brain. Pol J Pharmacol Pharm. **43**: 347–352.
17 Koe BK, Lebel LA (1995). Effects of serotoninergic agents on down regulation of beta-adrenoceptors by the selective serotonin reuptake inhibitor sertraline. Arch Int Pharmacodyn Ther. **329**: 231–244.
18 Kovářů H, Fišerová A, Španová A, Lisá V, Fišar Z, Velek J. (1998a): Antidepressants as neuroimmunomodulators at postreceptor level. J Neuroimmunology. **90**: 41–42.
19 Kovářů H, Kovářů F, Žižkovský V, Velek J. (1998b): Developmental changes of trimeric GTP-binding proteins in porcine brain and immune system. Acta Vet, Brno, **67**: 15–20.
20 Kovářů H, Fišerová A, Kovářů F, Paclt I, Lisá V (2000). Antidepressant or immunomodulator induced regulation of cell signalling in brain and immune system. Eur Neuropsychopharmacol. **Suppl. 3**, 269–270.
21 Kovářů H, Fišerová A, Kovářů F, Pospíšil M, Lisá V (2001). Modulation of heterotrimeric GTP-binding proteins in immune system and brain. Czech J Anim Sci. **46**: 62–67.
22 Kovářů H, Kovářů F (2005). Basis of neuroimmunomodulation. In: Základy neuroimmunomodulace. Galén, Praha, pp. 203–208.
23 Lee Y, Gaskins D, Anand A, Shekkar A (2007). Glia mechanisms in mood regulation: a novel model of mood disorders. Psychopharmacology (Berl). **19**: 55–65.
24 Lesch, KP, Manji HK (1992a). Signal transducing G proteins and antidepressant drugs: evidence for modulation of alpha subunit gene expression in rat brain. Biol Psychiat. **32**: 549–579.

25 Lesch KP, Hough CJ, Aulakh CS, Wolozin BL, Tolliver TJ, Hill JL Akioyoshi J, Chuang DM, Murphy DL (1992b). Fluoxetine modulates G protein alpha s, alpha q, and alpha 12 subunit mRNA expression in rat brain.Eur J Pharmacol. **277**: 233–237.
26 Luttrell LM (2006). Transmembrane signalling by G protein-coupled receptors. Methods Mol Biol. **332**: 3–49.
27 Maes M (2001). The immunoregulatory effect of antidepressants. Hum Psychopharmacol. **16**: 95–103.
28 Maes M (2008). The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuroendocrinol. Lett. **29**: 287–291.
29 Manev H, Uz T, Manev R (2003). Glia as a putative target for antidepressant treatments.J Affect Disord. **75**: 59–64.
30 Manji HK, Chen G, Shimon H, Hsiao JK, Potter WZ, Belmaker RH (1995). Guanine nucleotide-binding proteins in bipolar affective disorder. Effects of long-term lithium treatment. Arch Gen Psychiatry. **52**: 35–44.
31 Mareš V., Giordano P.A., Pellicciari C., Scherini E., Lisá V., Bottone M.G., Bottiroli G.(1991): Changes in cell cycle and chromatin distribution in C6 glioma cells treated by dibutyryl cyclic AMP. Cell Proliferation, **24**: 569–577.
32 Menkes DB, Rasenick MM, Wheeler MA, Bitensky MW (1983). Guanosine triphosphate activation of brain adenylate cyclase: enhancement by long-term antidepressant treatment. Science **219**: 65–67.
33 Miklič Š, Jurič DM,Čarman-Kržan M (2004). Differences in the regulation of BDNF and NGF synthesis in cultured neonatal rat astrocytes. Int. J. Dev. Neurosci. **22**: 119–130.
34 Milligan G (1988). Techniques used in the identification and analysis of function of pertussis toxin-sensitive guanine nucleotide binding proteins. Biochem J. **255**: 1–13.
35 Mori S, Zanardi R, Popoli M, Garbini S, Brunello N, Smeraldi E, Racagni G, Perez J (1998). cAMP-dependent phosphorylation system after short- and long-term administration of moclobemide. J Psychiatr Res. **32**: 111–115.
36 Nair A, Vaidya VA (2006). Cyclic AMP response element binding protein and brain-derived neurotrophic factor: molecules that modulate our mood? J Biosci. **31**: 423–434.
37 Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002). Neurobiology of depression. Neuron. **34**: 13–25.
38 Páv M, Kovářů H, Fišerová A, Havrdová E, Lisá V (2008). Neurobiological aspects of depressive disorder and antidepressant treatment: role of glia. Physiol Res. **57**: 151–64.
39 Pacheco MA, Stockmeier C, Meltzer HY, Overholser JC, Dille GE, Jope RS (1996). Alterations in phosphoinositide signalling and G-protein levels in depressed suicide brain. Brain Res. **723**: 37–45.
40 Peavy RD, Chang MS, Sanders-Bus E, Conn PJ (2001). Metabotropic glutamate receptor 5-induced phosphorylation of extracellular signal-regulated kinase in astrocytes depends on transactivation of the epidermal growth factor receptor. J Neurosci. **21**(24): 9619–9628.
41 Perea G, Araque A (2005). Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. J Neurosci. **25**: 2192–2203.
42 Rajkowska G, Miguel-Hidalgo JJ (2007). Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets. **6**(3): 219–233.
43 Ransnas LA, Insel PA (1989).Quantification of a guanine nucleotide binding regulatory protein by an enzyme-linked immunosorbent competition assay. Anal Biochem. **176**: 185–190.
44 Španová A., Kovářů H, Lisá V, Lukášová E, Rittich B (1997). Estimation of apoptosis in C6 glioma cells treated with antidepressants. Physiol Res. **46**: 161–164.
45 Spiegel AM (1996). Defects in G protein-coupled signal transduction in human disease. Ann Rev Physiol., **58**: 143–170.
46 Sun K, Alkon L (2005). Dual effects of bryostatin-1 on spatial memory and depression. Eur J Pharmacol. **512**: 43–51.
47 Tadokoro C, Kiuchi Y, Yamazaki Y, Nara K, Oguchi K, Kamijima K (1997). Behavioural stimulation without alteration of beta- and 5-HT receptors and adenylate cyclase activity in rat brain after sertraline administration. Psychopharmacology (Berl). **130**: 124–130.
48 Tadokoro C, Kiuchi Y, Yamazaki Y, Oguchi K, Kamijima K (1998). Effect of imipramine and sertraline on protein kinase activity in rat frontal cortex. Eur J Pharmacol. **342**: 51–54.
49 Tardito D, Perez J, Tiraboschi E, Musazzi L, Racagni G, Popoli M (2006). Signalling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. Pharmacol Rev. **58**: 115–134.
50 Tijjsen P (1993): Practice and theory of enzyme immunoassays, Elsevier, Amsterdam, London, N,York, Tokyo: 329– 447.
51 Tiraboshi E, Tardito D, Kasahara J, Morash S, Pruneri P, Gennarelli M, Racagni G, Popoli M (2004). Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. Neuropsychopharmacology. **29**: 1831–1840.
52 Tyeryar KR, Undie AS (2007). Tandem regulation of phosphoinositide signalling and acute behavioural effects induced by antidepressant agents in rats. Psychopharmacology (Berl). **193**: 271–282.
53 Wettschureck N, Offermanns S (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev. **85**: 1159–1204.
54 Young LT, Li PP, Kish SJ, Siu KP, Kamble A, Hornykiewicz O, Warsh JJ (1993). Cerebral cortex Gs alpha protein levels and forskolin stimulated cyclic AMP formation are increased in bipolar affective disorder. J Neurochem. **61**: 890–898.