Antidepressants elevate GDNF expression and release from C6 glioma cells in a β-arrestin1-dependent, CREB interactive pathway

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Abstract

Glial cell line-derived neurotrophic factor (GDNF), essential for neuronal survival, plasticity and development, has been implicated in the mechanism of action of antidepressant drugs (ADs). β-arrestin1, a member of the arrestin protein family, was found to play a role in AD mechanism of action. The present study aimed at evaluating whether the effect of ADs on GDNF in C6 rat glioma cells is exerted through a β-arrestin1-dependent, CREB-interactive pathway. For chronic treatment, C6 rat glioma cells were treated for 3 d with different classes of ADs: imipramine – a non-selective monoamine reuptake inhibitor, citalopram – a serotonin selective reuptake inhibitor (SSRI) or desipramine – a norepinephrine selective reuptake inhibitor (NSRI) and compared to mood stabilizers (lithium and valproic acid) or to the anti-psychotic haloperidol. Only ADs significantly elevated β-arrestin1 levels in the cytosol, while reducing phospho-β-arrestin1 levels in the cell nuclear fraction. ADs significantly increased both GDNF expression and release from the cells, but were unable to induce such effects in β-arrestin1 knock-down cells. Chronic AD treatment significantly increased CREB phosphorylation without altering the level of total CREB in the nuclear fraction of the cells. Moreover, treatment with ADs significantly increased β-arrestin1/CREB interaction. These findings support the involvement of β-arrestin1 in the mechanism of action of ADs. We suggest that following AD treatment, β-arrestin1 generates a transcription complex involving CREB essential for GDNF expression and release, thus enhancing GDNF’s neuroprotective action that promotes cellular survival and plasticity when the survival and function of neurons is compromised as occurs in major depression.

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Introduction

The biochemical mechanisms underlying the pathophysiology of mood disorders and the activities of antidepressant drugs (ADs) have been the subject of active research for more than 60 yr. ADs were shown to increase synaptic concentrations of monoamines, leading to hypotheses concerning the involvement of monoamines in the mechanisms of action of ADs and in the pathogenesis of major depression (Maes & Meltzer, 1995; Schatzberg & Schildkraut, 1995; Schildkraut, 1965). However, these hypotheses did not provide an adequate explanation for the lag period of 2–3 wk needed for ADs to exert therapeutic actions (Nestler et al. 2002). Thus emphasis was shifted from the acute presynaptic events of ADs to delayed postsynaptic adaptations at the receptor and post-receptor levels including modulations and alterations in G-protein-coupled receptor (GPCR) densities, GPCR-G protein coupling, post-receptor signalling cascades, and gene expression, leading to changes in neuroplasticity, cellular resilience and synaptic plasticity.
β-arrestins, a family of proteins initially known as negative regulators of GPCR-mediated signalling (Gainedinov et al. 2004; Lefkowitz, 2004; Lohse et al. 1990; Stephen & Lefkowitz, 2002), were discovered to play new roles as multi-protein scaffolds and adaptors in receptor endocytosis and signalling cascades in various MAP kinase modules, e.g. ERK, p38, JNK, independently of G protein activation (Gurevich & Gurevich, 2003; Kristen & Lefkowitz, 2001; Lefkowitz & Whalen, 2004; Luttrell & Lefkowitz, 2002; Shenoy & Lefkowitz, 2003). Moreover, it is now known that β-arrestin1 can translocate to the nucleus and associate with different transcription factors (Beaulieu & Caron, 2005; Kang et al. 2005; Ma & Pei, 2007).

Findings in various research models support a major role for β-arrestins in the pathophysiology of mood disorders as well as in the mechanism of action of ADs. β-arrestin1 levels are significantly elevated by serotonin selective reuptake inhibitors (SSRIs), noradrenaline selective reuptake inhibitors (NSRIs), non-selective monoamine reuptake inhibitors, and MAO inhibitor ADs in rat cortex and hippocampus. This process becomes significant within 10 d and takes 2–3 wk to reach maximal increase (Avissar et al. 2004) in accordance with the timetable of clinical improvement. β-arrestin1 protein and mRNA levels in mononuclear leukocytes of untreated patients with major depression are significantly lower than those of healthy subjects. The reduction in β-arrestin1 measures is significantly correlated with the severity of depressive symptomatology (Avissar et al. 2004; Matuzany-Ruban et al. 2005). The low β-arrestin1 protein and mRNA levels are alleviated by antidepressant treatment. Normalization of β-arrestin1 measures precedes, and thus predicts clinical improvement (Matuzany-Ruban et al. 2005). Although the ubiquitously expressed β-arrestins1 and 2 are structurally highly homologous, extensive investigation of the roles of these two proteins in receptor signalling revealed differential receptor affinities, differential cellular distribution, differential scaffolding functions, and differential regulation by phosphorylation (DeWire et al. 2007; Lefkowitz & Shenoy, 2005). In a mouse model of anxiety/depression induced by chronic corticosterone, mice deficient in the gene for β-arrestin2 displayed a reduced response to fluoxetine, suggesting that β-arrestin2 is necessary for fluoxetine effects (David et al. 2009). In C6 rat glioma cells, decreased β-arrestin2 cytosolic levels were shown to result from increased β-arrestin2 ubiquitinylation and degradation induced by ADs (Golan et al. 2010).

Evidence accumulated over the last decade has laid the grounds for the formulation of new hypotheses that assign the pathophysiological basis of major depression to impaired neuroplasticity (for review see Castren et al. 2007; Pittenger & Duman, 2008) which is restored by antidepressant-increased neurogenesis and gliogenesis (for review see Banasr & Duman, 2007; Racagni & Popoli, 2008; Schmidt et al. 2008). Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) were found to play a critical role in multiple molecular mechanisms (for review see aan het Rot et al. 2009; Racagni & Popoli, 2008; Tardito et al. 2006) and epigenetic mechanisms that contribute to neuronal plasticity, in relation to the pathophysiology of major depression and to the mechanism of action of ADs (Colvis et al. 2005; Duman & Newton, 2007; Racagni & Popoli, 2008).

GDNF, originally identified as a survival factor for midbrain dopaminergic neurons (Lin et al. 1993), is a member of the transforming growth-factor-β superfamily, essential for neuronal survival, plasticity and development (Huang & Reichardt, 2001). It is well documented that ADs increase GDNF levels (Hisaoka et al. 2001, 2007; Mercier et al. 2004) and recently it was found that depressed patients demonstrated lower levels of serum GDNF (Takebayashi et al. 2006; Zhang et al. 2008) which were increased following antidepressant treatment (Zhang et al. 2008). β-arrestin1 was previously reported to act as a nuclear scaffold that recruits the histone acetyltransferase p300 to the transcription factor CREB (cAMP response element-binding protein) leading to increased acetylation of histone H4, reorganization of chromatin, thereby increasing gene expression (Kang et al. 2005). CREB plays a major role in the transcription of neurotrophic factors in general (Baeker et al. 1999; Cen et al. 2006; Koyama et al. 2004) including BDNF (Lonze & Ginty, 2002) and GDNF (Cen et al. 2006), and also following antidepressant treatment (Hisaoka et al. 2008; Tardito et al. 2006, 2009). These reports led us to examine in the present study whether β-arrestin1, through interaction with CREB, plays a role in AD-induced transcription of GDNF in C6 glioma cells.

Methods

Materials

Mouse monoclonal anti-phospho-β-arrestin1 antibody (no. 2416, 1:1000) and rabbit monoclonal anti-phospho-CREB antibody (no. 9198, 1:500) were from Cell Signaling (USA). Mouse monoclonal anti-β-arrestin1 antibody (no. 610551, 1:250) was from Transduction Laboratories (USA). Mouse monoclonal...
anti-actin antibody (no. 691001, 1:50000) was from MP Biomedicals (USA). Rabbit polyclonal anti-GDNF antibody (sc-328, 1:200), rabbit polyclonal anti-CREB-1 antibody (sc-186, 1:100), rabbit polyclonal anti-GAPDH antibody (sc-25778, 1:400) and goat polyclonal anti-lamin B antibody (sc-6216, 1:1000) were from Santa Cruz Biotecnology (USA). Horseradish peroxidase-conjugated goat anti-mouse antibody (115-035-062) and DyLight-488 donkey anti-rabbit antibody (711-485-152) were from Jackson Immuno-Research Laboratories (USA). Horseradish peroxidase-conjugated donkey anti-rabbit antibody (NA934) was from Amersham (USA). Lipofectamine2000 (11668-027) and Opti-MEM I (31985-047) were from Invitrogen (USA). G418 disulfate salt (G8168), phorbol 12,13-didecanoate (PDD, P9018) and A23187 (C7522) were from Sigma (USA).

Cell culture and drugs treatments

C6 rat glioma cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Biological Industries, Israel) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin and 100 units/ml penicillin in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Twenty-four hours after seeding in 10 cm2 plates, growth medium was replaced with fresh medium containing the desired drug at the suitable concentration: 10 μM for all ADs, 1 mM lithium, 0.6 mM valproic acid and 0.1 mM haloperidol. Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). All measurements were performed following 72 h of drug treatment.

Subcellular fractionation

C6 cells were washed three times with ice-cold phosphate-buffered saline (PBS), scraped with a rubber policeman into lysis buffer [20 mM Hepes (pH 7.4), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 10% glycerol, 1 mM MgCl2, 25 mM NaF, 1 mM Na3VO4, 20 μl/1 ml protease inhibitor cocktail] and homogenized at 25000 rpm on a Polytron (PT 1200, Kinematica AG, Switzerland) for 1 min. Cell nuclei and debris were pelleted at 600 g, 10 min, 4°C. The supernatant was further centrifuged at 20000 g for 30 min at 4°C and the cytosolic fraction was collected. The pellet containing nuclei and debris was re-suspended in 0.25 M sucrose containing 10 mM MgCl2 and 20 μl/1 ml protease inhibitor cocktail and was gently laid on 0.88 M sucrose containing 0.5 mM MgCl2 and 20 μl/1 ml protease inhibitor cocktail. The sucrose gradient was centrifuged at 2800 g for 10 min at 4°C. Clean nuclei were re-suspended in RIPA buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 20 μl/1 ml protease inhibitor cocktail], sonicated for 50 s (Sonicator ultrasonic processor, Misonix Inc., USA) and centrifuged again as above to raise a clean nuclear extract. All fractions were diluted in sample buffer 1:3 [10% v/v glycerol, 20% v/v SDS 20%, 5% v/v β-mercaptoethanol, 0.05% w/v Bromophenol Blue (pH 6.8)], boiled for 5 min at 95°C and frozen at −80°C until assayed. Aliquots were taken for protein determination using the Bradford assay.

Immunoblotting

Cytosolic and nuclear fractions were thawed on the day of assay. Protein aliquots (30 μg/lane) were taken for protein separation by SDS-PAGE as previously described (Avissar et al. 2004). Semi-quantitative analysis was performed using a computerized image analysis system (EZQuant-Gel 2.1, EZQuanbt Biology Software Solutions Ltd, Israel). In order to ensure that equal amount of total protein was loaded to each lane, the housekeeping genes GAPDH, actin or lamin B were measured using the appropriate antibody.

Short hairpin RNA (shRNA) transfection

C6 cells were stably transfected with β-arrestin1 targeted shRNA or scrambled shRNA using SureSilencing™ shRNA kit (SuperArray Bioscience Corporation, USA) according to the manufacturer’s instructions. The pGeneClip-Neo expression vector, in which the desired shRNA was cloned conveys instructions. The pGeneClip-Neo expression vector in which the desired shRNA was cloned conveys neomycin resistance. Therefore, to establish stable expression in the C6 glioma cell line, 48 h after transfection, transfected cells underwent selection with full growth medium containing 800 μg/ml G418 disulfate salt solution (Sigma) for 14 d. After selection, individual cells were removed and plated in 24-well plates, maintaining antibiotic selection pressure. Individual colonies were screened for shRNA expression using Western blot analysis. Stably transfected cells were maintained by growing them in the presence of G418 at 400 μg/ml.

Enzyme-linked immunosorbent assay (ELISA)

GDNF protein levels in cell-conditioned media were determined using a GDNF ELISA kit (GDNF Emax® ImmunoAssay System; Promega, USA) according to the manufacturer’s instructions. In brief, Maxisorp 96-well, flat-bottomed ELISA plates were coated with anti-GDNF monoclonal antibody diluted 1:1000 in carbonate coating buffer (pH 8.2, 100 μl/well) and
incubated overnight at 4 °C. The next day plates were blocked for 1 h at room temperature with Block & Sample buffer (200 μl/well). GDNF standards, ranging from 1000 to 0 pg/ml, were prepared using recombinant human GDNF serially diluted 2-fold in Block & Sample buffer. One millilitre of cell-conditioned medium collected from a single 10 cm² plate of cultured cells (treated chronically with ADs) was lyophilized, re-suspended in 300 μl growth medium and assayed (100 μl/well). Standards were also added (in duplicate) to each ELISA plate. Samples and standards were incubated at room temperature for 6 h on a plate shaker (250 rpm) and rinsed five times with 300 μl washing buffer [20 mm Tris–HCl (pH 7.6), 150 mm NaCl, 0.05% (v/v) Tween-20]. The captured GDNF was incubated with 100 μl/well chicken anti-human GDNF polyclonal antibody, diluted 1:500 in Block & Sample buffer, overnight at 4 °C. After plates were washed five times as described above, horseradish peroxidase-conjugated anti-chicken IgY antibody, diluted 1:250 in Block & Sample buffer, was added to the plates (100 μl/well) and incubated at room temperature with shaking (250 rpm) for 2 h. Plates were washed five times as described above, and the enzyme substrate (TMB One Solution), was added (100 μl/well) and incubated for 15 min at room temperature in the dark. The enzyme reaction was stopped by adding 100 μl 1 N hydrochloric acid per well, and the absorbance at 450 nm was recorded on a microplate reader (ThermoMax microplate reader, Molecular Devices, USA). Cell-derived GDNF levels were normalized against the respective CREB bands. Blots were probed with a specific β-arrestin1 antibody and developed using a chemiluminescent method (ECL). β-arrestin1 bands were normalized against the respective CREB bands.

**Immunoprecipitation**

Following the desired treatment, C6 cells were lysed and homogenized in lysis buffer as described above. Polyclonal CREB antibody (5 μg, sc-186) was cross-linked to 50 μg Dynabeads protein G (Invitrogen, USA) according to the manufacturer’s protocol. Then 500 μg protein material from cell lysates was incubated with CREB Dynabeads for 10 min at room temperature on a rotating platform. CREB-immunoprecipitated complexes were washed three times with washing buffer and eluted by boiling in 30 μl sample buffer as described above. Immune complexes were resolved in 7.5% SDS–PAGE and transferred to nitrocellulose membranes. Blots were probed with a specific β-arrestin1 antibody and developed using a chemiluminescent method (ECL). β-arrestin1 bands were normalized against the respective CREB bands.

**Immunofluorescence microscopy**

For visualization of GDNF, C6 cells plated on cover slips were treated with the desired AD as described above. Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment. Cells were then fixed in absolute methanol for 8 min, −20 °C and stained with rabbit polyclonal anti-GDNF antibody (sc-328, 1:100) diluted in PBS containing 3% BSA, 0.05% Tween-20, 0.01% Na-Azide for 2 h at room temperature, followed by a 1:400 dilution of DyLight-488 donkey anti-rabbit antibody (711-485-152) in PBS containing 3% BSA, 0.05% Tween-20, 0.01% for 2 h at room temperature. Immunofluorescence was detected by an Olympus FV-1000 Spectral confocal laser-scanning microscope with excitation at 488 nm and emission at 520 nm. Image analysis was performed using ImageJ v. 1.40C software.

**Table 1. Dose-dependent citalopram effects on β-arrestin1 protein level in C6 rat glioma cells**

<table>
<thead>
<tr>
<th>Drug concentration (μg)</th>
<th>β-arrestin1 protein level (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>100.0 ± 3.0</td>
</tr>
<tr>
<td>1</td>
<td>98.7 ± 6.7</td>
</tr>
<tr>
<td>5</td>
<td>93.9 ± 5.2</td>
</tr>
<tr>
<td>10</td>
<td>137.7 ± 5.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>137.0 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
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Twenty-four hours after C6 cells seeding, growth medium was replaced with a fresh medium containing the desired dose of citalopram. Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). The cytosolic fraction was analysed by Western blot for effects on the specific protein. Data are means ± S.E.M. of three independent experiments.

<sup>a</sup> Protein levels are given as percent of untreated control cell levels.

<sup>b</sup> p < 0.001 compared to control untreated cells.
Statistical analysis

One-way ANOVA followed by Bonferroni corrections (for multiple comparisons among all groups) was used for the statistical analyses of AD effects on β-arrestin1, phospho-β-arrestin1, GDNF, CREB and phospho-CREB. Student’s t-test was used for the statistical analyses of citalopram effects on GDNF immunofluorescence staining.

Results

C6 rat glioma cells were treated with 10μM of one of the following ADs: imipramine (a non-selective monoamine reuptake inhibitor), citalopram (a SSRI), desipramine (a NSRI), or 1 mM lithium or 0.6 mM valproic acid or 0.1 μM haloperidol for 3 d. Drug concentrations were selected from dose–response experiments and represent the minimal concentrations inducing maximal response as previously described (Golan et al. 2010). Representative dose–response data for citalopram effects on cytosolic β-arrestin1 levels in C6 rat glioma cells is presented in Table 1. Drugs effects on cytosolic β-arrestin1 and phospho-β-arrestin1 levels in C6 rat glioma cells presented in Fig. 1 indicate that all ADs elevated β-arrestin1 levels (Fig. 1a) in the cytosolic fraction with no significant change in its

Fig. 1. AD-induced increase in β-arrestin1 activity in the cytosol of C6 rat glioma cells. C6 cells were treated chronically with imipramine (Imi), citalopram (Cit), desipramine (Des), lithium (Li), valproic acid (Val) or haloperidol (Hal). Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). The cytosolic fraction was analysed by Western blot for effects on: (a) β-arrestin1; (b) phospho-β-arrestin1. Proteins levels are given as percent of control levels. Data are means ± S.E.M. of at least five independent experiments. *** p < 0.001 compared to control cells.

Fig. 2. AD-induced increase in β-arrestin1 activity in the nucleus of C6 rat glioma cells. C6 cells were treated chronically with imipramine (Imi), citalopram (Cit), desipramine (Des), lithium (Li), valproic acid (Val) or haloperidol (Hal). Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). The nuclear fraction was analysed by Western blot for effects on: (a) β-arrestin1; (b) phospho-β-arrestin1. Proteins levels are given as percent of control levels. Data are means ± S.E.M. of at least four independent experiments. *** p < 0.001 compared to control cells.
nucleus of C6 thus inducing an increase in

**Discussion**

This report demonstrates for the first time that β-arrestin1 is essential for GDNF expression and release from C6 rat glioma cells following chronic antidepressant treatment. More specifically, it is shown that chronic treatment with different classes of ADs increases β-arrestin1 activity in C6 rat glioma cells in

**Fig. 3.** ADs increase GDNF release from C6 rat glioma cells. C6 cells were treated chronically with imipramine (Imi), citalopram (Cit) or desipramine (Des). Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). Conditioned medium was assayed by ELISA for GDNF release. GDNF release is given as percent of control release. Data are means ± S.E.M. of at least three independent experiments. ***p < 0.001 compared to control cells.

non-active form: phospho-β-arrestin1 (Fig. 1b), thus suggesting an increase in the active form of β-arrestin1 in the cytosol of C6 rat glioma cells. This effect was found to be AD-selective since neither the mood stabilizers lithium and valproic acid nor the antipsychotic haloperidol had a similar effect (Fig. 1). The effects of ADs on β-arrestin1 activity were also examined in the nuclear fraction of the cell. Although ADs did not change β-arrestin1 protein levels in this fraction (Fig. 2a), chronic exposure to ADs did significantly decrease the levels of phospho-β-arrestin1 (Fig. 2b) thus inducing an increase in β-arrestin1 activity in the nucleus of C6 rat glioma cells. Again, this effect was observed only for ADs but not for the mood stabilizers or the antipsychotic used (Fig. 2).

Chronic treatment with various types of ADs resulted in increased GDNF release from C6 glioma cells, as measured in the cell-conditioned media by GDNF ELISA kit (Fig. 3). We could not detect basal BDNF levels nor increased release by AD treatment in this cell system (data not shown).

In order to find out whether AD-induced elevation in GDNF release is β-arrestin1-dependent, we examined C6 rat glioma cells transfected with β-arrestin1 targeted shRNA (Fig. 4a). In β-arrestin1 knock-down cells no AD-induced increase in GDNF release was detected (Fig. 4b). Verity et al. (1998) demonstrated that diverse biological factors are capable of modulating GDNF protein levels and that multiple signal transduction systems can regulate GDNF synthesis and/or release. Thus, we further verified whether the inability of ADs to increase GDNF release in β-arrestin1 knock-down cells is specifically related to the low levels of β-arrestin1 in these cells and whether these cells retain their inherent capacity to release GDNF by pathways that are β-arrestin1-independent, by the use of established inducers of GDNF release: PDD (a PKC-activating phorbol ester) and A23187 (a Ca2+ ionophore) (Verity et al. 1998). Figure 4(c, d) demonstrates that PDD and A23187 succeeded in augmenting GDNF release from both wild-type C6 cells and β-arrestin1 knock-down cells strengthening our assumption that AD-induced GDNF release is indeed β-arrestin1-dependent.

Additionally, the effect of ADs on GDNF expression was measured in the cytosolic fraction of C6 glioma cells by Western blot analysis. While the various types of ADs were able to increase GDNF levels in the cytosolic fraction of wild-type cells (Fig. 5a) this effect was prevented in β-arrestin1 knock-down cells (Fig. 5b). Further support to these findings was obtained by immunocytochemistry of both wild-type and β-arrestin1 knock-down cells. Cells were stained with anti-GDNF antibody following chronic treatment with citalopram. Quantification of the acquired images revealed that while an increase in GDNF levels was observed in citalopram-treated wild-type C6 rat glioma cells (Fig. 6a), no such effect could be detected in β-arrestin1 knock-down cells (Fig. 6b), further supporting the role played by β-arrestin1 in AD-induced GDNF expression.

To determine whether β-arrestin1 effect on AD-induced GDNF expression involves the transcription factor CREB, we measured AD-induced alterations in CREB expression and activity in the nuclear fraction of C6 rat glioma cells and found that ADs increased CREB phosphorylation (Fig. 7a) without altering the level of total CREB (Fig. 7b). In order to verify the possibility that β-arrestin1 forms a complex with CREB hence enabling transcription of GDNF, co-immunoprecipitation analysis of the nuclear fraction of C6 cells was performed. Figure 8 clearly demonstrates that ADs indeed induced an increase in co-immunoprecipitation of β-arrestin1 and CREB.

![Fig. 3](http://ijnp.oxfordjournals.org/)

### Fig. 3.

**Fig. 3.** ADs increase GDNF release from C6 rat glioma cells. C6 cells were treated chronically with imipramine (Imi), citalopram (Cit) or desipramine (Des). Drug-containing medium was replaced every day for 3 d in order to achieve chronic treatment (Donati et al. 2001). Conditioned medium was assayed by ELISA for GDNF release. GDNF release is given as percent of control release. Data are means ± S.E.M. of at least three independent experiments. ***p < 0.001 compared to control cells.
both cytosolic (Fig. 1) and nuclear (Fig. 2) fractions, while neither the mood stabilizers lithium and valproic acid nor the antipsychotic haloperidol had a similar effect. Furthermore, ADs also elevate GDNF expression (Figs 5a, 6a) and release (Fig. 3) from these cells in a β-arrestin1-dependent manner (Figs 4b, 5b, 6b). All classes of ADs used in the present study also increased CREB activity (Fig. 7). Taken together, these results suggest that β-arrestin1 could be a primary target for antidepressant action through which ADs elevate GDNF release from glia cells and hence contribute to neurogenesis and neuron plasticity.

Glial cells, commonly known as neuroglia or simply glia, are non-neuronal cells that in addition to their traditional roles in neuronal migration and inflammatory processes are now acknowledged to have roles in providing trophic support to neurons, in neuronal metabolism, and in the formation of synapses and neurotransmission (Cotter et al. 2001). Thus, reduced glial cell numbers and activity could be responsible for some of the pathological changes in major depression. For example, low numbers of glial cells were found in the cerebral cortex of patients with major depression (Ongur et al. 1998; Rajkowska et al. 1999). These findings led to the assumption that the lower density of glial cells plays a role in the pathological changes seen in these patients. As astrocytes are also involved in energy supply to neurons, it is assumed that the decreased metabolism found in the prefrontal cortex of patients with major depression results from a decrease in astrocyte cell numbers or activity (Magistretti et al. 1999). Moreover, since neurotrophic factors are essential for the survival and differentiation of neurons during development and the survival and function of neurons in the adult (Schinder & Poo, 2000), it is not surprising that mood disorders could result from low levels of release of neurotrophic factors due to glial cell depletion (Connor & Dragunow, 1998). Further, a growing body of evidence from human post-mortem studies has shown modifications in cortico-limbic glial cell morphology and expression of glial fibrillary acidic protein (GFAP) (Rajkowska & Miguel-Hidalgo, 2007) associated with major depressive disorder. Importantly, studies on laboratory animal models of stress...
and mood disorders have also supported the concept of an astrocytic deficit in depression (Leventopoulos et al. 2007). Fluoxetine (Czeh et al. 2006) and clomipramine (Liu et al. 2009) treatments reversed glial pathology in a chronic unpredictable stress-induced animal model of depression. ADs were also found to normalize elevated S100B protein, suggested as the serum marker of glial pathology in major depression.
Taken together the above findings emphasize the importance of glial cells to normal cortical function.

GDNF plays an important role in neurogenesis and high-ordered brain function, such as learning and memory. Increased expression of GDNF leads to neuronal survival and thus could be of high importance when the survival and function of neurons is compromised as occurs in major depression. In 2001 Hisaoka and colleagues reported that long-term use of antidepressant treatment significantly increased GDNF mRNA expression and GDNF release in time- and concentration-dependent manners in rat C6 glioblastoma cells (Hisaoka et al. 2001). The present findings conform to this previous study while using different ADs and provide additional data that suggests a possible cellular pathway for this outcome. The expression of GDNF can be modulated by various biological and/or pharmacological agents such as calcium (Oh-hashi et al. 2006), growth factors and cytokines (Verity et al. 1998), neuropeptides, prostaglandins and other intracellular signalling agents (Verity et al. 1998). The present findings demonstrate that β-arrestin1 is essential for antidepressants effect on GDNF expression and release in C6 rat glioma cells. We have previously shown that β-arrestin1 is involved in the pathophysiology of major depression, as well as in the mechanism of action of ADs (Avisar et al. 2004; Matuzany-Ruban et al. 2005). The present findings in C6 glioma cells shed some light regarding the specific role β-arrestin1 might fulfil in these pathways. Recent reports regarding β-arrestin1 function as a transcription regulator (Beaulieu & Caron, 2005; Kang et al. 2005; Ma & Pei, 2007) along with known roles of CREB in the transcription regulation of GDNF (Baecker et al. 1999; Cen et al. 2006; Hisaoka et al. 2008; Koyama et al. 2004) support our hypothesis according to which β-arrestin1 and CREB form a transcription complex essential for the production of GDNF following...
chronic antidepressant treatment. Although C6 rat glioma cells are an established and useful model for studying the phenomena of GPCR desensitization and down-regulation (Fishman & Finberg, 1987), and appear to be a suitable model to study the mechanism of action of ADs (Donati & Rasenick, 2005), the present data that the effect of ADs on GDNF in C6 rat glioma cells is exerted through a β-arrestin1-dependent, CREB-interactive pathway, should be taken with considerable caution, as findings obtained in a model of glioma cell line cannot be directly extrapolated to living organisms. Further studies are needed to elucidate the precise mechanism leading to the formation of this complex and the identity of all its components.

Even though the antidepressant mechanism of action and the biological basis of major depression have been studied for more than 60 yr, they still remain unclear. Research focus progressed along the signal transduction cascade, from studies at the level of the primary messenger and its receptor (acute pre-synaptic events) to information transduction mechanisms beyond receptors (delayed post-synaptic adaptations). Nowadays new approaches focus on pathways involved in cellular survival and plasticity. Growing evidence endorses a role for increased trophic support in the treatment of depression. Thus, components of neurotrophic factor-mediated signalling cascades or the signal transduction pathways that regulate neurotrophic factor expression may provide additional targets for the development of novel, more efficacious ADs. Hopefully further studies will reveal new strategies that will eventually lead to better understanding of the pathophysiology of major depression and to improvement of treatment.

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Statement of Interest

None.

References


