Effect of fluoxetine and adenosine receptor NECA agonist on G alpha q/11 protein of C6 glioma cells

Hana Kovářů 1, František Kovářů 2, Věra Lisá 3
1 1st Faculty of Medicine in Prague, Charles University, Czech Republic
2 University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
3 Institute of Physiology, Academy of Sciences CR, Prague, Czech Republic

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

Submitted: 2012-09-28  Accepted: 2012-10-07  Published online: 2012-11-15

Key words: C6 glioma cells; 1,4,5 IP3; fluoxetine; selective serotonin reuptake inhibitor, SSRI antidepressant; trimeric GTP binding protein; NECA agonist; adenosine receptor; G alpha q/11 signalling; G protein coupled receptor

Abstract

OBJECTIVES: Trimeric G-proteins play a crucial role in the transmembrane signalling to intracellular pathways via effector phospholipase C (1,4,5 IP3) or adenylylcy clase (cAMP). G-protein modulation is considered to participate in the antidepressant mode of action by neurotransmitter G-protein coupled receptors (GPCR). Adenosine is naturally occurred nucleoside and adenosine receptor belongs to GPCR family. Properties and functions of ubiquitous adenosine receptor were described with number of agonists and antagonists.

METHODS: In C6 glioma cells, we studied acute administration of SSRI antidepressants – fluoxetine, sertraline and citalopram. We used immunochemical estimation (ELISA) of the main types of G-protein alpha subunits from isolated membranes of C6 glioma cells. We also estimated effect of NECA agonist on fluoxetine induced signalling via 1,4,5 IP3 and its levels.

RESULTS: Results show involvement of the antidepressant drugs in the C6 glioma signal transduction cascades and their modulation in dependence on the antidepressant of SSRI type. We measured main G alpha protein profiles after fluoxetine, sertraline and citalopram administration. We found significant changes as following: decreased G alpha Gq/11 for fluoxetine, low G alpha s for sertraline and both high G alpha q/11 and high G alpha s for citalopram. Furthermore the NECA (5’-N-ethylcarboxamido- adenosine) agonist of adenosine receptor alone evoked high decrease of G alpha q/11 levels. Whereas fluoxetine influenced G alpha q/11 decline was abolished by NECA in concentration manner, especially at 10^{-8} and 10^{-9} M concentrations. These results support abolishment NECA effect on fluoxetine influenced 1,4,5 IP3 signalling via PLC.

CONCLUSION: Main G alpha profiles are dependent on SSRI type antidepressant. Abolishing both fluoxetine evoked G alpha q/11 and and 1,4,5 IP3 signalling can indicate parallel interference between G-protein coupled receptors (GPCR) and the cell response. Presented data are first findings about adenosine receptor interaction with fluoxetine signalling. Thus in vitro studies contribute to the clarification of the molecular basis of antidepressant action.
INTRODUCTION

The selective serotonin reuptake inhibitor (SSRI) fluoxetine is one of the most widely used antidepressant. The other commercially available SSRIs include sertraline, citalopram, escitalopram, fluvoxamine and paroxetine (Páv et al. 2008; Sghendo & Mifsud 2012).

The SSRI antidepressants are used to augment the actions of serotonin (5-hydroxytryptamine, 5-HT) in the nervous system. Serotonin plays a pivotal role in the regulation of mood. Although it is generally accepted that 5-HT-mediated processes are fundamental in depressive aetiology, a consensus regarding the specific 5-HT transporter and 5-HT receptor subtype alterations has not been reached (Millan 2006; Tardito et al. 2006, Páv et al. 2008; Sghendo & Mifsud 2012).

Seven serotonin receptors, members of trimeric G protein-coupled receptor (GPCR) family have been discovered to date. All serotonin receptors, except 5-HT3, are trimeric G-protein coupled receptors that activate an intracellular second messenger cascade. The 5-HT3 receptor is a ligand-gated ion channel members of G protein-coupled receptor (GPCR) family (Wettschureck & Offermanns 2005; Avissar and Schreiber 2008; Sghendo & Mifsud 2012). Furthermore, 5-HT1A receptors are known as autoreceptors since their stimulation inhibits the release serotonin in nerve terminals. Transmembrane signalling systems consist of heptahelical GPCR receptors that are coupled with limited repertoire of G proteins and interact with few effector molecules, including adenylyl cyclase (AC), phospholipase C (PLC), phospholipase A2, cyclic-GMP phosphodiesterase and others (Wettschureck Offermanns 2005; Hubbard & Hepler 2006).

Among the GPCR of serotonin type, 5-HT2C receptors still raise particular attention in view of their implication in many physiological functions and a spectrum of psychiatric disorders (Millan 2006). Serotonin 5-HT2C receptor is a Gq-coupled receptor exhibiting a high degree of constitutive activity toward phospholipase C effector pathway, a process regulated by receptor mRNA editing (Labasque et al. 2010).

In the family of GPCR receptors, adenosine receptors, AR (P1 purinoceptors) are involved and are divided to A1, A2A, A2B, and A3 AR. Adenosine is a naturally occurring nucleoside, which exerts its physiological/biological effects by interacting with a family of adenosine receptors known as A1, A2A, A2B, and A3, (Cohen et al. 2010). The A(2B) subtype and A2A receptor couple to stimulation of adenyl cyclase and phospholipase C. The A(2B) receptors show a ubiquitous distributions. NECA is one of the most potent A(2B) adenosine receptor agonist (Baraldi et al. 2009). Furthermore, high-affinity A1 and A2A AR subtypes are known in contrast to low-affinity A2B and A3 AR subtypes (Baraldi et al. 2009). But the sensitivity of A2B adenosine receptors can be greatly increased by interaction with protein kinase C (Baraldi et al. 2009, Cohen et al. 2010).

Rat C6 glioma cells are an established and useful model for long time. C6 glioma cells are a suitable model to study the mechanism of antidepressant action because of no synaptic structures. The C6 glioma cells are also used in analyzes of antidepressant effects on Galpha subunit levels or production of growth factors, cytokines and gene expression (Hisaoka et al. 2007, Tsuchioka et al. 2008, Choi et al. 2011; Kovářů et al. 2000; 2011). Furthermore, C6 glioma cells have been shown to respond to antidepressants in a manner similar to primary astroglial cells (Hisaoka et al. 2007). In C6 glioma cells, role of 5HT2 receptors was examined in relationship ERK phosphorylation and the mechanism of 5-HT2 receptor-induced glia cell line-derived neurotrophic factor (GDNF) mRNA expression and a participation G alpha q/11 (Tsuchioka et al. 2008). Also adenosine receptors A1, A2A, A2B, and A3 were detected in C6 glioma cells (Castillo et al. 2007).

Here, we examined the acute effect of fluoxetine, sertraline and citalopram of SSRI group on G alpha subunit profiles of main G-protein types in C6 glioma cells. In other experiments, a possible role of adenosine receptor NECA agonist (5’-N-ethylcarboxamido-adenosine) on fluoxetine influenced G alpha q/11-protein signalling via PLC effector and second messenger 1,4,5 IP3 formation by the enzyme was also analysed.

MATERIAL AND METHODS

C6 glioma cells

Cells were cultured in MEM medium, pH 7.3 with 5% fetal calf serum under standard conditions. Confluent cultures were exposed to antidepressant (final 1 μM) for up to 24 h (acute model). Then cells were washed with PBS, harvested by scraping with rubber policeman in PBS, containing proteolytic inhibitors, and cholate membrane extracts were prepared as described previously (Kovařů et al. 2011).

G alpha subunit estimation

G alpha chains ganges were analysed by ELISA technique with our rabbit antibodies against synthetic C-terminal dekapptides of alpha chains of Gs, Gi1,2 and Gq/11. ELISA method of competitive inhibiton was performed and modified by use of Maxisorp microtitration plates (NUNC) for noncovalent peptide binding.
ELISA method assay estimation was compared with Western immunoblotting (Kovařů et al. 1998; 2011). Used monospecific antibodies against individual G alpha dekapeptides were without cross reactivity. Synthetic dekapeptides were prepared in the Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague, Czech Republic, and primary amino acid sequence of dekapeptides was checked by sequence analysis (Kovařů et al. 1998). For other details see Kovařů et al (2010).

The 1,4,5 IP3 was estimated according to protocol of TRK1000 assay system (Amersham Biosciences, UK).

**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.

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**RESULTS AND DISCUSSION**

Figure 1 summarizes the acute effect of fluoxetine, sertraline and citalopram of SSRI type on C6 glioma cells. Results indicate that SSRI antidepressants induce different G alpha subunit profiles as followed: significantly decrease of G alpha q/11 for fluoxetine, low G alpha s for sertraline and both significantly low G alpha q/11 and high G alpha s for citalopram. Results show that each SSRI antidepressant provoked various regulation of one or more G alpha subunits via both signalling effectors 1,4,5 IP3 or cAMP, 2nd messengers. On the other hand, general knowledge is traditional in relationship to a chronic mode of administration that despite the very different molecular structures of SSRIs, they all have similar mechanisms of action, albeit with slightly different pharmacokinetic properties (Sghendo and Mifsud 2012).

Results show highly significant reduction of membrane G alpha q/11 level confirmed by highly decreased 1,4,5 IP3 concentration (Figure 2), i.e. reduced signalling via G alpha q/11 to effector PLC. Beside this we determined in another study fluoxetine induced translocation of membrane G alpha q/11 subunit into cytosol of C6 glioma cells as a part of desensitization mechanisms (Kovařů et al. in preparation). Furthermore, in C6 glioma (astrocytoma) cells, fluoxetine effects can involve probably 5-HT2B receptor linked to G alpha q/11 pathway (Tsuchioka et al. 2008, Zhang et al. 2010, Choi et al. 2011). Possible role of 5-HT2B in various SSRI antidepressant-induced can be in accordance with participation of HT2B in multipathway cascades of transmembrane signalling (Baraldi et al. 2010, Cohen et al. 2010). However A3R role is not excluded because of G alpha q/11 signalling (Baraldi et al. 2009, Cohen et al. 2010, Labasque et al. 2010).

On the other hand, fluoxetine was able to induce via G-protein coupled receptors pro-apoptotic event leading to C6 glioma cell apoptosis, indicating distinct pathways in the cell response (Španova et al. 1997; Levkovitz et al. 2005, Kovařů et al. – in preparation). In contrast to G protein signalling (Figure 2) NECA agonist exhibited potentiation effect on fluoxetine induced apoptosis in C6 glioma cells (Kovářů et al. 2000). Beside this sertraline alone (signalling via G alpha s, Figure 1) or sertraline with NECA were not able to induce the apoptic response (Kovářů et al. 2000). Relationship could be linked to various signalling pathways for fluoxetine via major Gq/11 and sertraline via major Gs under these conditions.

Figure 2 show the acute effect of fluoxetine on C6 glioma cells measured by G alpha q/11 signalling and 1,4,5 IP3 levels. Results indicate highly significant reduction of membrane G alpha q/11 level confirmed by highly decreased 1,4,5 IP3 concentration i.e. reduced signalling via G alpha q/11 to effector PLC. The 5-HT2 receptors are present in C6 glioma cells and they are coupled via G alpha q/11 protein to the signalling via
PLC and stimulation of PI hydrolysis (Tsuchioka et al. 2008, Zhang et al. 2010). But A3R role is not excluded because of a role in G alpha q/11 signalling (Baraldi et al. 2009, Cohen et al. 2010).

The NECA AR agonist effect on G protein signalling (Figure 2). We estimated NECA agonist evoked marked decrease of G alpha q/11 level in C6 glioma cells. The G alpha q/11 pathway could indicate a role of A2B subtype of AR receptor (Tsuchioka et al. Cohen et al. 2010). NECA is a potent A2B adenosine receptor agonist and can be involved in the response (Baraldi et al. 2009).

Combined effect of fluoxetine and NECA AR agonist (Figure 2). Our results show that fluoxetine or NECA agents act via Gq/11 on effector PLC system, indicated by 2nd messenger 1,4,5 IP3 formation. Furthermore, fluoxetine induced decrease of signalling by NECA is regulated in NECA dose dependent manner. Abolished G alpha q/11 signalling by fluoxetine was dependent on increased NECA concentration and was confirmed by declined levels of 1,4,5 IP3 formation by PLC of C6 glioma cells. Data should suggest a possibility of a cooperation between fluoxetine and NECA induced GPCR receptors. It seems that NECA agonist of adenosine receptor (A2B) influenced fluoxetine transmembrane signalling. It was evident that both G alpha q/11 levels and 1,4,5 IP3 signalling were very sensitive to low NECA concentration at 10⁻⁸M and 10⁻⁹M to its strong abolishing effect.

To date there are very limited data in literature about possibility of adenosine receptor interference with 5-HT receptor or 5-HT transporter signalling. Recently, evidence was presented that A3 adenosine receptor AR was colocalized with 5-HT transporter (5-HTT) in mouse midbrain serotonergic neurons (Zhang et al. 2011). The A3AR can exist within 5HT transporter complexes and the abundance of A3AR/5-HTT complexes was enhanced in a protein kinase G of I type (PKGI) dependent manner. Mechanisms by which A3 adenosine receptor activity control activation of 5-HTT proteins require an understanding whether regulation is indirect or is mediated by more confined, physical interactions or pathways coordinating A3AR signalling to 5-HT transporter can be spatially restricted and regulated. In presence, compartmentalizing mechanisms by which GPCRs can target one or more of these modulators to regulate 5-HT transporter without influencing other cytosolic and membrane effectors are unknown (Zhang et al. 2011).

Finally, our experiments are first finding about adenosine receptor effect on fluoxetine signalling. Undoubtedly, more detailed knowledge remains to be elucidated in an area cooperation of GPCR receptors in transmembrane signalling. However, in vitro studies contribute to the clarification of the molecular basis of antidepressant action and its regulation.

