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ORIGINAL PAPER

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Correlations between negative symptoms and peripheral G protein levels in mononuclear leukocytes of deficit and nondeficit schizophrenics

Preliminary results

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Abstract Receptor-coupled G proteins were measured in mononuclear leukocytes (MNL) of 17 drug-treated patients with deficit schizophrenia (DS) and 16 drug-treated patients with nondeficit schizophrenia (NDS). No significant difference was found in MNL levels of $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$ and G_{β} proteins between the two groups; however, MNL levels of $G_{\alpha s}$ were inversely correlated to the severity of negative symptoms in DS patients, while MNL levels of $G_{\alpha q}$ were positively correlated to negative symptoms in NDS patients. Since $G_{\alpha s}$ and $G_{\alpha q}$ are coupled to D-1 and 5-HT_2 receptors, respectively, these findings may support the hypothesis that a prevalent dysfunction of D-1 receptors is involved in the pathophysiology of negative symptoms in DS, whereas a prevalent dysfunction of 5-HT_2 receptors underlies negative symptoms in NDS. These results must be regarded as preliminary because of the possible interference of antipsychotic drugs on the explored parameters.

Key words G proteins · deficit schizophrenia · nondeficit schizophrenia · negative symptoms

Introduction

Signal transducing G proteins are composed of α, β and γ subunits and transduce extracellular signals into various intracellular responses by coupling membrane cell receptors to intracellular second messengers. Since a variety of neurotransmitter and neurohormone receptors share similar membrane G proteins, these molecules provide an elegant mechanism to organize, integrate and amplify the signals from different neurotransmitter pathways. Given this critical role in signal processing, it is not surprising that abnormalities in the function and/or the expression of G proteins have been implicated in the pathophysiology of various psychiatric disorders, including schizophrenia (Manji 1992). However, post-mortem studies exploring G protein concentrations in brain areas of schizophrenic patients have provided conflicting results (Okada 1997; Nishino et al. 1997; Jope et al. 1998; Yang et al. 1998). It is plausible that at least part of such inconsistency is due to the extreme clinical heterogeneity of schizophrenia, possibly reflecting a multiplicity of etiopathogenetic mechanisms and biological backgrounds.

An opportunity to overcome this handicap is to select patients according to well-defined clinical characteristics. Recently, Carpenter et al. (1988) proposed that the primary enduring negative symptoms of schizophrenia, termed deficit symptoms, identify a relatively homogeneous subtype of schizophrenia, named deficit schizophrenia (DS), which is opposed to nondeficit schizophrenia (NDS). Based on Carpenter's criteria, Kirkpatrick et al. (1989) have developed the Schedule for the Deficit Syndrome (SDS) to reliably identify this syndrome in schizophrenia. The deficit versus nondeficit classification has excellent diagnostic stability (Amador et al. 1999).

Clinical, neuropsychological, oculomotor and brain imaging differences between deficit and nondeficit schizophrenics have been demonstrated (Carpenter et al. 1988; Kirkpatrick et al. 1989; Buchanan et al. 1994; Bryson et al. 2001; Thaker et al. 1989; Tamminga et al. 1992; Galderisi et al. 2002). However, to date, very few biochemical investigations of the deficit/nondeficit distinction have been performed (Ribeyre et al. 1994; Nibuya et al. 1995; Thibaut et al. 1998), and, to the best of our knowledge, no study has assessed the functional status of G proteins in deficit as opposed to nondeficit schizophrenics. Therefore, we measured receptor-coupled G proteins in mononuclear leukocytes (MNL) of chronic schizophrenic patients in an attempt to unravel

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a pattern of G protein measures in DS subjects distinctive from that in NDS patients.

Subjects and methods

Thirty-three patients regularly attending the outpatient unit, day-care and rehabilitation centers of our department were recruited for the study. Patients fulfilled DSM-IV diagnosis of schizophrenia, as confirmed by the Structured Clinical Interview for DSM-IV (SCID-I) (Spitzer 1994), had no history of alcohol or drug abuse or dependence, no previous electroconvulsive therapy, and did not exhibit significant changes in their clinical state and drug treatment in the three months preceding the enrolment. They were diagnosed as having either DS (N = 17) or NDS (N = 16), by using the SDS (Kirkpatrick et al. 1989). Their clinical and demographic characteristics are shown in Table 1. Daily doses of antipsychotic drugs were converted in chlorpromazine equivalents. There was no difference between the two patient groups as to the type of antipsychotic treatment they were receiving: 47% of DS patients and 50% of NDS patients were being treated with novel antipsychotics; 41.3% of DS vs 37.5% of NDS with standard neuroleptics; 11.7% of DS vs 12.5% of NDS with a combination of standard neuroleptics and novel antipsychotics. In particular, in the NDS group, 8 patients were treated with clozapine (mean daily dose ± SD = 318.7 ± 109 mg/day), 3 with haloperidol (6.0 ± 3.0 mg/day), 1 with haloperidol depot (200 mg every 4 weeks), 1 with clotiapine (80 mg/day), 1 with zuclopentixole (80 mg/day), 1 with olanzapine (10 mg/day) + thioridazine (200 mg/day) and 1 with risperidone (4 mg/day) + fluphenazine (4 mg/day). In the DS group, 8 patients were treated with clozapine (300 ± 125 mg/day), 3 with haloperidol (5 ± 1.7 mg/day), 1 with haloperidol depot (100 mg every 4 weeks), 1 with fluphenazine depot (25 mg every 2 weeks), 1 with thioridazine (300 mg/day), 1 with olanzapine (5 mg/day) + haloperidol (3 mg/day) and 1 with olanzapine (10 mg/day) + zuclopentixole (10 mg/day).

Patients' psychopathological state was assessed by the Expanded Brief Psychiatric Rating Scale, version 4.0 (EBPRS) (Ventura et al. 1993), the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen 1981) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen 1984). The presence of extrapyramidal signs was evaluated by the Simpson-Angus Scale (Simpson and Angus 1970).

MNL isolation

Blood samples (60 ml) were collected by venipuncture between 8:00 and 10:00 a. m. into a heparinized glass tube and processed immediately. MNL were isolated using Ficoll-Hypaque gradient. Mononuclear cells were washed with phosphate-buffer saline, lysed mechanically in AT buffer (60 mM KCl, 150 mM NaCl, 14 mM β-mercaptoethanol, 2 mM EDTA, 15 mM Hepes, pH 7.9, 0.3 sucrose, 5 μg/ml aprotinin, 10 μg/ml leupeptin, 2 μg/ml pepstatin and 1% Triton X–100) and frozen at –80 °C until assayed.

Table 1 Clinical characteristics of the study sample*

	Deficit schizophrenics	Nondeficit schizophrenics
Number of Subjects (men/women)	17 (15/2)	16 (13/3)
Age, yrs	36.1 ± 8.1	36.1 ± 8.1
Age at onset of the illness, yrs	21.4 ± 4.9	22.5 ± 6.8
Duration of the illness, yrs	15.6 ± 9.2	16.8 ± 7.1
SAPS Total Score	14.1 ± 2.3	11.9 ± 4.3
SANS Total Score	6.0 ± 2.7	5.7 ± 4.2
EBPRS Total Score	44.5 ± 11.3**	39.1 ± 11.4
Simpson-Angus Average Score	0.45 ± 0.33	0.32 ± 0.25
Antipsychotic dose (chlorpromazine equivalent, mg/day)	395.13 ± 318.4	557.5 ± 238.4

* Data are expressed as mean ± SD; ** p = 0.04 (Mann-Whitney Test)

Immunoblot analysis

On the day of assay, samples were thawed and the extracts were centrifuged at 100,000 × g for 30 min at 4 °C. Protein concentration was determined according to the method of Bradford (1976).

The linearity of protein concentration for Western blotting was ascertained by resolution of selected quantity of protein (between 5 and 50 μg). Subsequent assays were performed using a protein concentration within the linear range for immunolabeling of G protein subunits. An aliquot of 20 μg of proteins, in duplicate for each subject, was separated by 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis and transferred to nitrocellulose filter (0.45 mm Schleicher & Shuell). Blots were washed in TBS-T (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) and blocked by incubation with 5% non-fat dry milk in TBS-T (blocking buffer). After 3 washes in TBS-T, blots were incubated in blocking buffer overnight with each of the following policlonal antibodies directed specifically against $G\alpha_s$, $G\alpha_{i-3}$, $G\alpha_{q/11}$, Gβ (all diluted 1:100) (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and anti-actin monoclonal antibody (dilution 1:5000) (Sigma-Aldrich Corp, St. Louis, MO). After being washed by TBS-T, blots were incubated with the secondary antibody, horseradish peroxidase-linked anti rabbit IgG (Amersham Pharmacia Biotech, Buckinghamshire, England), in blocking buffer for 1 hour. Labeled blots were washed with TBS-T and immunoreactivity was detected with Enhanced Chemiluminescence Western Blot detection System (Amersham Pharmacia), followed by exposure to Kodak X-Omat film (Kodak, Rochester, NY). Quantification of immunoblots was performed by a densiometric scanning of the autoradiograms using an image analysis system.

An aliquot of pooled standard leukocyte proteins were run on one lane of every gel to minimize the inter-assay variation. The optical density units obtained from each subject were normalized against those of pooled standard leukocyte proteins. Furthermore, the optical density of each G protein band was normalized against respective actin immunoreactivity band to reduce the inter-lane variation.

Although anti-$G\alpha_s$ detects both 52- and 45-kDa $G\alpha_s$ species, only 45-kDa species is detected in leukocytes. The other G protein subunits were found to migrate at the expected molecular weight.

Statistical analysis

The BMDP statistical software package (Dixon 1995) was used for data analysis. Data distributions were examined for normality and homogeneity of variance. Since these assumptions were violated, nonparametric statistics were used. Differences among the groups were analyzed by the Mann-Whitney U-test. Correlations between G protein measures and clinical variables, such as EBPRS, SANS, SAPS total scores, the Simpson-Angus average score, and the chlorpromazine equivalents were assessed by the Spearman's rank correlation test.

Results

Clinical data

No significant difference emerged between DS and NDS patients in age, age at onset of the illness, duration of the illness, SANS and SAPS total scores, Simpson-Angus average score and the mean current daily dose of antipsychotic drug (Table 1). Deficit schizophrenics scored significantly higher than nondeficit schizophrenics on the EBPRS total score ($p = 0.04$).

Biochemical measures

Immunoblot analyses using policlonal antibodies against $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$ and G_{β} proteins showed that the immunoreactive levels of these proteins in MNL of patients with DS did not significantly differ from those in MNL of NDS patients (Table 2).

In the whole patient group, a significant negative correlation between MNL levels of $G_{\alpha s}$ and SANS total scores (rho = –0.35, $p = 0.04$) and a significant positive correlation between MNL levels of $G_{\alpha q}$ and SANS total scores (rho = 0.46, $p = 0.007$) emerged. When we analyzed these correlations in the two patient groups separately, we found that the negative correlation between $G_{\alpha s}$ and SANS total scores persisted only in DS patients (rho = –0.78, $p = 0.0005$), while the positive correlation between MNL levels of $G_{\alpha q}$ and SANS total scores was maintained only in NDS patients (rho = 0.53, $p = 0.02$) (Fig. 1). No significant correlation emerged between immunoreactive levels of G proteins and antipsychotic daily doses.

Table 2 Immunoreactivity levels of G proteins in MNL of DS and NDS patients*

	Deficit schizophrenics	Nondeficit schizophrenics
$G\alpha_s$	1355.7 ± 403.1	1355.5 ± 439.2
$G\alpha_i$	1464.5 ± 271.8	1493.2 ± 268.4
$G\alpha_q$	1508.3 ± 288.8	1501.0 ± 420.6
$G\beta$	1396.4 ± 412.2	1289.1 ± 428.8

* Data are expressed as mean ± SD optical density

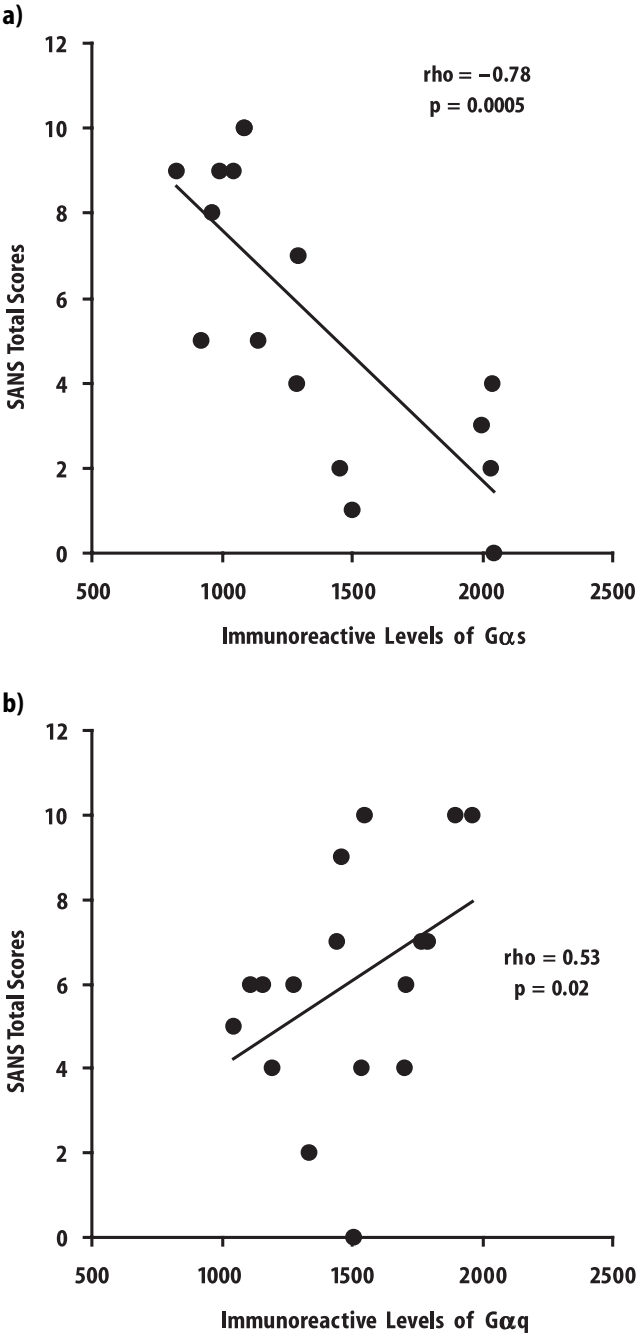

Fig. 1 Correlations between negative symptoms, as assessed by the Scale for the Assessment of Negative Symptoms (SANS), and G protein levels in mononuclear leukocytes of patients with deficit schizophrenia (**a**) or nondeficit schizophrenia (**b**)

Discussion

To our knowledge, this is the first study exploring receptor-coupled G protein levels in the MNL of DS and NDS patients, diagnosed according to the SDS.

We found no significant difference in both clinical characteristics and MNL levels of $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$ and G_{β} proteins between DS and NDS patients. However, we detected significant correlations between the severity of

negative symptoms, as assessed by the SANS, and immunoreactive levels of $G_{\alpha s}$, and $G_{\alpha q}$.

The major limitation of this study is that all patients were under chronic treatment with either classical or newer antipsychotic drugs. Even if there was no difference between DS and NDS patients in the daily dose and the type of antipsychotic they received, we cannot exclude that blood levels of these drugs could have been significantly different in the two groups. This could have been relevant for the expression levels of MNL G proteins and could have masked possible differences between the two patient groups. To this regard, studies exploring the effects of both typical and atypical antipsychotics on G protein levels in the rat brain provided conflicting results, with most of them showing no change or a decrease in the concentration of different G protein subtypes after chronic antipsychotic administration (See et al. 1993; Kaplan et al. 1999). In any event, in the 16 clozapine-treated patients (8 with DS and 8 with NDS), we were able to measure plasma levels of the drug and its major metabolites (data not shown). No significant differences emerged in the mean concentrations of plasma clozapine, N-desmethyl-clozapine and clozapine-N-oxide between DS and NDS patients. Therefore, at least for a subgroup of patients, if an effect of antipsychotic treatment had occurred on MNL G protein expression, this would have been the same and would not have affected possible difference in G protein measures between deficit and nondeficit subjects.

The finding of no quantitative change in MNL levels of G proteins in both DS and NDS schizophrenics does not exclude the possibility of an altered function of these proteins. To this regard, Avissar et al. (2001) recently reported an enhanced dopamine receptor-coupled $G_{\alpha s}$ protein activity in spite of no significant quantitative modification of this molecule in MNL of schizophrenic patients.

The negative correlation between MNL levels of $G_{\alpha s}$ and SANS total scores in our DS patients and the positive correlation between $G_{\alpha q}$ and SANS total scores in our NDS subjects are intriguing. It is known that $G_{\alpha s}$ is coupled to several neurotransmitter receptors, including the dopamine-1 (D-1) receptor subtype, while $G_{\alpha q}$ is linked, among others, to the serotonin-2 (5-HT_2) receptor. A hypofunction of D-1 receptors and a hyperactivity of 5-HT_2 receptors in the prefrontal cortex have been suggested to underlie the negative symptomatology of schizophrenia. Indeed, although post-mortem studies have failed to produce a consistent finding of abnormal D-1 receptor density in schizophrenic brains, recent PET investigations have found that, in the prefrontal cortex of schizophrenic subjects, the number of D-1 receptors was significantly reduced and correlated to both negative symptoms and cognitive impairments (Okubo et al. 1997; Sedvall 1995). Analogously, blockade of 5-HT_{2A} receptors by novel antipsychotics has been proposed as a mechanism mediating their ability to improve negative symptoms, thus suggesting a possible role for 5-HT_{2A} hyperactivity in the genesis of negative symptomatology (Moller 1999, 2001; Abi-Dargham and Krystal 2000). Thus, keeping in mind the possible interference of antipsychotic drug treatment on MNL G protein expression, our results seem to support the idea that a prevalent dysfunction of D-1 receptors could underlie negative symptoms in DS, whereas a prevalent dysfunction of 5-HT_{2A} receptors could be involved in the pathophysiology of negative symptoms of NDS. Future studies directly assessing the function of G protein subtypes in both DS and NDS patients will help confirm or disregard this hypothesis.

In conclusion, although no difference in the peripheral MNL levels of G proteins was found between DS and NDS patients, intriguing correlations between immunoreactive levels of $G_{\alpha s}$ or $G_{\alpha q}$ and negative symptoms emerged in these subjects. The relevance of these preliminary findings to the pathophysiology of negative symptomatology in DS and NDS remains to be determined.

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