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ARTICLE

THEMATIC SECTION
New Aspects in the Treatment of Affective Disorders

CINP

Normalization of GRK2 protein and mRNA measures in patients with depression predict response to antidepressants

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Abstract

G-protein-coupled receptor kinases (GRKs) interfere in receptor–G-protein coupling leading to desensitization of G-protein-mediated receptor signalling. G-protein-coupled receptor signalling and its desensitization were previously implicated in the pathophysiology, diagnosis and treatment of mood disorders. The present study aimed to evaluate alterations in GRK2 protein and mRNA levels in mononuclear leukocytes (MNL) of untreated patients with major depression and the effects and time-course of antidepressant treatments on these alterations. Repeated GRK2 protein and mRNA measurements were carried in MNL of 24 patients with major depression. Each patient was examined while untreated and after 1, 2, 3 and 4 wk of antidepressant treatment; 24 healthy subjects were also studied. GRK2 protein and mRNA levels were evaluated through immunoblot analyses using monoclonal antibodies against GRK2 and reverse transcriptase–polymerase chain reaction, respectively. GRK2 protein and mRNA levels in MNL of untreated patients with major depression were significantly lower than the measures characterizing healthy subjects. The decreased GRK2 protein and mRNA levels were alleviated by antidepressant treatment. Normalization of GRK2 measures preceded, and, thus, could predict clinical improvement by 1–2 wk. These findings support the implication of GRK2 in the pathophysiology of major depression and in the mechanism underlying antidepressant-induced receptor down-regulation and therapeutic effects. GRK2 measurements in patients with depression may potentially serve for biochemical diagnostic purposes and for monitoring and predicting response to antidepressants.

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Key words: Antidepressant, GRK2, mood disorders, post-receptor signal transduction, receptor desensitization.

Introduction

Our knowledge concerning the basic mechanisms underlying the regulation of G protein-coupled receptor (GPCR)–G-protein coupling has been greatly advanced during the last two decades. These mechanisms involve the activities of two families of proteins: G-protein-coupled receptor kinases (GRKs), and arrestins (Reiter & Lefkowitz, 2006). GRKs comprise a highly regulated cytosolic, multigene family of serine–threonine kinases, capable of specifically phosphorylating the agonist-occupied form of GPCRs. Receptor phosphorylation by GRKs has been ultimately identified as the initial and critical step in the uncoupling of receptors from G protein, leading to the attenuation or desensitization of GPCR signalling (Reiter & Lefkowitz, 2006). GRKs are translocated to the plasma membrane for their appropriate interaction with receptor domains. Following phosphorylation by GRKs, GPCRs bind to a family of soluble proteins named arrestins, which 'arrest' GPCR signalling. Arrestins bind to regions of GPCRs that are also primary determinants for G-protein interaction, thus uncoupling GPCR from G proteins (Benovic *et al*. 1986).

Seven mammalian genes encoding GRKs 1–7, have been cloned to date (Lefkowitz *et al*. 1990; Luttrell & Lefkowitz, 2002; Pitcher *et al*. 1998). GRK1 and GRK7

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are specific to the visual system, GRK4 is selectively present in sperm cells, while GRKs 2, 3, 5 and 6 are ubiquitously distributed. Based on sequence and functional similarities, the GRK family has been divided into three subfamilies: (*a*) the rhodopsin kinase subfamily (GRKs 1, 7); (*b*) the β-adrenergic receptor kinase subfamily (GRKs 2, 3); and (*c*) the GRK4 subfamily (GRKs 4, 5, 6) (Palczewski, 1997; Weiss *et al*. 1998).

Growing evidence suggests that GPCR–G-protein coupling, and its regulation, may be involved in the pathophysiology, diagnosis and treatment of mood disorders (Avissar *et al*. 2004; Metaye *et al*. 2005; Premont & Gainetdinov, 2007; Schreiber & Avissar, 2006, 2007). Using a convergent functional genomic approach a series of candidate genes involved in the pathogenesis of mood disorders were identified including GRK3, which was also found to be decreased in lymphoblastoid cell lines from a subset of bipolar patients (Niculescu *et al*. 2000). A single nucleotide polymorphism in the promoter region of GRK3 was found to be associated with bipolar disorder (Barrett *et al*. 2003). The findings concerning the GRK3 gene are in accord with evidence from genome-wide linkage surveys suggesting that the chromosome 22q12 region contains a susceptibility locus for bipolar disorder. Alterations of GRK2/3 levels were described in specimens of the prefrontal cortex collected from depressed subjects (Garcia-Sevilla *et al*. 1999; Grange-Midroit *et al*. 2003). Acute, but not prolonged, treatment with desipramine altered GRK2/3 in rat brain (Miralles *et al*. 2002). Reduced platelet GRK2 levels detected in patients with major depression were normalized by mirtazapine treatment (Garcia-Sevilla *et al*. 2004).

The existing knowledge concerning the involvement of alteration in receptor–G-protein coupling in the pathophysiology of mood disorders and in antidepressants' mechanism of action, together with the findings concerning the regulation of this coupling by GRKs raised the following research questions:

(*a*) Are GRK2 protein or mRNA levels altered in patients with depression compared to healthy subjects? Do these alterations correlate with the severity of clinical symptoms?
(*b*) Do GRK2 altered measures in patients with depression normalize under antidepressant treatment? Is this normalization correlated with clinical improvement?
(*c*) What is the time-course of GRK2 normalization? Can GRK2 measures predict future clinical response to antidepressant treatment?

Methods

Patients

All subjects were evaluated using Structured Clinical Interview for Axis I DSM-IV disorders (SCID DSM-IV). Inclusion criteria: (1) age $\geqslant$18 yr; (2) good general physical health; (3) normal laboratory test results for renal, hepatic, haematological, and thyroid function, and normal electrocardiogram; (4) willingness and ability to give informed consent. For the patient group only: (5) a current existence of a major depressive episode; (6) untreated yet with antidepressants; (7) 17-item Hamilton Rating Scale for Depression (HAMD) score of $\geqslant$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 wk) with psychopharmacological or other medications; (4) the existence of Axis II disorders; (5) Alcohol or drug dependency or abuse.

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the Institutional Review Boards of Soroka Medical Center and Barzilai Medical Center. The group of 24 untreated patients with major depressive episode [15 female, nine male subjects, age 33.1 yr, (S.D. = 11.6, range 19–57 yr), average duration of the current episode 2.9 months (S.D. = 1.4 months), 17 patients with first depressive episode and seven patients with a second episode previously successfully treated with antidepressants] were blindly assigned in advance to either 20–40 mg/d citalopram (12 patients) or 150–225 mg/d venlafaxine (12 patients). None of the patients was under current or recent treatment with psychological strategies. The healthy volunteer group consisted of 24 subjects [15 female; age 34.3 yr (S.D. = 11.8, range 20–57 yr)], from the students and staff of Ben Gurion University of the Negev. Blood was drawn between 08:00 and 10:00 hours.

Mononuclear leukocytes (MNL) isolation and immunoblot analysis

MNL were isolated from 50 ml heparinized fresh blood using Ficoll–Paque gradient. Cells were homogenized in 25 mM Tris–HCl (pH 7.4), 1 mM Mg^{2+}, 1 mM EGTA, 1 mM dithiothreitol, and antiprotease cocktail (1:100) (Sigma). The cytosolic fraction was separated from the membrane fraction by centrifugation at 18 000 *g* for 20 min. Membranes were suspended in homogenization buffer, and both fractions were frozen at $-80\,^{\circ}C$ until assayed. Protein concentration was determined using the Lowry assay.

Immunoblot analysis

The cytosolic or membrane fraction was thawed on the day of assay. Proteins (10 μg total) were separated by SDS (10%)–polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose paper by electroblotting apparatus. Blots were incubated overnight with a monoclonal antibody to GRK2 (Santa Cruz Biotechnology, USA).

In a preliminary screening assay two commercially available anti-GRK2 monoclonal antibodies were compared: (*a*) rabbit monoclonal ab32558 (Abcam plc, UK), raised against residues in the C-terminus of human GRK2; (*b*) mouse monoclonal sc-13143 (Santa Cruz Biotechnologies), raised against amino acids 468–689 of GRK2 of human origin. Both antibodies showed cross-reactivity with both mouse and rat GRK2 protein. In both cases, one single band was detected in the Western blots at $\sim$82 kDa as has been previously shown by others in human leukocytes using a variety of anti-GRK2 antibodies. All data presented in the present study were obtained using Santa Cruz anti-GRK2 sc-13143 antibody. This specific antibody has been previously described and used by others for GRK2 detection in leukocytes (Fan & Malik, 2003; Wu *et al*. 2007).

The immune bands were visualized by ECL followed by exposure to Kodak X-Omat film, and quantified by densitometry on a BioImaging System 202D (Pharmacia & Dynco-Renium, Israel). Semiquantitative analysis was performed using a computerized bioimaging system: Tina 2.0 software by Ray Test (Staubenhardt, Germany). Preliminary and repeated assays using healthy volunteer MNL membranes and cytosolic fractions were performed across the study, for the determination of the range of linearity of the assay in respect of protein concentration in each fraction. Linearity was determined and repeatedly ascertained between 2.5 and 20 μg total protein for the cytosolic fraction and 2.5–25 μg total protein for the membrane fraction. Thus aliquots of 10 μg membrane or cytosolic total protein were loaded on each PAGE well to ascertain that linearity is kept.

β-Actin, detected using mouse monoclonal anti-actin antibody (cat. no. 691001, MP Biomedicals, USA), served as an internal protein reference. In each blot, 10 μg rat cortical membranes were used as a standard reference. The within- and between-blot coefficients of variation for 10 μg rat cortical membrane reference were 0.025 and 0.051, respectively. The monoclonal anti-GRK2 used in all Western blot measurements (sc-13143, Santa Cruz Biotechnologies), shows cross-reactivity with both mouse and rat GRK2 protein and thus was effective also for the detection of GRK2 in rat cortical membranes that were used as a standard reference in all blots.

Isolation of RNA and reverse transcriptase–polymerase chain reaction (RT–PCR)

Isolation and purification of total RNA from MNL was carried out using EZ-RNA kit. One-step RT–PCR was performed with oligonucleotide primers selected from the highly conserved nucleotide sequences of β-actin. β-Actin RNA served as an internal control for cDNA normalization. Normalized cDNAs were subjected to analysis of GRK2. GRK-2 (forward primer: 5′-ACCTGACCATGAATGACTTC-3′; reverse primer: 5′-CTTCTTGGAGAAGTCACAGG-3′; amplified product 482 bp). β-actin (forward primer: 5′-CTACAATGAGCTGCGTGTGG-3′, reverse primer: 5′-CGGTGAGGATCTTCATGA-3′; amplified product 295 bp). Both primers were synthesized by Sigma Genosys (Israel). Sequencing of the PCR products confirmed the expected sequences.

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. The number of PCR cycles was within the exponential phase of amplification: 30 cycles for β-actin and 32 cycles for GRK2 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.

Statistical analyses

In the 24 patients who completed the treatment, ANOVA with repeated measures (before, and during various time-periods of antidepressant treatment) allowed an assessment of drug effects (basal *vs*. protein change in immunoreactivity) and time (changes with the duration of treatment). Significant interactions revealed by ANOVA were further examined with Bonferroni's multiple comparison test to determine when significant effects occurred and the magnitude of the changes. The Bonferroni *t* test was applied for multiple comparisons of patients before and after the various treatment periods against a single control group of healthy volunteers. Paired *t* tests were applied for comparisons in the same patients before and after treatment. Pearson's correlation was applied for

Table 1. Normalization of mononuclear leukocyte (MNL) GRK2 protein and mRNA levels in patients with depression precedes clinical improvement

Subjects	HAMD score	GRK2 protein level (%)[a]		GRK2 mRNA level (%)[a]
		Cytosolic fraction	Membrane fraction	
Untreated depressed	25.9 (6.1)	41.2% (18.6)	56.3% (26.8)	44.4% (29.8)
AD treatment				
1 wk	21.0 (5.6)	66.8% (26.5)	82.9% (31.2)	61.4% (34.9)
2 wk	12.3 (7.2)	87.4% (30.7)	100.1% (14.3)	91.9% (20.4)
3 wk	4.7 (3.7)	100.3% (10.5)	103.5% (21.5)	98.9% (12.0)
4 wk	4.5 (7.2)	99.6% (16.7)	104.0% (19.1)	99.2% (11.7)

AD, Antidepressant; HAMD, Hamilton Depression Rating Scale.
Values in parentheses are standard deviations.
In the 24 patients who went through a course of antidepressant treatment (0, 1, 2, 3, and 4 wk of treatment) ANOVA with repeated measures detected significant drug effects on cytosolic GRK2 protein levels ($F=31.84$, $p<0.001$), membrane GRK2 levels ($F=17.81$, $p<0.001$), and GRK2 mRNA levels ($F=26.47$, $p<0.001$), as well as on the severity of depressive symptomatology evaluated through HAMD score ($F=58.49$, $p<0.001$).
[a] GRK2 protein and mRNA levels in MNL of patients are expressed as percent of the respective protein and mRNA levels measured in healthy subjects.

correlations between biochemical measures and clinical ratings.

Results

GRK2 protein and mRNA levels in MNL obtained from the healthy volunteer group were independent of age (for cytosolic and membrane GRK2 protein levels Pearson's $r=0.101$, $t=0.050$, $n=24$ $p>0.50$, and $r=0.030$, $t=0.14$, $n=24$, $p>0.5$, respectively; for GRK2 mRNA levels $r=0.053$, $t=0.013$, $n=24$, $p>0.5$) or gender [average cytosolic GRK2 levels for female and male subjects: 100.9% (S.D. = 5.3%) and 99.1% (S.D. = 6.8%), respectively, $t=0.725$, d.f. = 22, $p>0.50$; average membrane GRK2 levels for female and male subjects: 100.3% (S.D. = 5.4%) and 99.7% (S.D. = 5.0%), respectively, $t=0.659$, d.f. = 22, $p>0.5$; average membrane GRK2 mRNA levels for female and male subjects: 98.3% (S.D. = 7.1%) and 101.7% (S.D. = 8.1%), respectively, $t=1.090$, d.f. = 22, $p>0.5$].

As shown by Bonferroni t tests in comparison with the age- and gender-matched healthy volunteer subjects, patients with depression, while untreated, had significantly lower levels of MNL GRK2 protein for cytosolic GRK2: $t=13.711$, d.f. = 115, $p<0.001$; for membrane GRK2: $t=7.186$, d.f. = 115, $p<0.001$, and significantly lower GRK2 mRNA levels $t=8.153$, d.f. = 115, $p<0.001$ (Table 1). The extent of reduction in GRK2 protein and mRNA levels in untreated patients with depression were found to be correlated with the severity of depressive symptoms assessed by HAMD score (for cytosolic GRK2 protein: Pearson's $r=-0.677$, $n=24$, $t=3.679$, $p<0.002$; for membrane GRK2 protein: Pearson's $r=-0.833$, $n=24$, $t=5.628$, $p<0.001$; for GRK2 mRNA levels: Pearson's $r=-0.807$, $t=5.107$, $n=24$, $p<0.001$).

Figure 1 depicts representative examples of: (i) immunoblots of cytosolic (Fig. 1*b*, top panel), membrane (Fig. 1*b*, bottom panel) GRK2 protein; (ii) RT–PCR of GRK2 (Fig. 1*a*, top panel), β-actin mRNA (Fig. 1*a*, bottom panel), measured in MNL obtained from a patient with major depression undergoing antidepressant treatment. The figure shows normalization of GRK2 measures in the course of treatment.

The low GRK2 measures in patients with depression were normalized by 4 wk treatment according to paired t tests (for cytosolic GRK2 protein level: $t=7.787$, d.f. = 23, $p<0.001$; for membrane GRK2 protein level: $t=4.338$, d.f. = 23, $p<0.001$; for GRK2 mRNA level: $t=4.989$, d.f. = 23, $p<0.001$). GRK2 measures after 4 wk treatment did not differ from healthy subjects measures, according to Bonferroni t test (cytosolic GRK2: $t=0.084$, d.f. = 115, n.s.; membrane GRK2: $t=0.720$, d.f. = 115, n.s.; GRK2 mRNA: $t=0.186$, d.f. = 115, n.s.).

GRK2 measures were significantly increased after 1 wk antidepressant treatment according to paired t tests (for cytosolic GRK2: $t=6.236$, d.f. = 23, $p<0.001$;

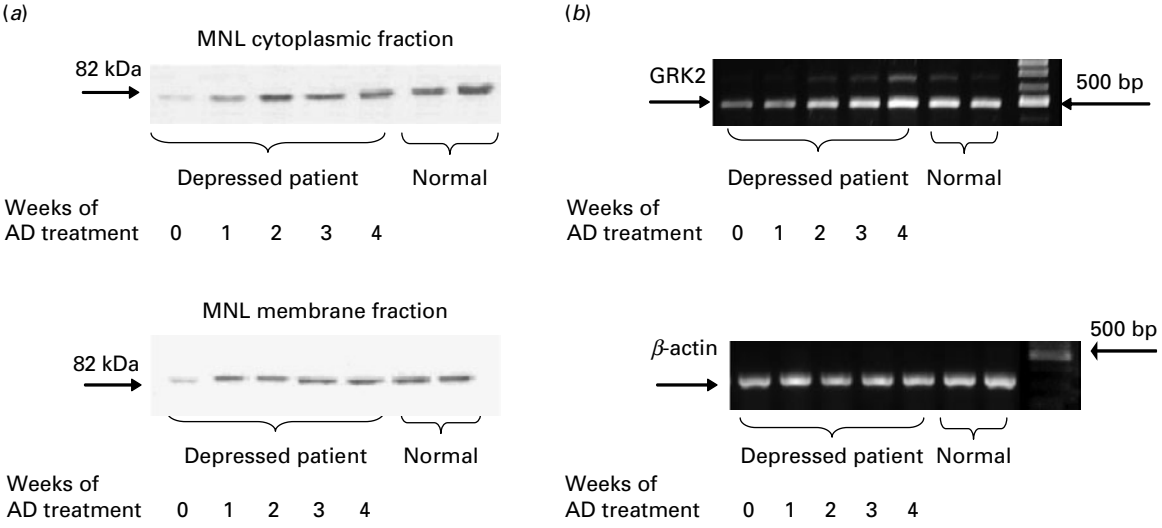

Fig. 1. Representative immunoblot (*a*) and RT–PCR analysis (*b*) of GRK2 in mononuclear leukocytes (MNL) of a depressed patient in the course of antidepressant (AD) treatment. The data in the presented example refer to treatment with citalopram. The exemplary data for GRK2 normalization under venlafaxine were similar.

for membrane GRK2: $t=4.851$, d.f. $=23$, $p<0.001$; for GRK2 mRNA: $t=3.258$, d.f. $=23$, $p<0.005$). In contrast with the significant GRK2 normalization, which appears after 1 wk antidepressant treatment, HAMD score after 1 wk antidepressant treatment did not differ from the measure before treatment ($t=1.215$, d.f. $=23$, $p>0.2$, n.s., paired t test). After 2 wk antidepressant treatment GRK2 measures did not significantly differ from healthy subjects' measures, according to Bonferroni t test (cytosolic GRK2: $t=1.970$, d.f. $=115$, n.s.; membrane GRK2: $t=0.032$, d.f. $=115$, n.s.; GRK2 mRNA: $t=1.874$, d.f. $=115$, n.s.). Normalization of GRK2 measures preceded clinical improvement by 1–2 wk (Fig. 2). The dynamics of GRK2 normalization did not differ between SSRI (citalopram) and SNRI (venlafaxine) treatments. Moreover, no significant difference in clinical response to the two types of antidepressants was observed.

Incremental alterations in cytosolic and membrane GRK2 protein levels (Fig. 3) (Pearson's $r=-0.900$, $n=24$, $t=9.684$, $p<0.001$, and $r=-0.946$, $n=24$, $t=13.686$, $p<0.001$, respectively), and GRK2 mRNA levels (Pearson's $r=-0.953$, $n=24$, $t=14.737$, $p<0.001$) (not shown) in MNL of patients with depression induced by 3 wk antidepressant treatment were significantly correlated with decrements in depressive symptoms evaluated by HAMD score.

Figure 4 depicts a correlation between the extent of increase in membrane GRK2 protein level after 1 wk antidepressant treatment and the extent of decrease in HAMD score after 3 wk treatment (Pearson's

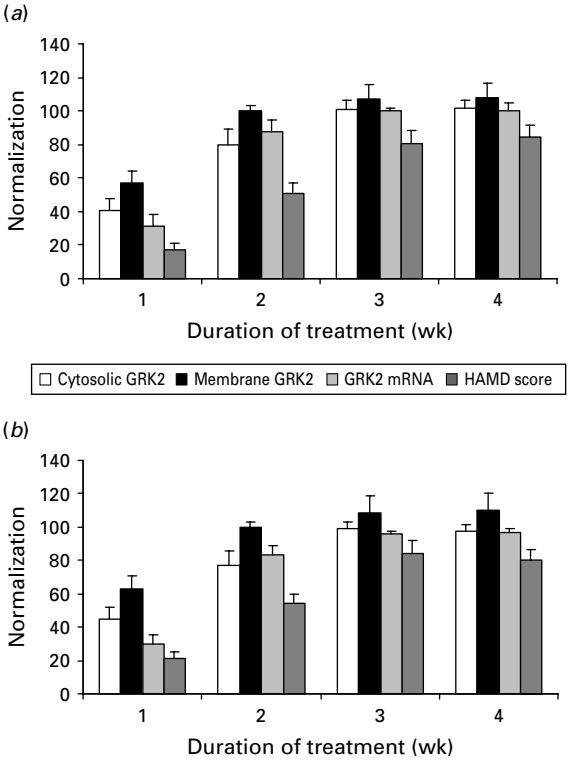

Fig. 2. Dynamics of antidepressant-mediated GRK2 protein and mRNA normalization in mononuclear leukocytes of depressed patients compared to the rate of clinical improvement. (*a*) citalopram (*b*) venlafaxine. GRK2 measures under antidepressant treatment were normalized against the respective patient's pre-treatment values. HAMD, Hamilton Depression Rating Scale

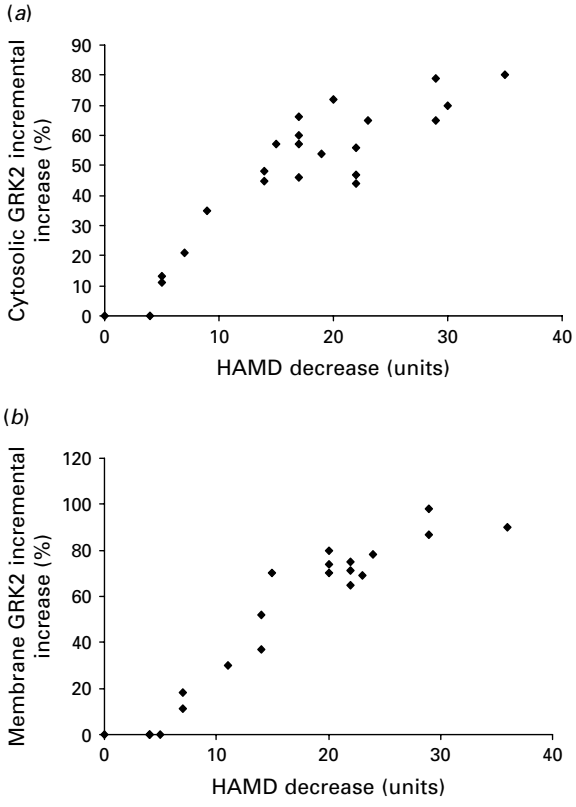

Fig. 3. Correlation between 3-wk antidepressant-induced incremental alterations in GRK2 and the decrements in depressive symptoms measured by the Hamilton Depression Rating Scale (HAMD). (*a*) Increments in cytosolic mononuclear leukocyte (MNL) GRK2 protein *vs*. decrements in HAMD score (Pearson's $r = -0.900$, $n = 24$, $t = 9.684$, $p < 0.001$) after 3 wk antidepressant treatment. (*b*) Increments in membrane MNL GRK2 protein *vs*. decrements in HAMD score ($r = -0.946$, $n = 24$, $t = 13.686$, $p < 0.001$) after 3 wk antidepressant treatment.

$r = -0.931$, $n = 24$, $t = 11.964$, $p < 0.001$) segregating responders from non-responders.

Discussion

The present study describes two major findings: (*a*) reductions of GRK2 protein and mRNA levels in MNL of untreated patients with depression; (*b*) normalization of the reduced MNL GRK2 measures in patients with depression by antidepressant treatments, which precedes clinical improvement by 1–2 wk.

GRK2 is a dynamic cytosolic regulatory protein, recruited to the plasma membrane upon agonist stimulation of the receptor. Alterations in GRK2 protein levels in depression and/or by antidepressants may reflect dynamic cytosolic-membrane translocations, or

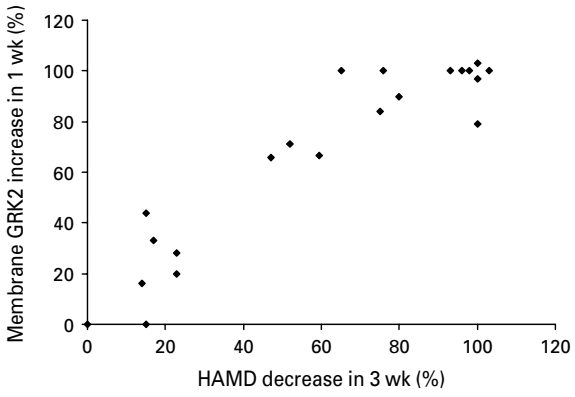

Fig. 4. Relative increase in membrane GRK2 levels in mononuclear leukocytes (MNL) of patients with depression after 1 wk antidepressant treatment segregates responders from non-responders. Relative increase in MNL membrane GRK2 levels after 1 wk antidepressant treatment correlates with relative decrease in HAMD score after 3 wk treatment (Pearson's $r = -0.931$, $n = 24$, $t = 11.964$, $p < 0.001$).

alterations in expression. Our findings show that both cytosolic and membrane GRK2 protein levels are reduced in MNL of patients with depression, suggesting that the protein is under-expressed in depression. Indeed, the reduction in GRK2 mRNA levels in MNL of patients with depression supports this suggestion. Similarly, the effects of antidepressants treatment of elevating both cytosolic and membrane GRK2 protein and GRK2 mRNA levels point to a possible biochemical mechanism of action of antidepressants through increased expression of GRK2 protein.

Comparing the dynamics of GRK2 normalization to β-arrestin1 normalization under antidepressant treatments (Matuzany-Ruban *et al*. 2005) shows that the extent of normalization of GRK2 after 1 wk antidepressant treatment is 88.9% of the β-arrestin1 normalization rate in the cytosolic MNL fraction, and 153.4% of the β-arrestin1 normalization rate in MNL membrane fraction. After 2 wk treatment normalization rates for cytosolic and membrane GRK2 relative to β-arrestin1 rates become similar, 91.8% and 93.3%, respectively. It can, thus, be suggested that two processes take place in GRK2 normalization: (*a*) GRK2 cytosol-to-membrane translocation; (*b*) increased expression of GRK2. The suggestion that the mechanism for the normalization of GRK2 involves cytosolic-to-membrane translocation could not be supported by analysis of the present data. As no increase in ratio of membrane to cytosolic GRK2 over the weeks of treatment and response was observed, our data supports the second mechanism, which is increased GRK2 expression as probably the predominant one.

The fact that the extent of membrane MNL GRK2 normalization after 1 wk antidepressant treatment is relatively higher than cytosolic MNL GRK2 protein and GRK2 mRNA normalization and relatively higher than MNL β-arrestin1 protein and mRNA normalization, suggests membrane GRK2 protein level as a better predictor for antidepressant responsiveness after 1 wk treatment. GRK2 measurements after 1 wk antidepressant treatment can predict responsiveness and unresponsiveness to an antidepressant, enabling a distinct segregation between responders and non-responders (Fig. 4).

MNL from humans with rheumatoid arthritis as well as multiple sclerosis, both associated with high prevalence of depression (Mohr *et al*. 2007; Schiffer & Wineman, 1990; Wells *et al*. 1988, 1989), have decreased GRK2 expression (Lombardi *et al*. 1999; Vroon *et al*. 2005). Treating normal lymphocytes with interferon-γ (increased production in depression, decreased production by antidepressants; Mohr *et al*. 2001), or interleukin-6 (increased plasma concentrations in depression; Alesci *et al*. 2005; Maes *et al*. 1995), both lead to decrease in GRK2 expression (Premont & Gainetdinov, 2007), rendering further support for the involvement of GRK2 in the pathophysiology of depression.

We are aware that the involvement of GRK2 protein in the pathophysiology of depression as implicated from the data presented here should be taken with considerable caution: findings obtained in peripheral blood cells cannot be directly extrapolated to the central nervous system. We have discussed this issue at length previously (Avissar *et al*. 1997, 1999). In this regard, it should be noted that the findings reported in the present study on antidepressant- induced increases in MNL GRK2 measures in patients with depression are in partial accord with previous reports on increased membrane-associated GRK2/3 in rat brain induced by acute treatment with desipramine (Miralles *et al*. 2002). However, the reported findings with imipramine were limited to acute treatment and were not found with fluoxetine. In contrast to the present study and with previous findings in MNL of patients with depression by Garcia-Sevilla *et al*. (2004), in posmortem studies in the prefrontal cortex drug-free depressed subjects presented an increase in the density of membrane-associated GRK2. Further, prefrontal cortex GRK2 levels were found to be reduced in antidepressant drug-treated subjects (Garcia-Sevilla *et al*. 1999; Grange-Midroit *et al*. 2003). An alternative explanation might be that there are differences between the brain and peripheral cells. This would reduce the significance of the presently described findings in terms of understanding the biochemical mechanisms that underlie depression and antidepressants' mechanism of action. However, the possible empirical-applicative significance of the findings for future use of the assays for biochemical diagnostic purposes of patients with depression as well as for biochemical monitoring and/or predicting responses to antidepressant treatments still remains relevant.

As we use a mixed-cell MNL preparation for our assays, there remains a possibility that alterations observed in GRK2 measures reflect, at least in part, alterations in the white cell subpopulation induced by the depressive state and/or by antidepressant treatment. Blood cell distribution may be affected by a variety of factors including stress and physical activity, as well as secondary effects of the antidepressants on peripheral neurohormonal dynamics, among others. However, previous findings on decreased GRK2 levels in platelets of patients with depression and depressed suicide attempters, and its up-regulation by treatment with the antidepressant mitrazapine (García-Sevilla *et al*. 2004), suggest that the findings reported in the present study might characterize various peripheral blood elements in general, and thus, probably, cannot be explained by alterations in subpopulations of peripheral blood elements.

Interestingly, GRKs were found to interact with an increased number of signalling proteins (Ribas *et al*. 2007) hypothesized to be relevant to neuronal plasticity including ERK, MAP kinases and Akt. Activation of ERK proceeds through a pathway requiring Ras, Raf, and MEK. Activation of Akt requires phosphatidylinositol 3-kinase and the phosphoinositide-dependent protein kinases 1 and 2 (Cowen, 2007). Both signalling pathways were implicated in the mechanism of action of antidepressants hypothesized to induce neuroprotection and neurogenesis (Tanis & Duman, 2007). Thus, the involvement of GRK2 in depression and in the mechanism of action of antidepressants may have implications concerning possible post-receptor signalling related to these pathways.

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Statement of Interest

None.

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