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Dysfunction of Protein Kinase FA/GSK-3α in Lymphocytes of Patients with Schizophrenic Disorder

Shiaw-Der Yang, Jau-Song Yu, Tsong-Tze Lee, Chuan-Ching Yang, Mei-Hui Ni, and Yong-Yi Yang

Institute of Basic Medicine and Department of Cell and Molecular Biology, Chang Gung Medical College (S.-D.Y., J.-S.Y., C.-C.Y., M.-H.N.); and Department of Psychiatry, Chang Gung Medical Center, Lin-Kou, Tao-Yuan 33332, Taiwan, ROC (Y.Y.Y.); Tao-Yuan and Institute of Life and Biomedical Sciences, National Tsing Hua University, Hsinchu (S.-D.Y., T.-T.L.)

Abstract As compared to normal people, the lymphocytes of patients with schizophrenia were found to have an impairment of ATP.Mg-dependent protein phosphatase activation. More importantly, the impaired protein phosphatase activation in the lymphocytes of schizophrenic patients could be consistently and completely restored to normal by exogenous pure protein kinase FA/glycogen synthase kinase-3α (kinase FA/GSK-3α) (the activating factor of ATP.Mg-dependent protein phosphatase), indicating that the molecular mechanism for the impaired protein phosphatase activation in schizophrenic patients may be due to a functional loss of kinase FA/GSK-3α. Immunoblotting and kinase activity analysis in an anti-kinase FA/GSK-3α immunoprecipitate further demonstrate that both cellular activities and protein levels of kinase FA/GSK-3α in the lymphocytes of schizophrenic patients were greatly impared as compared to normal controls. Statistical analysis revealed that the lymphocytes isolated from 37 normal people contain kinase FA/GSK-3α activity in the high levels of 14.8 ± 2.4 units/mg of cell protein, whereas the lymphocytes of 48 patients with schizophrenic disorder contain kinase FA/GSK-3α activity in the low levels of 2.8 ± 1.6 units/mg, indicating that the different levels of kinase FA/GSK-3α activity between schizophrenic patients and normal people are statistically significant. Taken together, the results provide initial evidence that patients with schizophrenic disorder may have a common impairment in the protein levels and cellular activities of kinase FA/GSK-3α, a multisubstrate protein kinase and a multisubstrate protein phosphatase activator in their lymphocytes.

Key words: schizophrenic disorder, lymphocytes, kinase FA/GSK-3α, impaired protein phosphatase activation, immunoblotting

An ATP.Mg-dependent multisubstrate/multifunctional protein phosphatase has been identified and characterized in most mammalian nervous and nonnervous tissues [Yang et al., 1980; Stewart et al., 1981; Jurgensen et al., 1984; Yang and Fong, 1985]. The phosphatase is inactive as isolated but can be activated in the presence of ATP.Mg and an activating factor termed FA which is identical to glycogen synthase kinase-3α (GSK-3α) [Vandenheede et al., 1980; Hemmings et al., 1981; Woodgett, 1990]. After kinase FA/GSK-3α-mediated activation, the activated phosphatase becomes capable of dephosphorylating and modulating many key regulatory enzymes and proteins involved in the regulation of diverse cell functions [Stewart et al., 1981; Ingebritsen and Cohen, 1983; Merlevede et al., 1984; Yang, 1986, 1991; Yang et al., 1991]. Kinase FA/GSK-3α has further been identified as a multisubstrate/multifunctional protein kinase that could act on many substrates including the R_{II} subunit of cAMP-dependent protein kinase [Hemmings et al., 1982], phosphatase inhibitor-2 [Depaoli-Roach, 1984; Jurgensen et al., 1984], myelin basic protein [Yang, 1986], the nerve growth factor [Taniuchi et al., 1986], neural cell adhesion molecule [Mackie et al., 1989], ATP-citrate lyase [Ramakrishna et al., 1990], neurofilament proteins [Guan et al., 1991], proto-oncogene products such as c-myc and c-jun [Boyle et al., 1991], synapsin I [Yang et al., 1992a,b], acetyl-CoA carboxylase [Hughes et al., 1992], microtubule associated protein-2

Abbreviations: GSK-3α, glycogen synthase kinase 3α; kinase FA, ATP.Mg-dependent type-1 protein phosphatase activating factor; MBP, myelin basic protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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Address reprint requests to Dr. S.-D. Yang, Institute of Basic Medicine and Department of Cell and Molecular Biology, Chang Gung Medical College, Tao-Yuan 33332 Taiwan, Republic of China.

and tau protein [Yang et al., 1991, 1993a–c, 1994; Hanger et al., 1992; Mandelkow et al., 1992], and brain clathrin-coated vesicles [Yu and Yang, 1993a]. By its dual role as a multisubstrate protein kinase and as a multisubstrate protein phosphatase activator, kinase FA/GAK-3α may simultaneously modulate the phosphorylation and dephosphorylation states of many diverse key regulatory proteins involved in the regulation of cell metabolism and functions.

Only recently, kinase FA/GSK-3α was found to be involved in the regulation of more complicated cell functions than was previously conceived. Kinase FA/GSK-3α has further been identified as a potent brain kinase most abundantly existing in the brain [Yu and Yang, 1993b] and could be involved in the regulation of diverse brain functions including the regulation of brain myelin functions [Yang, 1986; Yang et al., 1990; Yu and Yang, 1994a], brain microtubule assembly-disassembly [Yang et al., 1991, 1993a,b] and regulation of brain neurotransmission [Yang, 1991; Yang et al., 1992a,b]. Most importantly, kinase FA/GSK-3α was further identified as a potent tau kinase capable of phosphorylating tau on sites of Thr^{231}, Ser^{235}, Ser^{262}, Ser^{324}, Ser^{356} and Ser^{404} which are abnormally phosphorylated in Alzheimer disease brain and responsible for reducing microtubule binding involved in neuronal degeneration [Hanger et al., 1992; Mandelkow et al., 1992; Yang et al., 1993c, 1994], suggesting that the intrinsic defect of brain functions and the related psychiatric diseases may possibly be due to deregulation of this unique kinase. In an attempt to clarify the underlying mechanism, we have now examined the activity changes of kinase FA/GKS-3α using lymphocyte as a testing model isolated from patients with schizophrenia. In this report, we found that patients with schizophrenia statistically have a common defect in the capability to activate exogenous inactive ATP.Mg-dependent protein phosphatase in their lymphocytes and the molecular mechanism for this impaired protein phosphatase activation is due to a functional loss of protein kinase FA/GSK-3α, suggesting an association of schizophrenic disorder with protein kinase FA/GSK-3α in human lymphocytes.

EXPERIMENTAL PROCEDURES

Materials

[γ-^{32}P]ATP was purchased from Amersham (UK). ATP, heparin, N-hydroxysuccinimide biotin, and bovine serum albumin were obtained from Sigma (St. Louis, MO). Sucrose, dithiothreitol, and Triton X-100 were from Merck (Rahway, NJ). Ficoll-Paque, protein A-Sepharose CL-4B, and CNBr-activated Sepharose 4B were from Pharmacia (Uppsala, Sweden). RPMI medium was from Gibco. Polyvinylidene fluoride (PVDF) membrane (Immobilon-P) was from Millipore (Bedford, MA). Strepavidin conjugated with alkaline phosphatase, molecular weight marker proteins, and Lumingen PPD were from Boehringer Mannheim (Mannheim, Germany).

Protein Purification

Phosphorylase b [Fischer and Krebs, 1958] and phosphorylase b kinase [Cohen, 1973] were purified from rabbit skeletal muscle. ^{32}P-phosphorylase a [Krebs et al., 1958] was prepared from phosphorylase b, phosphorylase b kinase and [γ-^{32}P]ATP. Myelin basic protein (MBP) was purified from pig brain [Yang et al., 1987]. The ATP.Mg-dependent protein phosphatase [Yang and Fong, 1985] and its activating factor (kinase FA/GSK-3 α) [Yang, 1986; Yu and Yang, 1993b] were purified from pig brain. When analyzed by gel electrophoresis in the presence of SDS and Coomassie blue staining, the purified kinase FA/GSK-3 gave a single major protein band at a Mr = 53,000. Analysis of the radioactively autophosphorylated kinase FA/GSK-3 on the autoradiogram also revealed a single major phosphorylated protein band at a Mr = 53,000. Immunoblot analysis further revealed that the purified kinase FA/GSK-3 could be specifically recognized by anti-kinase FA/GSK-3α antibody which can not recognize GSK-3β [Yu and Yang, 1993b, 1994b,c]. Therefore, the preparations of kinase FA/GSK-3 used in this report belong to the category of GSK-3α according to the definition of Woodgett [1990].

Human Subjects

This study was conducted in 48 patients with schizophrenic disorder that have not taken any medication within one month. Another 37 medical staff members or students with matched sex, height, age, and body mass index (BMI) were enrolled as normal controls. The diagnosis of schizophrenic disorder was assured and classified according to the definition of DSM III (*Diagnostic and Statistical Manual of Mental Disorders*) which was edited by American Psychiatric Association [1987].

Isolation of Lymphocytes and Preparation of Cell Extracts

Human lymphocytes were isolated from 10 ml venous blood placed in a heparin-filled syringe and purified by Ficoll-Paque method basically according to Boyum [1968] and Fotino et al. [1971]. Briefly, after informed consent, 10 ml venous blood were drawn by a syringe rinsed with heparin. Within 3 h, 5 ml of each blood sample was diluted with 5 ml of phosphate buffer saline (PBS) and then carefully layered onto 10 ml lymphocyte separation medium (Ficoll-Paque, Pharmacia Co.) in two centrifuge tubes. After centrifugation at 480*g* (1,600 rpm using Kubota KS-5200C centrifuge) at 25°C for 40 min, the lymphocytes which appeared as an opalescent band at the interface layer were then carefully removed with a Pasteur pipette and suspended in 4 ml RPMI medium and further centrifuged at 480*g* at 25°C for 5 min. After centrifugation, the supernatant was discarded and the cell pellets were resuspended in 4 ml RPMI medium and processed as described above. The cell pellets were washed two more times with 4 ml of PBS to remove the residual medium. The lymphocytes ($\sim 2 \times 10^7$ cells) were finally pooled and mixed with 0.3 ml solution A (20 mM Tris-HCl at pH 7.0, 1% Triton X-100, 0.1 mM phenylmethanesulfonyl fluoride, 0.5 mM benzamidine and 0.1 mM L-1-chloro-3-tosylamido-7-amin-2-hepanone) and sonicated at 50% power at 4°C for 15 times (each cycle time was 2 s). After centrifugation at 72,000*g* at 4°C for 7 min, the supernatant was used as the cell extract. Under this condition, the cell extracts obtained from either patients or normal people all contained similar cell protein levels (~ 2.0 mg/ml) for the following experiments. It is important to note that if the patients contain lower levels of lymphocytes number ($< 80\%$) in their blood or lower levels of cell protein ($< 80\%$) in their lymphocytes as compared to normal controls, we simply did not use them for any further experiments. It is also important to note that all the patients tested were diagnosed to have clinical characteristics of schizophrenic disorder but have no other clinical complications such as hypertension and hyperglycemia in order to avoid misinterpretation of the results [Yang et al., 1992c].

Enzyme Assays

The activities of ATP.Mg-dependent protein phosphatase and kinase FA were determined by methods described in the previous reports [Yang et al., 1980; Yang and Fong, 1985; Yang, 1986]. Briefly, the activity of ATP.Mg-dependent protein phosphatase was measured after a 7-min preincubation at 30°C with 0.1 mM ATP, 0.5 mM Mg^{2+} ions and saturating amount of kinase FA in the buffer containing 20 mM Tris-HCl at pH 7.0 and 1 mM dithiothreitol for full activation of the phosphatase. ^{32}P-phosphorylase a (final concentration was 1 mg/ml) was next added to initiate the phosphatase action. The assay time was 8 min. The activity of kinase FA assayed as the activating factor of ATP.Mg-dependent protein phosphatase was measured by the formation of activated ATP.Mg-dependent protein phosphatase at 30°C. The assay mixture contained 20 mM Tris-HCl at pH 7.0, 1 mM dithiothreitol, appropriate dilutions of kinase FA, 0.1 mM ATP, 0.5 mM Mg^{2+} ions and excess amount of inactive ATP.Mg-dependent protein phosphatase to ensure the linear activation of the phosphatase activity. The preincubation time was 7 min. ^{32}P-phosphorylase a (final concentration was 1 mg/ml) was used as the substrate and assay time was 8 min. The activity of spontaneously active protein phosphatase was assayed under the same conditions except that ATP.Mg and exogenous inactive phosphatase were removed from the reaction mixtures. A unit of protein phosphatase activity is that amount of enzyme that catalyzes the release of 1 nmol of phosphate/min from ^{32}P-phosphorylase a. A unit of kinase FA assayed as the activator of ATP.Mg-dependent protein phosphatase is that amount of enzyme that produces one unit of activated ATP.Mg-dependent protein phosphatase after a 1-min preincubation.

Production of Anti-Kinase FA/GSK-3α Antibody

The anti-kinase FA/GSK-3α antibody was produced by using the peptide, TETQTGQDWQAPDA, corresponding to the carboxyl-terminal regions from amino acids 462-475 of the sequence of kinase FA/GSK-3α [Woodgett, 1990] as the antigen. Production, affinity-purification, identification and characterization of anti-kinase FA/GSK-3α antibody were detailed in previous reports [Yu and Yang, 1993b, 1994b,c]. In this report, the antibody can potently and specifically immunoblot kinase FA/GSK-3α from the human lymphocyte extracts on SDS-PAGE. The antibody can also efficiently immunoprecipitate all the kinase FA/GSK-3α from the lymphocyte extracts without blocking the kinase activ-

ity essentially as described in previous reports [Yu and Yang, 1994b,c] (data not further illustrated).

Biotinylation of Anti-Kinase FA/GSK-3α Antibody

Biotinylation of anti-kinase FA/GSK-3α antibody was performed according to the method described by Harlow and Lane [1988]. Briefly, 1 ml of purified anti-kinase FA/GSK-3α antibody as described above (3 mg/ml in 0.1 M sodium borate buffer at pH 8.8) was mixed with 0.1 ml of N-hydroxysuccinimide biotin (10 mg/ml in dimethyl sulfoxide) and incubated at room temperature for 4 h. The coupling reaction was stopped by incubating with 80 μl of 1 M NH4Cl for 10 min. After extensive dialysis against PBS to remove uncoupled biotin, the biotinylated anti-kinase FA/GSK-3α antibody was concentrated by dialysis against PBS containing 50% glycerol and stored at −20°C.

Immunoprecipitation and Kinase FA/GSK-3α Activity Assays in the Immunoprecipitates

Before immunoprecipitation, protein concentrations of the lymphocyte extracts were first diluted to equal amounts with solution A. For immunoprecipitation, 300 μl of lymphocyte extracts (~2.0 mg protein/ml) were incubated with 2 μl of affinity-purified kinase FA/GSK-3α antibody (10 mg/ml pure IgG) at 4°C for 1 h and then with 100 μl of protein A-Sepharose CL-4B (20% v/v, in solution A) for another 1 h with shaking. The immunoprecipitates were collected by centrifugation, washed three times with 1 ml of 0.5 M NaCl, once with 1 ml solution B (20 mM Tris-HCl at pH 7.0, 0.5 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride and 0.5 mM benzamidine), and resuspended in 100 μl solution B. For kinase FA/GSK-3α activity assay in the immunoprecipitate, 15 μl of immunoprecipitate prepared as described above was incubated with 30 μl of a mixture containing 20 mM Tris-HCl at pH 7.0, 0.5 mM dithiothreitol, 0.2 mM [γ-^{32}P]ATP, 20 mM $MgCl_2$, and 4 mg/ml MBP at 30°C for 10 min. ^{32}P incorporation into MBP was measured by spotting 30 μl of reaction mixture on phosphocellulose paper (1 × 2 cm), washing three times with 75 mM H_3PO_4, and counting in liquid scintillation analyzer (Model 1600CA, Packard) essentially as described in previous reports [Yang, 1986; Yu and Yang, 1994b,c]. A unit of protein kinase FA/GSK-3α assayed as MBP kinase is defined as that amount of kinase that incorporates 1 pmol of phosphate/min into the kinase substrate.

Immunoblot

Proteins were transferred from SDS-gels to Immobilon-P membrane in a Transphor (Hoefer) at 350 mA in transfer buffer (10 mM 3-[cyclohexylamino]-1-propane-sulfonic acid (CAPS) at pH 10 containing 20% methanol) at 4°C for 2 h. The membrane was incubated in TTBS buffer (20 mM Tris-HCl at pH 7.4, 0.5 M NaCl and 0.05% Tween 20) containing 5% non-fat dried milk at room temperature for 1 h to block the free protein binding sites. After washing 3 times with TTBS buffer, the membrane was incubated with 1 μg/ml biotinylated anti-kinase FA/GSK-3α antibody in TTBS buffer containing 3% non-fat dried milk at room temperature for 4 h, washed 3 times in TTBS buffer, and then incubated with strepavidin conjugated with alkaline phosphatase diluted at 1:2,000 in TTBS buffer containing 3% non-fat dried milk at room temperature for 40 min and washed 3 times in TTBS buffer. The kinase FA/GSK-3α protein was visualized by chemiluminescence detection using Lumingen PPD as a chemiluminescent substrate.

Analytic Methods

Protein concentration was determined by the method of Lowry et al. [1951]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [1970] using 10% gels. Molecular weight markers used are as follows: α_2-macroglobulin (170,000), β-galactosidase (116,400), fructose-6-phosphate kinase (85,200), glutamate dehydrogenase (55,600), and aldolase (39,200).

Statistical Analysis

The results are expressed as means ± SD for the indicated case number (n) of observations. The two-tailed Student's *t*-test was used to calculate the statistical significance of the differences. The cellular activity of protein kinase FA/GSK-3α was assayed as a protein kinase using MBP as substrate in an anti-kinase FA/GSK-3α immunoprecipitate from the lymphocyte extracts of patients and of normal controls as described above throughout all the statistical study.

RESULTS

Addition of exogenous protein kinase FA/GSK-3α together with or without 0.1 mM ATP/0.5 mM Mg^{2+} ions or with inactive ATP.Mg-dependent protein phosphatase was found to have no significant effect on the protein phospha-

tase activity in the lymphocytes of normal subject (Fig. 1A). However, when the inactive ATP.Mg-dependent protein phosphatase together with 0.1 mM ATP and 0.5 mM Mg^{2+} ions was added to the same lymphocyte extracts, the protein phosphatase activity could be dramatically stimulated (Fig. 1A), suggesting the existence of protein kinase FA (an activating factor of ATP.Mg-dependent protein phosphatase) [Vandenheede et al., 1980; Yang et al., 1980] in human lymphocytes. In sharp contrast, when the inactive ATP.Mg-dependent protein phosphatase and ATP.Mg were added to the schizophrenic patient lymphocytes and processed under *identical* conditions, the phosphatase activity was only slightly stimulated as compared to normal control (Fig. 1A,B). It is noted that the lymphocytes isolated from schizophrenic patient and from normal subject appeared to contain similar activity level of spontaneously active protein phosphatase (Fig. 1). Taken together, the results indicate that schizophrenic patient may have an impairment in the capability to activate the exogenous ATP.Mg-dependent protein phosphatase in the lymphocytes and the molecular mechanism for this defect may possibly be due to a functional loss of protein kinase FA/GSK-3α, the activating factor of protein phosphatase [Yang et al., 1980; Vandenheede et al., 1980] in the lymphocytes of schizophrenic patient.

To elucidate the molecular mechanism for the impaired protein phosphatase activation in the lymphocytes of patient with schizophrenia, we next used the exogenous pure protein kinase FA/GSK-3α to titrate the crude extracts of patient lymphocytes. As shown in Figure 2, the

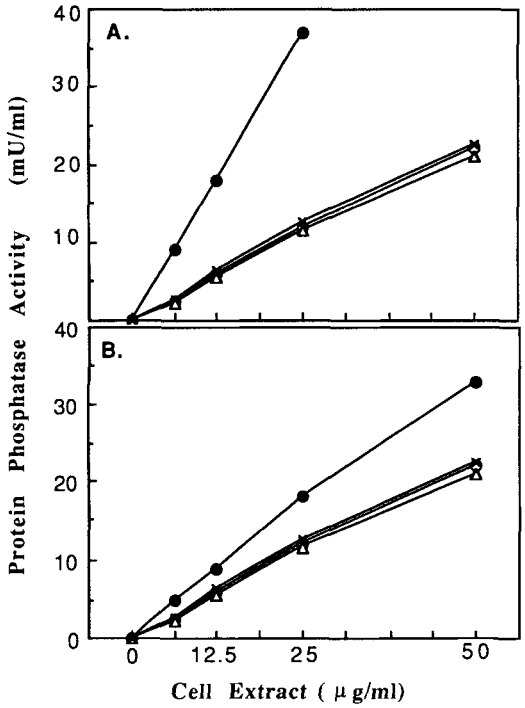

Fig. 1. Comparisons of protein phosphatase activation in the lymphocytes of normal subject and of schizophrenic patient. **A:** The lymphocytes isolated from fresh blood of normal human subject were homogenized in 0.3 ml solution A. After centrifugation, the supernatant was diluted to the appropriate concentrations as indicated. The cell extracts were assayed for protein phosphatase activity in the absence (○) and presence (●) of 0.1 mM ATP/0.5 mM Mg^{2+} ions and 10 mU inactive protein phosphatase. Control experiments were also performed in ATP.Mg with (x) and without (△) kinase FA. Assay conditions were as described under "Experimental Procedures." Data were taken from the average of 3 independent experiments. **B:** The same as in A except that the lymphocytes were isolated from schizophrenic patient. A mU of protein phosphatase activity is that amount of enzyme that catalyzes the release of 1 pmol of phosphate/min from ^{32}P-phosphorylase a.

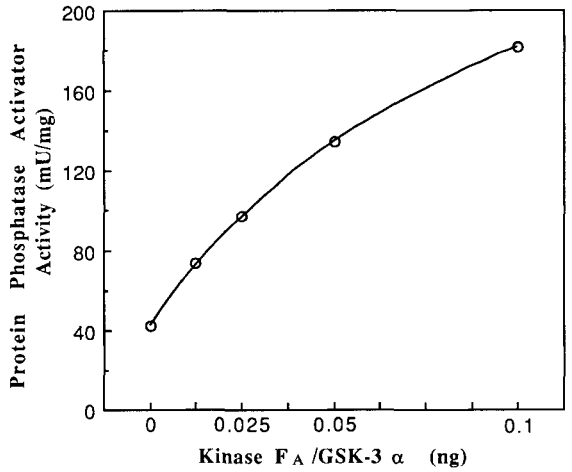

Fig. 2. Identification of kinase FA/GSK-3α as a missing component in the impaired protein phosphatase activation in the lymphocytes of schizophrenic patient. The lymphocyte cell extracts of schizophrenic patient as described in Figure 1B was diluted 80-fold (final concentration was ~0.025 mg/ml of cell protein) and then incubated with 0.1 mM ATP, 0.5 mM Mg^{2+} ions, 10 mU inactive protein phosphatase and titrated amounts of exogenous pure kinase FA/GSK-3α as indicated at 30°C for 7 min. ^{32} P-phosphorylase a was added to initiate the phosphatase action at 30°C for another 8 min. The total assay volume was 30 μl. The detailed assay conditions was as described under Experimental Procedures. The activity of kinase FA/GSK-3α was assayed as an activating factor of protein phosphatase and expressed as the protein phosphatase activator activity. A unit of kinase FA assayed as the activator of ATP.Mg-dependent protein phosphatase is that amount of enzyme that produces one unit of activated ATP.Mg-dependent protein phosphatase after a 1-min preincubation. Data were taken from the average of 3 independent experiments.

exogenously added pure kinase FA/GSK-3α could restore the protein phosphatase activation in the lymphocytes of schizophrenic patient. The results further support the notion that the molecular mechanism for the impaired protein phosphatase activation in lymphocytes of schizophrenic patient could be due to a functional loss of protein kinase FA/GSK-3α. For further demonstration, we finally subjected the lymphocyte extracts of schizophrenic patient and of normal control to immunoblotting and kinase activity analysis in an anti-kinase FA/GSK-3α immunoprecipitate using a potent and immunospecific anti-kinase FA/GSK-3α antibody produced and affinity-purified as described under Experimental Procedures. As shown in Figure 3A, the anti-kinase FA/GSK-3α antibody was found to be very potent and specific toward immunoprecipitating and immunoblotting kinase FA/GSK-3α at a molecular weight of 53,000 from lymphocytes of schizophrenic patient and of normal control, demonstrating the existence of kinase FA/GSK-3α in human lymphocytes. In agreement with the results as described in Figures 1 and 2, the immunoblotted protein level of kinase FA/GSK-3α in the lymphocytes of schizophrenic patients was greatly reduced as compared to normal controls, demonstrating that the impaired protein phosphatase activation could be due to a loss of kinase FA/GSK-3α in the lymphocytes of schizophrenic patients. In close correlation, the cellular activity of kinase FA/GSK-3α which is detectable in the immunoprecipitates from the lymphocytes of schizophrenic patient was also decreased down to less than 20% of normal control (Fig. 3B). Taken together, the results demonstrate that indeed there is a functional loss of protein kinase FAGSK-3α in the lymphocytes of schizophrenic patients.

To further demonstrate that the patients with schizophrenia may statistically have a functional loss of kinase FA/GSK-3α in their lymphocytes, we further tested 37 normal people and 48

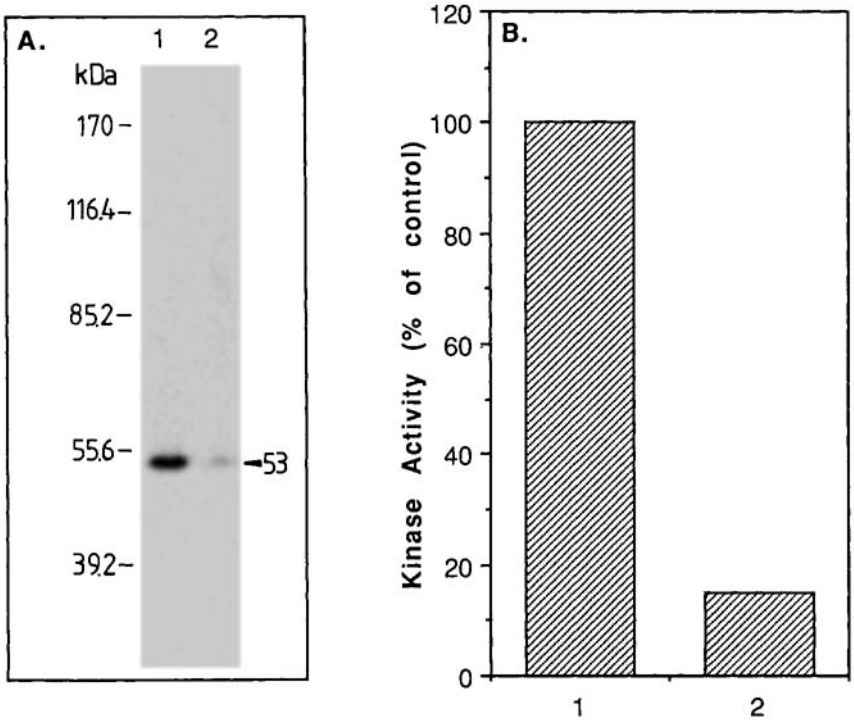

Fig. 3. Immunoblot and immunoprecipitate kinase activity analysis of kinase FA/GSK-3α from lymphocyte extracts of schizophrenic patient and of normal control. The lymphocyte extracts (~2.0 mg/ml of cell protein in 0.3 ml solution A) obtained from schizophrenic patient and from normal control were subjected to immunoprecipitation with 20 μg of anti-kinase FA /GSK-3α antibody. **A:** The immunoprecipitated proteins eluted from the immunoprecipitates with Laemmli sample buffer were immunoblotted on 10% SDS-PAGE using the biotinylated anti-kinase FA/GSK-3α antibody followed by chemiluminescent detection. **B:** The cellular activity of kinase FA/GSK-3α was assayed in the immunoprecipitates using MBP as substrate. Lane 1: Normal control. Lane 2: Schizophrenic patient. Detailed conditions for immunoprecipitation, immunoblotting and kinase activity assay in the immunoprecipitates were as described under Experimental Procedures. The immunoblot and cellular activity data shown are representative results from 3 independent experiments.

TABLE I. Statistical Dysfunction of Kinase FA/GSK-3α in Lymphocytes of Schizophrenic Patients†

Lymphocytes	Cell number	Cell protein (mg)	Kinase FA/GKS-3α activity (units/mg)
Normal (N = 37)	~2 × 10^7	0.65 ± 0.08	14.8 ± 2.4
Schizophrenia (N = 48)	~2 × 10^7	0.63 ± 0.07	2.8 ± 1.6*

†The cellular activity of kinase FA/GSK-3α in lymphocytes was measured in the anti-kinase FA/GSK-3α immunoprecipitate from the cell extracts using MBP as the kinase substrate as described under "Experimental Procedures." The results are expressed as means ± SD for the indicated case No. (n) of observations. The diagnosis of schizophrenic disorder was assured according to the definition of DSM III. The patients have no other clinical complications. A unit of protein kinase FA/GSK-3α assayed as a MBP kinase is defined as that amount of enzyme that incorporates 1 pmol of phosphate/min into the MBP substrate.

*$P < 0.01$ vs. normal control by Student's t-test.

patients that have been diagnosed to have clinical characteristics of schizophrenic disorder. It is important to note that both patients and normal subjects selected all contain similar numbers of lymphocytes in their blood and similar cell protein levels in their lymphocytes for these experiments. As summarized in Table I, we found that the cellular activity levels of kinase FA/GSK-3α assayed in the anti-kinase FA/GSK-3α immunoprecipitate from the lymphocytes of normal subjects tested fell in the range of 14.8 ± 2.4 units/mg of cell protein, whereas the levels of kinase FA/GSK-3α activity in the lymphocytes of the schizophrenic patients tested were found to be consistently low and fell in the range of 2.8 ± 1.6 units/mg of cell protein, demonstrating that patients with schizophrenia may statistically have a common dysfunction of protein kinase FA/GSK-3α in their lymphocytes.

DISCUSSION

This is the first report to demonstrate dysfunctions of protein kinase FA-GSK-3α in the lymphocytes of patients with schizophrenic disorder. First, kinase FA/GSK-3α was found to exist in human lymphocytes as demonstrated by immunoblotting analysis of the immunoprecipitates obtained from the lymphocyte extracts using a potent and immunospecific anti-kinase FA/GSK-3α antibody. Second, the impaired protein phosphatase activation in the lymphocytes of schizophrenic patients could be consistently and completely restored to normal by the exogenous pure protein kinase FA/GSK-3α in a dose-dependent manner. Third, among the 48 patients with schizophrenic disorder but with normal lymphocyte numbers and normal cell protein concentrations and without any other clinical complications tested, all the patients statistically and consistently have a common functional loss of kinase FA/GSK-3α in their lymphocytes as compared to normal controls. By using biotinylated anti-kinase FA/GSK-3α antibody to immunoblot the immunoprecipitates from the lymphocytes of schizophrenic patients and of normal people followed by chemiluminescent detection and quantification, we finally demonstrate that the molecular mechanism for the dysfunction of kinase FAGSK-3α could be due to underexpression of this kinase in the lymphocytes of schizophrenic patients. Although the pathophysiologic role of kinase FA/GSK-3α in human lymphocytes remains to be established, it is tempted to believe that kinase FA/GSK-3α may possibly be involved in the regulation of the lymphocytes function through phosphorylation and dephosphorylation in human lymphocytes. On the other hand, the results as presented in this report that patients with schizophrenia consistently have a functional loss of kinase FA/GSK-3α further support the notion that kinase FA/GSK-3α can be involved in more complicated pathophysiological roles than was previously conceived [Yang, 1991; Woodgett, 1991; Plyte et al., 1992; Yang et al., 1992c, 1994].

Although the pathologic role of lymphocytes in the schizophrenic disorder remains obscure, the results that patients with schizophrenia statistically have a functional loss of kinase FA/GSK-3α in their lymphocytes as presented here together with the previous reports that kinase FA/GSK-3α may play a potential role in the regulation of diverse brain functions [reviewed in Yang, 1991] strongly implicate an important connection among kinase FA/GSK-3α, lymphocyte and schizophrenic disorder. For instance,

the pathogenic mechanisms for the onset of schizophrenic disorder can be in part due to biological dysfunction of kinase FA/GSK-3α in the body especially in the brain. Conversely, the pathogenic mechanism for the immunological dysfunction in the schizophrenic patients can be in part due to the functional loss of kinase FAGSK-3α in the lymphocytes. Although the imbalance of dopamine/serotonin ratio and dysfunction of dopamine receptors were reported to be associated with schizophrenic disorder, however, to our knowledge, there are no biochemical markers currently available which are specific for schizophrenic disorder. Protein kinase FA/GSK-3α turned out to be one of the most potential protein markers specific for schizophrenia as reported here. From the clinical viewpoints, since the protein levels and cellular activities of kinase FA/GSK-3α in the lymphocytes of schizophrenic patients were greatly impaired as compared to normal control levels in a statistical manner, kinase FA/GSK-3α may possibly be used as a specific marker protein for clinical diagnosis of the status of schizophrenic disorder during pre- and post-diagnostics of the disease and may also be used as a specific marker protein of early diagnosis for screening patients that may have potential tendency for schizophrenic disorder. This important issue is under current investigation in this laboratory. Nevertheless, the present study clearly demonstrates that patients with schizophrenic disorder may statistically have a common defect in the capability to activate protein phosphatase in their lymphocytes and the molecular mechanism for this defect is due to dysfunction of multisubstrate/multifunctional protein kinase FA/GSK-3α.

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