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Decreased S100-beta protein in schizophrenia: preliminary evidence

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Abstract

The S100 proteins are a family of calcium-binding proteins found in the central and peripheral nervous systems of vertebrates. S100β, the most abundant member of this family in the CNS, mediates calcium signal transduction, and shows neurotrophic, gliotrophic and mitogenic actions that influence the development and maintenance of the nervous system. Another member of the S100 family (S100A10) was found to modulate phospholipid turnover by inhibiting the activity of enzyme phospholipase A2 (PLA2). We determined the concentration of S100β protein in the plasma of 23 medicated schizophrenic patients and 23 healthy controls. S100β protein accounts for 96% of the total S100 in the brain. Schizophrenic patients showed reduced S100β concentrations ($p=0.003$), and this finding was not related to clinical variables or to intake of antipsychotic medication. Decreased S100β could be related to the findings of increased PLA2 activity and to brain maldevelopment in schizophrenia. These results are discussed further with respect to the role of adenosine in S100β release.

Keywords: Brain development; Phospholipase A2; S100β protein; Schizophrenia

1. Introduction

An accelerated breakdown of membrane phospholipids has been suggested by our findings of increased cytosolic phospholipase A2 (cPLA2) activity in schizophrenia (Gattaz et al., 1987, 1990, 1995b). These findings were replicated by other laboratories (Noponen et al., 1993; Ross et al., 1997) and are in line with the results of ^{31}P-spectroscopy studies that show decreased resonances of the phosphomonoesters and increased phosphodiesters (precursors and metabolites of phospholipid turnover) in the frontal lobe of patients with schizophrenia (review in Deicken, 1999). Since, in rats, intracerebral injections of PLA2 inhibited dopaminergic activity (Brunner and Gattaz 1995a,b), we hypothesized that in schizophrenia increased PLA2 activity may account for the accelerated phospholipid turnover and for reduced dopaminergic activity in the frontal lobe (Gattaz and Brunner, 1996).

The increment in PLA2 activity in schizophrenia was not related to an increase in the concentrations of the enzyme, suggesting that the increased enzyme activity could result from an alteration in some endogenous modulators of the enzyme activity (Gattaz et al., 1990).

S100 proteins are a family of low molecular mass (10–12 kDa) calcium-binding proteins

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involved in a wide variety of cellular processes, including calcium signal transduction, cell proliferation and differentiation. Specifically, S100β is found mainly in the brain, where it is the most abundant member of this family (Zimmer et al., 1995; Schäfer and Heizmann, 1996). Due to its trophic actions on neurons and astrocytes, S100β has been implicated in the development and maintenance of the nervous system (Azmitia et al., 1992; Marshak, 1990).

Recently, a member of the S100 family (S100A10 or P11) was found to inhibit the activity of the 85 kDa cytosolic PLA2 (Wu et al., 1997). In the face of these findings, we speculated that decreased S100 protein concentration could contribute to increased PLA2 activity in schizophrenia.

In this preliminary study, we determined the plasma concentrations of the S100β protein, which accounts for 96% of total brain S100 proteins (Fagnart et al., 1988), in patients with schizophrenia compared with healthy controls. The concentration of S100 proteins in the blood was shown to reflect injury in the brain (Ingebrigtsen et al., 1995; Ingebrigtsen and Romner 1996; Missler et al., 1997) and there is a correlation between blood and CSF levels of S100β (Zimmer et al., 1995).

2. Material and methods

Twenty-three outpatients with schizophrenia (16 male and 7 female; mean age $\pm$SD = 36 ± 9 years) and 23 healthy controls (16 male and 7 female; age 44 ± 17 years) were recruited for this study. Patients were diagnosed according to DSM-IV criteria and their psychopathological state was assessed by means of the Brief Psychiatric Rating Scale (BPRS) and the Negative Symptoms Rating Scale (NSRS; Iager et al., 1985). Patients were recruited from the Schizophrenia Program at the Institute of Psychiatry of the University of São Paulo. Total BPRS and total NSRS scores were 25.8 ± 15.4 and 17.7 ± 13.6, respectively. All patients were medicated with antipsychotic drugs (mean 710 ± 340 mg CPZ equivalents/day) and 16 of the 23 patients were administered clozapine. The mean duration of the disease was 17 ± 7 years and the mean number of prior psychiatric hospitalizations was 5.3 ± 4.9.

Ten milliliters of citrate blood were collected, and plasma was separated and kept frozen at -70°C (for less than 3 months) until the day of the assay. S100β protein concentrations were measured using a highly specific and sensitive immunoluminometric assay (detection limit 0.02 μg/l (LIA-mat® Sangtec® 100, Sangtec Medical, Bromma, Sweden) according to Hansson et al. (1998). All determinations were performed in duplicate with 100 μl plasma. Chemiluminescence was measured in a MAGIC® Lite Analyser II luminometer after an automatic injection of 300 μl alkaline peroxide solution and 300 μl of catalyst solution into the test tubes. The S100β standard curve was linear within 0.02–20 μg/l concentrations, and variations of duplicate values were within 5%.

Data were analyzed by non-parametric tests (Mann–Whitney, Kruskall–Wallis one-way Anova and Spearman correlation coefficients, all two-tailed). The level of significance was 0.05. Values are given as mean $\pm$SD.

3. Results

The concentration of S100β was significantly lower in schizophrenic patients (0.44 ± 0.27 μg/l) than in healthy controls (0.55 ± 0.14μg/l, $p = 0.003$) (Fig. 1). As can be seen in the scattergram, there are two outliers among the patients, one on haloperidol and the other on clozapine treatment. Since they could bias the results, all statistical analyses were performed with and without these subjects.

3.1. Comparisons of mean levels of S100β in controls and patients given neuroleptic treatment

Plasma levels of S100β had a mean of 0.42 ± 0.28 μg/l in patients receiving clozapine ($n = 16$), 0.49 ± 0.29 μg/l in patients treated with other neuroleptics ($n = 7$) and 0.55 ± 0.14 μg/l in controls ($n = 23$). No significant difference of plasma levels

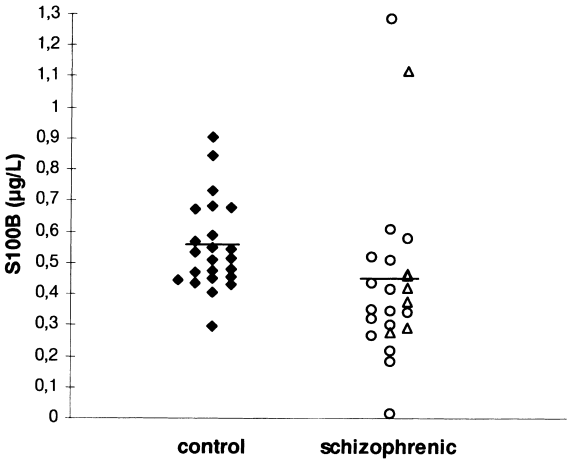

Fig. 1. Mean plasma concentrations of S100 beta protein (in µg/l) in 23 healthy controls (◆) and in 23 schizophrenic patients either on clozapine (○) or typical antipsychotics (△).

of S100β was found between patients on clozapine and those on other neuroleptics ($p=0.60$). The Kruskall–Wallis test for comparison of means showed that the levels of S100β were significantly lower in both patient groups ($p=0.011$). The Mann–Whitney statistic revealed significantly lower S100β in the seven patients treated with other neuroleptics ($p=0.047$) and in the 16 patients treated with clozapine ($p=0.007$) compared with controls. As could be expected, the significance of the difference between patients and controls increased in a new analysis without the outliers ($p=0.001$).

3.2. Correlations of plasma levels of S100β with age, age at onset, duration of illness, number of hospitalizations, chlorpromazine equivalents and psychopathology

Spearman correlation coefficients and significance levels are given in Table 1. Analyses were performed with the whole sample and with the outliers excluded. No significant correlations were found between plasma levels of S100β and age, psychopathological scores, duration of the disease, number of hospitalizations and the dose of antipsychotic drugs in CPZ equivalents.

4. Discussion

Plasma concentration of S100β was significantly reduced in patients with schizophrenia. Our results are in line with the finding of reduced autoantibodies to human brain S100 protein in schizophrenic patients (Jankovic and Djordjijevic, 1991). However, they contrast with the findings of Wiesmann et al. (1999), who reported elevated plasma levels of S100β in 20 schizophrenic patients, *all on neuroleptic medication*. We are presently unable to explain this contradiction. Wiesmann et al. (1999) used an immunofluorometric assay that detects three subtypes of S100 proteins; thus it is possible that they may have measured subfractions of S100 different from those measured in the present study.

The lack of correlation between S100β concen-

Table 1
Correlation coefficients between plasma levels of S100β and individual variables

Variables	Whole sample	Outliers excluded
Age	0.099 ($N=46$, $p=0.51$)	0.191 ($N=44$, $p=0.21$)
Age at onset	−0.227 ($N=23$, $p=0.29$)	−0.239 ($N=21$, $p=0.29$)
Duration of the disorder (years)	0 ($N=23$, $p=1$)	0.211 ($N=21$, $p=0.36$)
Number of hospitalizations	−0.132 ($N=23$, $p=0.55$)	−0.046 ($N=21$, $p=0.84$)
Chlorpromazine equivalents	0.216 ($N=23$, $p=0.32$)	0.156 ($N=21$, $p=0.50$)
BPRS (total)	0.017 ($N=23$, $p=0.94$)	0.044 ($N=23$, $p=0.85$)
Negative symptoms (total)	0.017 ($N=23$, $p=0.94$)	0.167 ($N=23$, $p=0.47$)

trations and duration of the disease suggests that decreased S100β in schizophrenia may not be a consequence of the disease itself. S100β was also uncorrelated with agitation or arousal, or non-specific motor activity.

Wu et al. (1997) found that a member of the S100 family (S100A10 or P11) interacts with the carboxyl region of the cytosolic PLA2, inhibiting its activity; conversely, the inhibition of the S100 expression by antisense RNA resulted in increased PLA2 activity. Thus, it is tempting to speculate that reduced S100β may be responsible for the increased PLA2 activity reported in schizophrenia. It is of interest that S100β was found to be increased in the brain (Griffin et al., 1989) and in the blood (Singh, 1994) of patients with Alzheimer's disease, a condition that has been associated with decreased PLA2 activity (Gattaz et al., 1995a, 1996).

Besides the possible effect of S100β on PLA2 activity, there are other mechanisms by which S100β could be involved in the neurobiology of schizophrenia. The release of S100β is stimulated by adenosine (Ciccarelli et al, 1998). Reduced adenosinergic activity has recently been proposed to be a common factor underlying many findings in schizophrenia (Lara and Souza, 1999), and the observed reduction in S100β concentration is consistent with this hypothesis.

Neurodevelopmental alterations have been proposed in schizophrenia (Weinberger, 1995). S100β is produced and released by astrocytes (Van Eldik and Zimmer, 1987), which are the most abundant cell type in the CNS and are clearly implicated in brain development (Tacconi, 1998). Thus, as previously pointed out (Lara and Souza, 1999), the neurodevelopmental alterations in schizophrenia could to be at least partly attributed to the role of astrocytes in the brain. Furthermore, if trophic factors are implicated in the pathogenesis of the disorder, rather than being specifically neurotrophic, they are likely to be pleiotrophic (Lara and Souza, 1999), which is the case of S100β. Therefore, it is conceivable that reduced levels of S100β could be related to CNS maldevelopment in schizophrenia. In this regard, Slater et al. (1998) found an abnormal persistence of serotonin-1A (5-HT1A) receptors in brains of adult schizophrenic patients, suggesting that this receptor population, in schizophrenia, undergoes misdirected reshaping during brain development. This is of interest, because 5-HT1A receptors stimulate the release of S100β in the brain (Whitaker-Azmitia et al., 1990).

Sixteen of our 23 patients were on clozapine treatment. Whereas clozapine antagonizes 5HT2 receptors, it shows a partial agonist activity at 5-HT1A receptors (Newman-Tancredi et al., 1998). Therefore, it is unlikely that reduced S100β in our sample is a direct effect of clozapine. Increased S100β would be predicted with clozapine, rather than decreased S100β as in our study. Moreover, no difference in S100β was found between our patients on clozapine and patients on typical neuroleptics, which have 'neutral' activity at 5HT1A receptors (Newman-Tancredi et al., 1998). Nevertheless, our results must be interpreted with caution until replication is completed in a medication-free sample.

However, another problem is that the patients on clozapine were non-responders to other conventional neuroleptics; thus, the present sample might overrepresent a subgroup of more severely impaired patients with schizophrenia, not representative of a broader group of schizophrenic psychoses. To address these problems we are repeating the investigation in a sample of first-onset, drug-naive schizophrenics, compared with healthy controls and with a group with other psychiatric disorders.

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