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

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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 treatment and depression etiopathogenesis.

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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. 1992b). 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 transduction 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; Wettshallreck and Offermanns 2005). These receptors are coupled to limited repertoire of G proteins and interact with few effector molecules that include adenylylcyclase, 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; Wettshallreck and Offermanns 2005).

Many data suggest that long-term antidepressant treatment is facilitating signalization cascade initiated by the G alpha s subunit, activation of adenylylcyclase, 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 Ca2+/calmodulin-dependent kinases signalling to cell nucleus (Shaywitz and Greenberg 1999; Tiraboschi 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 adenylylcyclase 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 and Offermanns 2008). 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.
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 G alpha 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 25M 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 humidified atmosphere of 95% of air and 5% of CO2 at 37°C under standard conditions 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 1000xg, and cholate membrane extracts were prepared with anti-proteolytic solution and stored at –80°C. 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 dekapptides 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 dekapptides 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 inhibition 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 G alpha q/11 and G alpha s level estimation and Maxisorp microtitration plates (NUNC) for Ga i1,2 subunit. Shortly, first antibody was monospecific rabbit antibody against G alpha subunit tested.

Immuochemical staining with alkaline phosphatase conjugated with goat antirabbit IgG and p-nitrophenyl phosphate as substrate was used (Tijjsen 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, melatonoine, 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-
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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 – 2fluoxetine 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; Kovářová 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 (Kovářová 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 Ca2+ from intracellular stores and Ca2+-dependent kinases CaMK I and IV, and p38-MAPK are subsequently activated, etc (Shaywitz and Greenberg 1999; Hisaoka et al. 2008). Ca2+-dependent kinase activation in the antidepressant induced CREB phosphorylation via G alpha q/11 cascade activation was observed (Tiraboschi 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. (Tyryar 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 signifi-
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(Hisaoka et al. 2001; 2008). In the production of neurotrophins, such a NGF or BDNF by glia is also involved. Gaq/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 (Pav 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.

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