Brief report

Dynamics of beta-arrestin1 protein and mRNA levels elevation by antidepressants in mononuclear leukocytes of patients with depression

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Abstract

Background: Beta-arrestins interfere in G protein-receptor interaction leading to desensitization of G protein-mediated receptor signaling. G protein-receptor signaling and its desensitization were previously implicated in the pathophysiology, diagnosis and treatment of mood disorders. The present study aims at evaluating alterations in beta-arrestin1 protein and mRNA levels in mononuclear leukocytes of untreated patients with major depression and the effects and time course of antidepressant treatments on these alterations.

Methods: Repeated beta-arrestin1 protein and mRNA measurements, through immunoblot analyses using monoclonal antibodies against beta-arrestin1 and reverse transcriptase polymerase chain reaction, respectively, were carried in mononuclear leukocytes of 18 patients with major depression and compared with 18 healthy subjects. Each patient was examined while untreated and after 1, 2, and 4 weeks of antidepressant treatment.

Results: Beta-arrestin1 protein and mRNA levels in mononuclear leukocytes of untreated patients with major depression were significantly lower than those of healthy subjects. The low beta-arrestin1 protein and mRNA levels were alleviated by antidepressant treatment. Normalization of beta-arrestin1 measures preceded, and thus predicted clinical improvement.

Conclusions: These findings support the implication of beta-arrestin1 in the pathophysiology of major depression and in the mechanism underlying antidepressant-induced receptor down-regulation and therapeutic effects. Beta-arrestin1 measurements in patients with depression may potentially serve for biochemical diagnostic purposes and for monitoring and predicting response to antidepressants.

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Keywords: Beta-arrestin; Antidepressant; Post-receptor signal transduction; Mood disorders; Receptor desensitization; Mononuclear leukocytes

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1. Introduction

Growing evidence suggests that receptor-G protein coupling, and its regulation, may be involved in both the pathogenesis and treatment of mood disorders (Avissar and Schreiber, 2002; Schreiber and Avissar, 2003; Avissar et al., 2004). Our knowledge concerning the basic mechanisms underlying the phenomenon of desensitization, internalization, down-regulation, and resensitization of GPCRs has been far advanced during the last decade. Following receptor phosphorylation by G protein coupled receptor kinase, beta-arrestin binding results in desensitization of G protein-mediated signaling by preventing interaction of receptors with G proteins (Lohse et al., 1990; Stephen and Lefkowitz, 2002; Lefkowitz, 2004; Gainetdinov et al., 2004). This process regulates the function of many GPCRs, including α and β-adrenergic, muscarinic cholinergic, serotonergic, and dopaminergic receptors (Kristen and Lefkowitz, 2001; Luttrell and Lefkowitz, 2002). Beta-arrestins interact with proteins of the endocytic machinery such as clathrin, to promote internalization of receptors via clathrin-coated vesicles (Goodman et al., 1996; Laporte et al., 1999), and are also involved in both receptor down-regulation (Gagnon et al., 1998) and resensitization (Zhang et al., 1997; Oakley et al., 1999).

To study the possible involvement of beta-arrestin1 in the pathophysiology of depression and/or in the mechanism of action of antidepressant medications and to characterize the dynamics of normalization of beta-arrestin1 measures by antidepressants, we undertook repeated measures of beta-arrestin1 protein and mRNA levels in MNL of patients with major depression before and during antidepressant treatment in parallel with their clinical evaluation.

2. Methods

2.1. Patients

All subjects were evaluated using SCID DSM-IV. Inclusion criteria: (1) >18 years; (2) physically healthy; (3) normal laboratory tests and electrocardiogram; (4) willingness to give informed consent. For the patient group only: (5) Diagnosis of a major depressive episode; (6) untreated yet with antidepressants; (7) 17 item Ham-D: >18. Exclusion criteria: (1) evidence for significant physical disorders; (2) diagnosis of a major psychiatric disorder (for the patient group only: other than a major depressive episode); (3) treatment (within the past 20 weeks) with psychopharmacological or other medications; (4) the existence of axis II disorders; (5) alcohol or drug dependency or abuse. The study was approved by the Institutional Review Board. The group of 18 untreated patients with depression (of 25 patients assessed for inclusion in the study), average duration of the current episode 2.9 months SD=1.4 months, 16 patients with first depressive episode and two patients with a second episode previously successfully treated with citalopram (11 female) (age 36.9, SD=15.1, range 18–58) were blindly assigned in advance to either citalopram 20–40 mg/d (9 patients) or venlafaxine 150–225 mg/d (9 patients). None of the patients was under current or recent treatment with psychological strategies. The healthy volunteer group consisted of 18 subjects (11 female) age 37.4 (SD=12.8, range 19–57) years, from the students and staff of Ben Gurion University.

2.2. Mononuclear leukocytes isolation and immunoblot analysis

MNL isolation, homogenization, fraction separation, and immunoblot analysis were performed as previously described (Avissar et al., 2004).

2.3. Isolation of RNA and reverse transcriptase polymerase chain reaction (RT-PCR)

Isolation and purification of total RNA from MNL was carried with EZ-RNA Kit. One-step RT-PCR was performed with oligonucleotide primers selected from the highly conserved nucleotide sequences of beta-actin. Beta-actin RNA served as an internal control for cDNA normalization. Normalized cDNAs were subjected to analysis of beta-arrestin1. Primers were synthesized by Sigma Genosys, Israel. One microgram of total RNA was used for RT-PCR in 25 µl reaction volume. After a denaturation step for 5 min at 94 °C, thermal cycling was performed at 94 °C for 20 s, 50 °C for 30 s, 72 °C for 1 min, with a total number of 30 cycles for both beta-actin and
beta-arrestin1 gene products. After staining with ethidium bromide, amplified DNA fragments were separated by gel electrophoresis in 1% agarose. The relative density of the bands imprinted on the autoradiographic films was measured using a computerized image analysis system. PCR products were sequenced in both directions.

2.4. Statistical analyses

Bonferroni t-tests: for comparisons between patients and controls. Paired t-tests: for comparisons in the same patients before and after treatment. Pearson’s correlation: for correlations between biochemical measures and clinical ratings.

3. Results

Beta-arrestin1 protein levels in the healthy volunteers group have previously been found to be independent of age or gender (Avissar et al., 2004). As shown by Bonferroni t-tests in comparison with the age- and gender-matched healthy subjects (cytoplasmic beta-arrestin1: 100.0%, SD = 5.98%; membrane beta-arrestin1: 100.0%, SD = 8.29%; mRNA: 100.0%, SD = 5.66%), patients with depression, while untreated, had significantly lower levels of MNL beta-arrestin1 protein (cytoplasmic beta-arrestin1: 46.4%, SD = 22.4%, t = 9.809, df = 68, p < 0.001; membrane beta-arrestin1: 36.7%, SD = 25.7%, t = 9.159, df = 68, p < 0.001) and significantly lower mRNA levels (39.5% SD = 24.8%, t = 7.361, df = 68, p < 0.001) (Table 1). Beta-arrestin1 measures in untreated patients with depression were correlated with Ham-D rating (cytoplasmic beta-arrestin: Pearson’s r = −0.775, n = 18, t = 3.95 p < 0.005; membrane beta-arrestin1: r = −0.791, n = 18, t = 4.34 p < 0.002; beta-arrestin1 mRNA: r = −0.663, t = 2.93 n = 18, p < 0.02). The low beta-arrestin1 measures in patients with depression were normalized by 4 weeks of treatment according to paired t-tests (cytoplasmic beta-arrestin1: 93.6%, SD = 21.9%; t = 6.399, df = 17, p < 0.001; membrane beta-arrestin1: 101.0%, SD = 17.7%; t = 9.215, df = 17, p < 0.001; beta-arrestin1 mRNA: 115.2%, SD = 10.8%; t = 4.910, df = 17, p < 0.001). Beta-arrestin1 measures after 4 weeks of treatment did not differ from healthy subjects measures, according to Bonferroni t-test (cytoplasmic beta-arrestin1: t = 0.910, df = 68, N.S.; membrane beta-arrestin1: t = 0.129, df = 68, N.S.). Beta-arrestin1 mRNA levels after 4 weeks of treatment were significantly higher in comparison with healthy volunteers (t = 3.176, df = 68, p < 0.01).

Fig. 1A and B is a representative example of: (a) immunoblots of cytoplasmic (Fig. 1A upper panel) and membrane (Fig. 1A lower panel) beta-arrestin1 protein; (b) RT-PCR of beta-arrestin1 and beta-actin mRNA (Fig. 1B), measured in MNL obtained from a patient with major depression undergoing antidepressant treatment. The figure depicts normalization of beta-arrestin1 measures in the course of treatment. Normalization of beta-arrestin1 measures preceded clinical improvement by 1–2 weeks (Fig. 2). The dynamics of beta-arrestin1 normalization did not differ between SSRI and SNRI treatments (not shown). Beta-arrestin1 measures significantly increased already after one week of treatment according to paired t-tests (cytoplasmic beta-arrestin1: 72.8%, SD = 23.3%; t = 3.987, df = 68, p < 0.001; membrane beta-arrestin1: 88.8%, SD = 25.4%; t = 6.459, df = 17, p < 0.001).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Ham-D score</th>
<th>Beck score</th>
<th>Beta-arrestin1 protein level (%)</th>
<th>Beta-arrestin1 mRNA level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytoplasmic fraction</td>
<td>Membrane fraction</td>
</tr>
<tr>
<td>Untreated depressed</td>
<td>26.2±4.7</td>
<td>29.8±10.9</td>
<td>46.4±22.4%</td>
<td>36.7±25.7%</td>
</tr>
<tr>
<td>Antidepressant treatment 1 week</td>
<td>25.3±7.6</td>
<td>28.7±11.6</td>
<td>72.8±23.3%</td>
<td>88.8±25.4%</td>
</tr>
<tr>
<td>Antidepressant treatment 2 weeks</td>
<td>19.4±9.2</td>
<td>21.3±10.5</td>
<td>81.5±20.3%</td>
<td>91.3±28.3%</td>
</tr>
<tr>
<td>Antidepressant treatment 4 weeks</td>
<td>7.9±8.0</td>
<td>7.5±5.0</td>
<td>93.6±21.9%</td>
<td>101.0±17.7%</td>
</tr>
</tbody>
</table>

* Beta-arrestin1 protein and mRNA levels in MNL of patients are expressed as percent of the respective protein and mRNA levels measured in healthy subjects.
4. Discussion

Beta-arrestin1 is a dynamic cytoplasmic regulatory protein, recruited to the plasma membrane upon agonist stimulation of the receptor. Alterations in beta-arrestin1 protein levels in depression and/or by antidepressants may reflect dynamic cytoplasmic-membrane translocations, or alterations in expression. Our findings show that both cytoplasmic and membrane beta-arrestin1 protein levels are reduced in MNL of patients with depression, suggesting that the protein is under-expressed in depression. Indeed, the reduction in mRNA levels in MNL of patients with depression.

\[ t = 2.576, df = 17, p < 0.05 \]

\[ p < 0.001; \text{beta-arrestin}1 \text{ mRNA: 78.5\%, SD = 25.5\%;} \]

Fig. 1. Representative immunoblot (A) and RT-PCR analysis (B) of beta-arrestin1 in MNL of a depressed patient in the course of antidepressant treatment.

Fig. 2. Normalization of beta-arrestin1 protein and mRNA levels by antidepressant treatment in MNL of 18 depressed patients in comparison with the rate of clinical improvement.
depression confirms under-expression of beta-arrestin1 protein in MNL of patients with depression. Similarly, the effects of antidepressant treatment of elevating both cytoplasmic and membrane beta-arrestin1 protein and mRNA levels point to a possible biochemical mechanism of action of antidepressants through increased expression of beta-arrestin1 protein. It should be acknowledged that the current changes in MNL of subjects may not be represented centrally. Indeed no difference in beta-arrestin2 levels was detected in postmortem prefrontal cortices between depressed and control subjects (Grange-Midroit et al., 2003).

The normalization of beta-arrestin1 measures, which was significant after one week, preceded clinical improvement by 1–2 weeks. Since clinical response to antidepressant treatments is due both to the specific biochemical antidepressant effects of the medication agent, as well as placebo effects, and since the placebo effect is usually more pronounced during the early period of treatment initiation, it is very difficult to assess in these early days the specific antidepressant effects of antidepressant treatments. Beta-arrestin1 measurements in peripheral blood cells of patients with mood disorder, as a state dependent characteristic, may afford biochemical monitoring of antidepressant effects and prediction of clinical response to antidepressant by 1–2 weeks in advance.

Beta-arrestin1 can induce a switch in receptor signaling from classical second messenger-generating G protein-mediated pathways by conferring tyrosine kinase activity upon the receptor leading to activation of mitogen-associated protein (MAP) kinase cascade (Luttrell et al., 1999). Beta-arrestins function as GPCR-regulated scaffolds for mitogen-activated protein kinase modules such as apoptosis signal-regulating kinase (ASK)-mitogen activated protein kinase kinase (MKK)-C-Jun N-terminal kinases (JNK) and RAF-(MAPK/ERK kinase) MEK-extracellular signal-regulated protein kinases (ERKs) (McDonald et al., 2000; Luttrell et al., 2001). The findings described here on the involvement of beta-arrestin in the pathophysiology and treatment of depression may have extended implications concerning possible switch in postreceptor signalling related to tyrosine kinases, Src, and MAP kinase that may characterize major depressive disorder or be induced by antidepressant treatment. Recent finding on reduced activation and expression of ERK1/2 MAP kinase in post-mortem brain of depressed suicide subjects (Dwivedi et al., 2001) and on MAP kinase activation by fluoxetine in cultured rat astrocytes (Mercier et al., 2004) supports this suggestion.

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**References**


