Proteins of the Akt1/GSK-3β Signaling Pathway in Peripheral Blood Mononuclear Cells of Patients with Affective Disorders

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Received March 20, 2014

Abstract—The Akt1/GSK-3β signaling pathway is involved in the regulation of biological processes in nerve cells. Here we studied the intracellular proteins Akt1 and GSK-3β and their phosphoforms in patients with affective disorders. Sixty patients with diagnoses of depressive episodes, major depressive disorder, and bipolar affective disorder (BAD) and 34 mentally and somatically healthy subjects were examined. Proteins of the Akt1/GSK-3β signaling pathway (total glycogen synthase kinase 3β (GSK-3β), phospho-serine-9-GSK-3β, total protein kinase Akt1, phospho-serine-473-Akt1) were measured in peripheral blood mononuclear cells using the immunoblotting technique. High levels of total GSK-3β were found in groups of patients with affective disorders. Patients with recurrent depressive disorder had an increased level of total GSK-3β as compared with the level in depressive episode. The level of total Akt1 in patients decreased as compared with the control group. Patients with depressive disorder had decreased levels of phospho-serine-473-Akt1. Our results confirm the hypothesis of the involvement of glycogen synthase kinase-3β in the pathogenesis of affective disorders.

Keywords: glycogen synthase kinase-3β (GSK-3β), Akt1, depressive disorders, bipolar disorders

DOI: 10.1134/S1819712414030106

INTRODUCTION

Affective disorders are a serious socioeconomic and medical problem of modern society [1]. The incidence of this disease in the general population is 5–20% [2]. The neurobiological mechanisms that underlie the pathogenesis of affective disorders are not fully explored as yet [3]. Currently, much attention is given to studies on the deregulation of intracellular signaling pathways, as one of the factors of the pathogenesis of mental disorders [4–7]. We believe that protein kinases, as participants of neurobiological processes, may act as markers for the diagnostics and prognosis of affective disorders or targets for new pharmacotherapy [8, 9].

The Akt1/GSK-3β signaling pathway is involved in the regulation of a large number of cellular functions. Intracellular protein Akt1 (an isoform of protein kinase B) is activated by phosphorylation on the serine residue at position 473 and regulated by such molecules as serotonin, dopamine, BDNF (brain-derived neurotrophic factor), NGF (nerve growth factor), and GNF (glial growth factor). Active Akt1, in turn, inhibits glycogen synthase kinase 3β (GSK-3β) by its phosphorylation at serine-9 [10]. GSK-3β is an important protein involved in neuronal processes. By changing the activities of more than 50 target proteins, this enzyme participates in the regulation of metabolic processes, cell proliferation, apoptosis, cell cycle, embryogenesis, neurotransmission, neurodegeneration, formation of neuronal polarity, synaptic plasticity, and circadian rhythms [11, 12]. The study of the role of GSK-3β in the pathogenesis of affective disorders began from the finding of the ability of the mood stabilizer lithium to effectively block GSK-3 and its isoforms in 1996 [13]. Later, it was shown in animal models that the mood-stabilizing effects of lithium and other psychotropic drugs (antidepressants, anticonvulsants, and antipsychotics) are determined by the inhibition of GSK-3β. Moreover, increased activity of this protein is involved in the formation of both depression- and mania-like symptoms in animals [14].

A few recent reports were devoted to the study of the Akt1/GSK-3β signaling pathway in patients with affective disorders. Some of them were performed on postmortem material, which complicates interpretation of the results due to the fact that the activity of GSK-3β begins to decrease in the first few minutes after death [15]. The activity of these proteins in depressive disorders is virtually unexplored; their relationship with a variety of clinical manifestations of affective disorders also was not found.

This study aimed at the investigation of the intracellular proteins Akt1 and GSK-3β and their phosphoforms in patients with affective disorders. Peripheral
blood mononuclear cells were used as the target, since they are a convenient and adequate model for the evaluation of various molecular and biochemical processes in patients [16, 17].

MATERIALS AND METHODS

Sixty patients with affective disorders, who were treated at the Department of affective states of clinics of Research Institute of Mental Health of the Siberian Branch of the Russian Academy of Medical Sciences, were examined. The main criteria of inclusion were clinically verified diagnoses of a depressive episode (ICD—10: F32), recurrent depressive disorder (ICD-10: F33), bipolar affective disorder (BAD) (ICD-10: F33), and the absence of organic or neurological disorders. Additionally, for the group of patients with BAD, intake of the drug lithium was a criterion for exclusion from the study, because it is known that lithium is able to increase the phosphorylation of GSK-3β at serine-9 [18]. The group of patients with depressive episodes included 26 patients (mean age 47.5 years (37.5–58.6), 6 men and 20 women), the group with a major depressive disorder included 18 patients (mean age 50.0 years (44.0–58.5), 3 men and 16 women). Bipolar affective disorder was observed in 16 patients (mean age 37.5 years (27.2–58.0), 6 men and 10 women). The control group comprised 34 healthy mental and somatic donors (mean age 27.5 years (24.0–40.2), 9 men and 25 women). The study was performed in accordance with the requirements of the Helsinki Declaration of the World Medical Association on the Ethical Principles for Medical Research Involving Human Subjects (2000).

Venous blood of the examined individuals that was taken on an empty stomach in the morning from the cubital vein prior to the beginning of therapy into tubes containing EDTA was used as the material for the study. Mononuclear cells were isolated from the blood using Ficoll density gradient centrifugation (ρ = 1.077 g/cm³) (Sigma-Aldrich, United States) according to a standard technique [19]. Further, aliquots of 2 × 10⁶ mononuclear cells were made to prepare cell lysates. An aliquot of mononuclear cells isolated from peripheral blood was treated with 4 μL of a mixture of protease inhibitors (Fermentas, United States), 2 μL of a mixture of serine–threonine phosphatase inhibitors (Sigma-Aldrich, United States) and 200 μL of lysing buffer (50 mM Tris–HCl (pH 6.5), 100 mM of dithiothreitol, 0.1% of bromophenol blue, 10% of glycerol, 2% of sodium dodecyl sulfate). Then, the mixture was thoroughly stirred and incubated for 15 min at 95°C. Upon being cooled to room temperature, lysates were again mixed and centrifuged for 10 min at 12000 rpm, and the supernatant was collected. The cell lysates were used for SDS–polyacryla-
mide gel electrophoresis in a volume of 20 μL following the standard technique [20].

For subsequent studies the proteins were transferred to a PVDF (Polyvinylidene fluoride) membrane (Bio-Rad, United States) using Trans-Blot Turbo equipment (Bio-Rad, United States). The membranes were then incubated with primary (1 : 1000 dilution) and secondary antibodies (1 : 300 dilution) (Abcam, United Kingdom). Chromatographic detection using an Opti-4CN Substrate Kit (Bio-Rad, United States) was performed during the following step. Calculation of the intensity band of the protein we studied and image processing was performed using Alliance 2.7 gel documentation system (Uvitech Cambridge, United Kingdom). The level of the protein in samples was normalized to the reference signal of β-actin (total GSK-3 β/β-actin and total Akt1/β-actin). The relative level of phosphoforms of GSK-3β and Akt1 was assessed with respect to the level of their total fractions (phospho-serine-9 GSK-3β/total GSK-3β and phospho-serine-473 Akt1/total Akt1).

Statistical analysis was carried out using SPSS 20.0 software. The normality test for the distribution of the studied parameters was performed using the Kolmogorov–Smirnov criterion. The median and 25% and 75% quartiles were calculated for each sample; the significance of differences between the groups was determined by the Mann–Whitney and Kruskal–Wallis tests. The differences were considered as significant at p < 0.05.

RESULTS

We studied the levels of Akt1 and GSK-3β and their phosphorylated forms: phospho-serine-473-Akt1 and phospho-serine-9 GSK-3β. The average age in the control group was significantly lower than in the group of patients with depressive disorder (p = 0.001); therefore, we performed correlation analysis to establish the relationship between age and the levels of the proteins we investigated. According to the analysis, significant correlations between the studied parameters were absent (p > 0.05). Measurements of intracellular levels of the Akt1 and GSK-3β proteins and their phosphoforms showed the following results. The level of total GSK-3β significantly differed between the groups. In patients with depressive and bipolar disorders, the total GSK-3β significantly exceeded its value in the healthy donors (p = 0.001) (Fig. 1a). The intracellular level of the protein in patients with recurrent depressive disorder was higher than in the group of patients with depressive episodes (p = 0.042) (Fig. 2a); note that the level of total GSK-3β in patients with depressive episodes was comparable with the level in healthy donors. No differences were found between the level of