European Journal of Neuroscience, Vol. 12, pp. 3721–3728, 2000

Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex *in vivo*

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Keywords: dopamine, glutamate, human, magnetic resonance spectroscopy, oxidative stress

Abstract

Schizophrenia is a major psychiatric disease, which affects the centre of the personality, with severe problems of perception, cognition as well as affective and social behaviour. In cerebrospinal fluid of drug-free schizophrenic patients, a significant decrease in the level of total glutathione (GSH) by 27% ($P < 0.05$) was observed as compared to controls, in keeping with the reported reduced level of its metabolite γ-glutamylglutamine. With a new non-invasive proton magnetic resonance spectroscopy methodology, GSH level in medial prefrontal cortex of schizophrenic patients was found to be 52% ($P = 0.0012$) lower than in controls. GSH plays a fundamental role in protecting cells from damage by reactive oxygen species generated among others by the metabolism of dopamine. A deficit in GSH would lead to degenerative processes in the surrounding of dopaminergic terminals resulting in loss of connectivity. GSH also potentiates the *N*-methyl-D-aspartate (NMDA) receptor response to glutamate, an effect presumably reduced by a GSH deficit, leading to a situation similar to the application of phencyclidine (PCP). Thus, a GSH hypothesis might integrate many established biological aspects of schizophrenia.

Introduction

Schizophrenia is an endogenous psychosis characterized by an array of symptoms classically dichotomized into positive symptoms (delusions, hallucinations, thought disorder, incoherence of speech and behaviour) and negative ones (deficits in cognitive and social abilities, poverty of speech, affective flattening, etc.). The lifetime prevalence is 0.85% in the general population. While two theories have been proposed as the biological bases of schizophrenic disorders, its aetiology has not been clearly established. Evidence for a dopamine system dysfunction includes the psychosis-inducing effects of dopaminergic agonists, and the antipsychotic potency of antagonists (Matthysse, 1973; Carlsson, 1988; Davis *et al*., 1991). The glutamate hypofunction hypothesis relies on the fact that phencyclidine (PCP), a psychotomimetic drug, blocks the *N*-methyl-D-aspartate (NMDA) glutamate receptor (Kim *et al*., 1980; Deutsch *et al*., 1989; Carlsson & Carlsson, 1990; Javitt & Zukin, 1991; Olney & Farber, 1995). Moreover, evidence is increasing to support an impaired antioxidant defence and increased oxidative injury in schizophrenia (Mahadik & Mukherjee, 1996). An impaired activity of an antioxidant enzyme (superoxide dismutase) in red blood cells from never-medicated first-episode patients (Mukherjee *et al*., 1996) and an elevated plasma lipid peroxides at the onset of non-affective psychosis (Mahadik *et al*., 1998) have been reported. However, whether or not the plasma lipid peroxides as well as the other peripheral indices of oxidative cell injury reflect some of the brain pathophysiologic abnormalities in schizophrenic patients remain unclear. Glutathione (GSH), the major intracellular non-protein thiol, is known as a nucleophilic scavenger and an enzyme-catalysed antioxidant, and plays an important role in protecting the brain against oxidative stress and harmful xenobiotics (Meister & Anderson, 1983; Cooper, 1997). The present study examined the levels of GSH directly in both cerebrospinal fluid (CSF) and brain of schizophrenic patients. An important deficit in GSH was observed, thus supporting a hypothesis involving an impaired antioxidant defence system in the pathophysiology of schizophrenia.

We have previously investigated the concentrations of amino acids, dopamine and serotonin metabolites, *N*-acetylaspartate and *N*-acetylaspartylglutamate in the CSF of 26 drug-naive ($n = 21$) or drug-free ($n = 4$ for 1 years and $n = 1$ for 8 years) schizophrenic patients in whom long-term changes secondary to previous antipsychotic treatment could be excluded. Among the 26 compounds analysed, we reported a decrease in γ-glutamylglutamine (γ-Glu-Gln, Do *et al*., 1995). This γ-glutamyl dipeptide is most probably synthesized from GSH by the enzyme γ-glutamyl-transpeptidase, which transfers the γ-glutamyl moiety of GSH to an amino acid (Meister & Anderson, 1983). We therefore determined the GSH concentration of the same CSF samples by high-performance liquid chromatography (HPLC) and mass spectrometry. In addition, a method was developed for magnetic resonance spectroscopy (MRS) in order to determine the level of GSH in the brain in a non-invasive way.

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Received 13 April 2000, revised 15 June 2000, accepted 22 June 2000

Materials and methods

Subjects and the CSF sampling procedure were as described in Do *et al.* (1995). Briefly, 26 inpatients recruited in the Max Planck Institute of Munich (15 women, 11 men; age range, 21–53 years) with a diagnosis of a schizophreniform ($n = 9$) or a schizophrenic disorder ($n = 17$; DSM-III-R, American Psychiatric Association 1987) participated in the study. Twenty-one patients were drug naive, four free of psychoactive medication for at least 1 year and one for 8 years. Fourteen age- and gender-matched subjects (eight women, six men; 19–58 years) served as the control group. None of these subjects, both patients and controls, had received any medication during the 2 months before lumbar puncture. In these patients, who were recruited during a more than 6-year lasting period, possible concurrent medical disorders and drug abuse were ruled out by a thorough medical examination and laboratory tests including electrocardiogram, blood analysis and urinary drug screening. The CSF was collected from subjects after one night of bed rest and fasting, immediately frozen and stored at –80 °C until analysis. The experimental protocol was approved by the Ethics Committee for Human Experiments of the Max Planck Institute of Psychiatry.

Subjects in the MRS study

GSH measurements were made in 14 male inpatients (age range: 18–43 years) who satisfied DSM-III-R diagnostic criteria for schizophreniform ($n = 5$) or schizophrenic disorder ($n = 9$) who were recruited from the Psychiatric Department of Zurich University Hospital. Five patients were treated with neuroleptic medication in the past, five were drug naive, four drug free for at least 6 months. One was a first-episode patient. The first symptoms were detected before MRS measurement for 8 months to 1 year in four patients, 2–3 years in four patients, and 5–14 years in five patients. AMDP (Association for Methodology and Documentation in Psychiatry 1997), PANSS, SANS, SSCL-16, SCL 90 served as psychopathological rating scales. Fourteen male, age-matched subjects served as control group.

N-acetylaspartate (NAA), creatine + phosphocreatine and choline MRS were performed in eight out of the 14 patients, which were recruited in the GSH-MRS study. The control group consisted of 10 other age-matched subjects (three females and seven males).

CSF analysis

The samples were thawed slowly on ice and deproteinated by ultrafiltration at 18 300 ***g*** (4 °C) in Millipore Ultrafree-MC filter units (10 000 molecular weight cutoff). Fifty-microlitre aliquots of samples containing norvaline (internal standard; 100 pmol) were treated with 10 µL of 10 mM dithiothreitol to convert all glutathione to its reduced form. Each sample was then diluted to 100 µL by addition of water and derivatized at pH 8 by vortexing with 25 µL borate buffer (1 M, pH 6.2) and 150 µL *N*-9-fluorenylmethyl-chloroformate (FMOC-Cl, 15.5 mM in acetone) for 1 min. The excess of reagent was extracted three times with 0.5 mL *n*-pentane, and 60-µL aliquots of the water phase were injected onto the HPLC column in duplicate.

An analytical column (125 × 4 mm), packed with Lichrospher 100, RP-18, 5 µm, 100 Å (Merck), was used. The compounds were eluted at 40 °C with a linear gradient of 0–65% mobile phase B [0.1% trifluoroacetic acid (TFA) in acetonitrile/methanol/water (70/20/10% v/v/v)] in mobile phase A (0.1% TFA in water) during 80 min at a flow rate of 0.8 mL/min. Fluorescence was monitored at 315 nm (emission) and 260 nm (excitation). The FMOC derivative of authentic GSH eluted at the retention time of 75 min. The quantification was based on peak area measurements and the internal standard method.

Identification of GSH in CSF by micro HPLC-continuous flow-fast atom bombardment-mass spectrometry (CF-FAB-MS)

To show that the derivatized CSF component termed P75 was indeed the FMOC derivative of glutathione, as suggested by its retention time, the eluent containing P75 was collected, and subjected to micro-HPLC-CF-FAB-MS (Do *et al.*, 1995). The instrumentation consisted of the following components: a dual syringe gradient system Model 140 A (Applied Biosystems, Foster City, CA, USA), an injector (Rheodyne 8125), a 150 × 0.32 mm fused silica column packed with Lichrospher 100, RP-18, 5 µm, 100 Å stationary phase (LC Packings, Amsterdam, The Netherlands) connected in series to a UV detector (Model 785 A, Applied Biosystems) and then to a Finnigan MAT (Bremen, Germany) CF-FAB-interface, coupled to an MAT 90 (Finnigan MAT) double-focusing mass spectrometer. Compounds were eluted with a gradient of water against acetonitrile/water 83/15 (v/v), both containing 2 vol.% glycerol and 0.025% TFA. The total flow rate was reduced from 75 µL to 4–5 µL/min by a splitting device placed between the pump and the injector. The FAB gun (Ion Tech, Teddington, UK) was operated with xenon at ~ 9 kV. The ion source temperature was 60 °C. Negative ion spectra were taken between m/z 300 and 900 at a resolution of ~ 1000.

^{1}H-MR spectroscopy

From the three amino acid components of GSH, cysteine was found to be the most suitable for the identification of GSH by means of NMR spectroscopy. Cysteine forms a strongly coupled ABX spin system. In the ^{1}H-NMR spectrum, the cysteine compound shows two separated multiplets centred in the 4.564 p.p.m. and 2.95 p.p.m. regions. The focus was on the 2.95 p.p.m. resonance of GSH as it is located in a spectrally more convenient region. Other resonances found in this frequency region which potentially contribute to the observed *in vivo* spectrum are creatine (singlet at 3.03 p.p.m.), aspartate (multiplet at 2.82 p.p.m.) and γ-aminobutyric acid (GABA, triplet at 3.01 p.p.m.). For the selective detection of the cysteinyl compound of GSH we used a double quantum coherence filter (DQC) technique based on coherence pathway filtering with static field gradients (Bax *et al.*, 1980) in combination with spatial selection of a single volume by means of the PRESS technique (Bottomley, 1987). In addition, the radio frequency read pulse was made frequency selective for the sake of a higher signal yield. To secure optimal and reproducible phase correlation between the radio frequency pulses, a calibration procedure was used. The sequence was implemented on a Philips Gyroscan ACS NT (Philips Medical Systems, Best, The Netherlands) 1.5 Tesla whole body scanner as described in Trabesinger *et al.* (1999). The overall examination time is ~ 1 h.

Statistical analysis

In addition to descriptive statistics (mean ± SD) a two-factors analysis of covariance (ANCOVA) was performed in the CSF study samples [factor 1, groups (patients, controls); factor 2, gender (women, men); covariable, age]. In case of significant main group effects, group-by-group comparisons then were calculated using Student's *t*-tests (method, modified least significant difference tests to control for the increased type I error rate). In addition, we calculated a linear canonical discriminant analysis as reported earlier (Do *et al.*, 1995; method, minimizing Wilks' lambda; set of independent variables: Asp, Glu, γ-Glu-Gln, Ile, Tau; correctly classified subjects, 83%) and now included GSH as an additional independent variable. Finally, we calculated Pearson correlation coefficients to assess

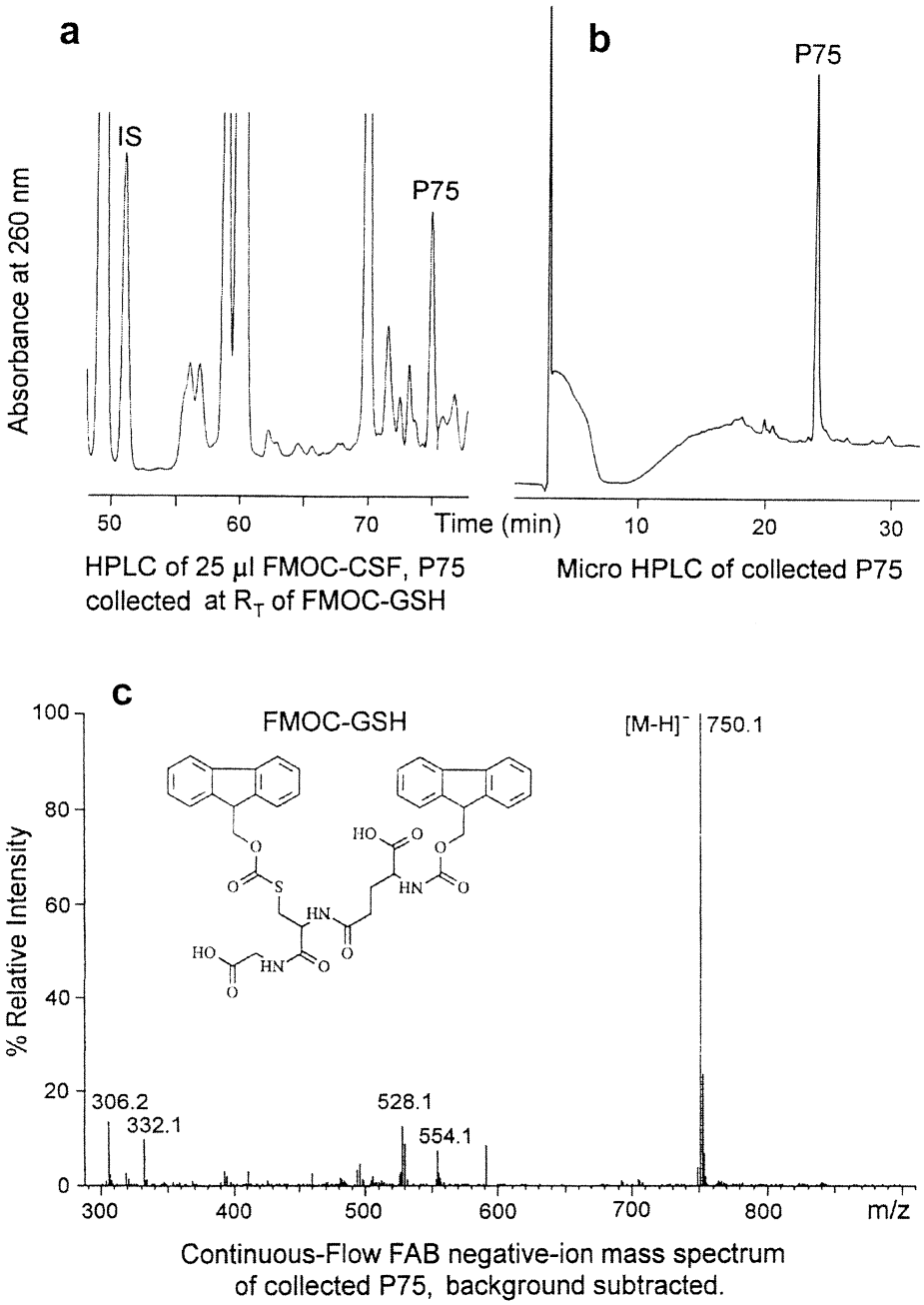

FIG. 1. Analysis of CSF samples. (a) Typical HPLC chromatogram of FMOC derivatives from a CSF sample. The FMOC derivative of authentic GSH eluted at the retention time of 75 min. (b) Micro-HPLC of collected P75. To show that the derivatized CSF component termed P75 was indeed the FMOC derivative of glutathione, as suggested by its retention time, the eluent containing P75 was collected, and subjected to micro-HPLC-CF-FAB-MS (Do *et al.*, 1995). The analysis of the collected fraction revealed predominantly only one component that eluted at the retention time of FMOC-GSH. (c) CF-FAB-MS (negative ion spectra taken between m/z 300 and 900 at a resolution of 1000) of this principal component: deprotonated intact molecule at m/z 750 and major characteristic fragment ions at m/z 554, 528, 332 and 306. This spectrum was indistinguishable from the corresponding spectrum of synthetic FMOC-GSH.

associations among the various compounds and the duration of illness. In the MRS study, we applied non-parametric statistical tests due to the small sample size. The level of significance was set at 5% (two-tailed).

Results

Biochemical analysis of cerebrospinal fluid

As the concentration of GSH in CSF is at the nanomolar range, which lies at the detection limit of the enzymatic recycling methods, we have developed a sensitive and specific analytical method to quantify GSH. The samples were analysed by HPLC following derivatization with FMOC-Cl. The FMOC derivative of GSH was then identified by micro-HPLC-CF-FAB-MS (Fig. 1). Figure 1A shows a typical HPLC chromatogram of FMOC-derivatized CSF. The derivative eluting at the retention time of FMOC-glutathione (75 min), termed P75, was well resolved. P75 was collected and subjected to micro-HPLC-CF-FAB-MS. In this system which is endowed with a high resolution of separation, the analysis of the collected fraction revealed mainly one component, which eluted at the retention time of FMOC-glutathione (Fig. 1B). The CF-FAB-MS of this principal component (Fig. 1C) showed the deprotonated intact molecule at m/z 750 and major characteristic fragment ions at m/z 554, 528, 332 and 306. This spectrum was indistinguishable from the corresponding spectrum of synthetic FMOC-glutathione. The chromatographic and mass spectral data provided solid evidence that P75 represents FMOC-glutathione.

The total GSH content (GSH reduced + GSSG) in CSF of schizophrenics and control subjects was quantified. Owing to the very low concentration of the oxidized form of glutathione and to the well-known instability of GSH in biological specimens (Cooper,

TABLE 1. Concentrations of various compounds in the CSF of schizophrenic patients and control subjects

	Schizophrenic patients ($n = 26$)	Control subjects ($n = 14$)	ANCOVA: effects of		
			Group [$F_{1,39}$]	Age [F_1]	Sex [F_1]
Age (year)	32.2 ± 7.8	35.6 ± 12.9	1.05	—	—
Women/men	15/11	8/6	$\chi^2 = 0.00$	—	—
GSH	327 ± 127	445 ± 206	5.80*	1.33	0.17
Asp	245 ± 106	193 ± 100	1.89	0.08	5.26*
Glu	323 ± 90	381 ± 184	4.26*	5.44*	0.93
γ-Glu-Gln	2077 ± 522	2444 ± 480	5.81*	0.11	11.68**
Ile	5996 ± 650	5462 ± 628	6.37*	0.87	0.24
Tau	4988 ± 865	5838 ± 640	13.47***	4.19*	1.54
5-HIAA	19.9 ± 9.4	26.6 ± 12.3	2.65	0.40	1.37
HVA	38.6 ± 16.0	48.4 ± 29.1	1.02	0.43	1.23
NAA	800 ± 620	830 ± 390	0.07	0.07	0.61
NAAG	2110 ± 900	2210 ± 900	0.01	0.79	0.03

CSF samples were analysed as described in Fig. 1 and Materials and methods section. All data (pmol/mL) are mean ± SD values. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data on 5-HIAA, HVA, NAA, NAAG, Asp, Glu, γ-Glu-Gln, Ile and Tau are from Do *et al.* (1995). In the GSH measurements, there is in the original control group ($n = 15$) an outlying very high GSH value (2253 pmol/mL) bringing the average control to 565 ± 508 pmol/mL and to a decrease of 42% ($F = 4.78$, $P < 0.05$) when compared with the patients. By excluding this outlier from the control group ($n = 14$), the average becomes 445 ± 206 pmol/mL and the difference with the patients 27% ($F = 5.80$, $P < 0.05$), i.e. smaller, but with higher significance. As a consequence, all the values concerning the 'outlying' control subject have been taken out from the Do *et al.* (1995) data, leading to new averages for all substances analysed. The new averages for some substances were presented here. The decrease in glutamate level is becoming significant ($P < 0.05$), while it did not quite reach significance when the outlier was included (Do *et al.*, 1995). In addition to descriptive statistics (mean ± SD), a two-factors analysis of covariance (ANCOVA) was performed in the CSF study samples [factor 1, groups (patients, controls); factor 2, gender (women, men); covariable, age]. In addition, linear canonical discriminant analysis (minimizing Wilks' lambda) was performed using the original set of independent variables (Asp, Glu, γ-Glu-Gln, Ile, Tau; Do *et al.*, 1995) together with GSH.

1997), we decided against determining single GSSG concentrations. Total GSH level was significantly decreased by 27% ($P < 0.05$) in the patients compared to controls (Table 1). As reported earlier, isoleucine was significantly higher, and both taurine and γ-Glu-Gln (–15%) were significantly lower in the patients. In order to ascertain that the group differences (e.g. regarding GSH and γ-Glu-Gln) are not due to age and/or gender effects, we explicitly performed an analysis with age as covariate and gender as a second factor. While we found no significant age and gender effects regarding GSH, the gender effect was significant for γ-Glu-Gln, i.e. the concentration of this compound differs between women and men irrespective of whether or not the subjects are patients or controls, as described in Do *et al.* (1995). Furthermore, Glu was lower in the patients (–15%). The levels of dopamine and serotonin metabolites [homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA)] as well as those of NAA and *N*-acetylaspartylglutamate (NAAG) were not different from controls (Do *et al.*, 1995).

Pearson correlation coefficients were calculated to evaluate the possible association between GSH and the duration of the illness (range, 2 weeks to 23 years) or the duration of the current episode (range, 2–80 weeks). No significant association of these parameters was found ($r = –0.03$ and $r = –0.20$, respectively). We also performed a discriminant analysis using the original set of independent variables (aspartate, glutamate, γ-Glu-Gln, isoleucine, taurine; Do *et al.*, 1995) and including GSH. The discriminant function (DF) correctly classified 87.5% of the subjects with a specificity of 96.2% (per cent correctly classified patients) and a sensitivity of 71.4% (per cent correctly classified controls).

GSH determination in brain by MRS

The possibility that the observed changes in CSF composition are a reflection of changes in brain tissue was investigated using a new, non-invasive proton magnetic resonance spectroscopy (^{1}H-MRS) method that allows detection of GSH with a high selectivity (Trabesinger *et al.*, 1999). In conventional *in vivo* ^{1}H-MR spectra, GSH is not visible due to its complicated spectral pattern and spectral overlapping with other resonance lines. From the three amino acid components of GSH, cysteine was most suitable for the identification of GSH by means of ^{1}H-MRS. For the selective detection of cysteine we used a DQC filter technique based on coherence pathway filtering with static field gradients in combination with spatial selection of a single volume. This MRS technique developed detects only cysteinyl compound in small molecules, i.e. in cysteine and glutathione. Cysteinyl compounds in macromolecules (protein thiols) are not detected due to their fast relaxation. In addition, it is known that the concentrations of GSH and cysteine in the human brain are 1–3 mM and 30–70 μM, respectively (Cooper, 1997), i.e. GSH level is 15–100 times higher than that of cysteine. Therefore, GSH contributes predominantly to the MRS signal and cysteine should represent only 1 to maximally 7% of GSH.

Quantification was accomplished using tissue water content as an internal standard. Indeed, when comparing patients to controls, no change was observed in the water levels in the selected volume of interest from which the MRS signals were acquired. Due to the complex spin dynamics of the cysteine spin system, the ratio GSH signal : water signal does not directly reflect the ratio [GSH] : [water]. Absolute concentrations of GSH in brain tissue may not therefore be derived from our data. However, an average GSH concentration in the range of 2–5 mM could be estimated for the control group (Trabesinger *et al.*, 1999), in keeping with biochemical measurements (Cooper, 1997).

In *in vitro* experiments where 13 brain metabolites in physiological concentrations were mixed with GSH (0–10 mmol/L, 'phantom', Fig. 2A), a linear dependence ($R^2 = 0.9989$) of the ratio GSH signal : water signal upon the GSH concentration was seen in DQC filtered spectra (Fig. 2B). Figure 2C and D showed the comparison of *in vivo* spectra in occipital area (Fig. 2C) and prefrontal area (Fig. 2D) of the cortex with spectra of phantom containing 2.5 mM GSH

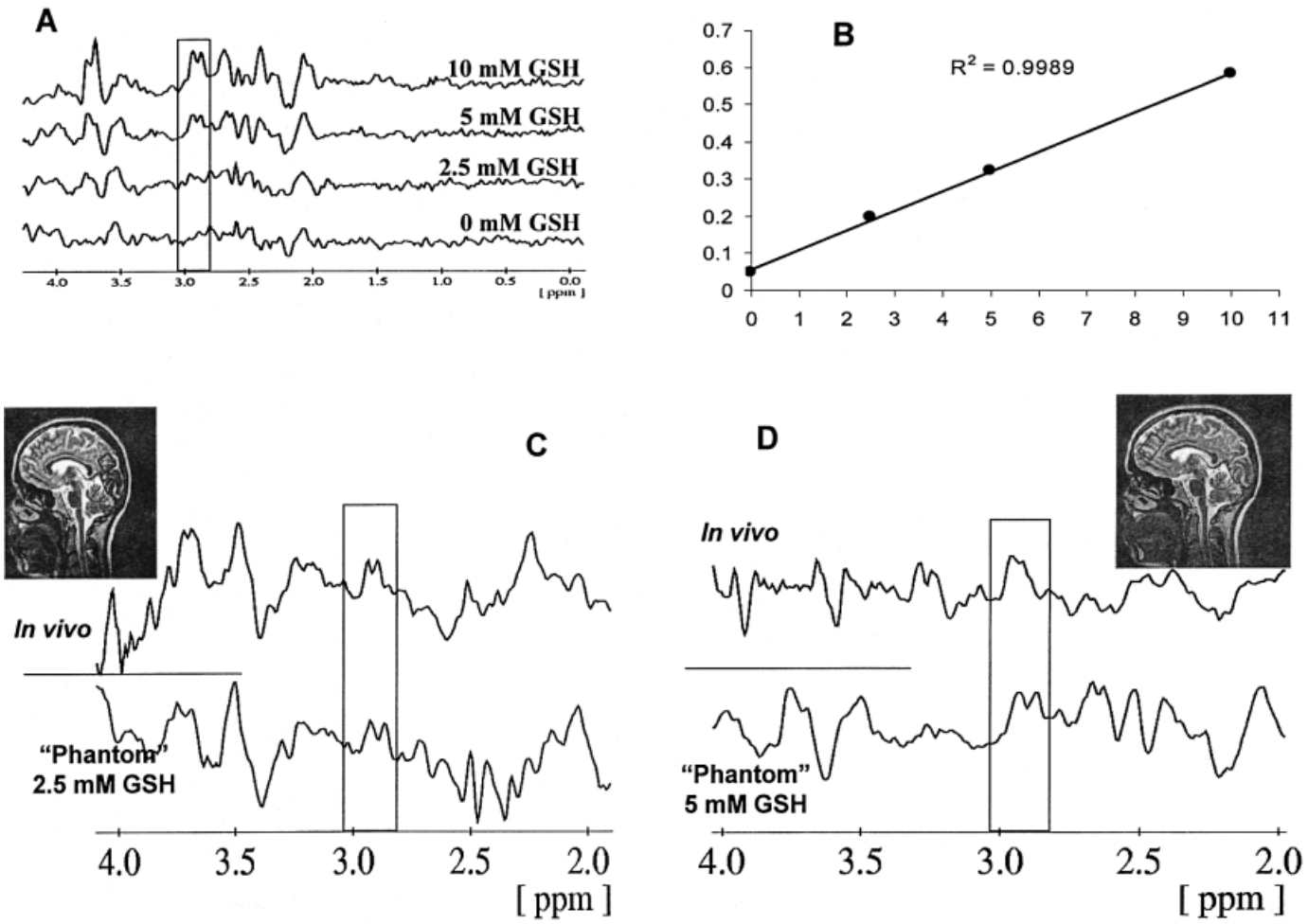

FIG. 2. ^{1}H-MR spectroscopy of GSH. (A) ^{1}H-MR spectroscopy of GSH in 'phantom' which is an aqueous solution containing various concentrations of GSH (0–10 mM) in the presence of 13 brain metabolites in physiological concentrations – (in mM): choline, 3; creatine, 7; NAA, 11; glucose, 1; aspartate, 1; alanine, 0.5; glycine, 1; glutamate, 7; GABA, 1; lactate, 0.5; taurine, 1; glutamine, 3; myo-inositol, 5. For the quantification of GSH, the peak at 2.9 p.p.m. (frame) was integrated. (B) Quantification of GSH. Plot showing a linear correlation ($R^2 = 0.9989$) between the peak area at 2.9 p.p.m. and the concentration of GSH. Comparison of *in vivo* spectra, (C) in the occipital area and (D) in the prefrontal area, with 'phantom' spectra (C, 2.5 mM GSH; D, 5 mM GSH). The inset shows the localization of the volume (17.4 mL) measured. Note that a change of 50% in GSH levels can be reliably quantified.

(Fig. 2C) and 5 mM GSH (Fig. 2D). These data also showed that a change of 50% of GSH concentration could be reliably quantified.

Fourteen male patients participated in this study. The selection was limited in a first step to male patients as this group is more susceptible to first episodes at early age. The control group consisted of 14 age-matched male subjects. The volume of interest (VOI) that comprised 17.4 mL ($24 \times 22 \times 33$ mm) was placed mid-sagittally in the prefrontal cortex, an established site of dysfunction in schizophrenia (Andreasen *et al.*, 1992). Although it would be of great interest to investigate other brain regions, time constrains only allow psychotic patients to be placed in the magnet for a single measurement. In the control group a mean ratio GSH signal : water signal of 6.12 (± 2.82) $\times 10^{-5}$ was found compared to 2.95 (± 1.48) $\times 10^{-5}$ in the patients (Fig. 3). The decrease in GSH level in the prefrontal cortex of patients compared to controls was calculated to be 52% ($P = 0.0012$; Mann–Whitney test).

Moreover, in the same MRS examination of patients, a non-edited spectrum was acquired, enabling the assessment of absolute concentrations of NAA, creatine + phosphocreatine and choline (Duc *et al.*, 1998). No significant change was seen in creatine and choline levels. NAA concentrations, however, were 31% ($P = 0.0005$) lower in schizophrenics (6.0 ± 1.1 mM; $n = 8$) than in controls (8.7 ± 1.4 mM; $n = 10$). This is consistent with the NAA decrease reported in the frontal lobe of schizophrenics (Bertolino *et al.*, 1998).

Discussion

Significant decreases in concentration of GSH and of its metabolite γ-Glu-Gln were observed in CSF of schizophrenic patients mostly drug naive. As revealed by MRS, this decreased level of GSH probably reflects a GSH deficit in the nervous tissue. The convergence of these three findings observed with different highly reliable methods (HPLC-MS, MRS), in different biological materials (CSF, cortex *in vivo*) and in two different pools of patients, indicates that they are likely to be reproducible and solid. It should be noted that out of 27 compounds analysed in CSF, only five were significantly different from control values: GSH (–27%), γ-Glu-Gln (–15%), Glu (–15%), Tau (–15%) and Ile (+10%). For all other values, the variations reported oscillated around the control values in both directions (Do *et al.*, 1995). The predictive character of these values is particularly important; the use, as independent variables, of these compounds (GSH, γ-Glu-Gln, Glu, Tau and Ile) plus Asp is necessary and

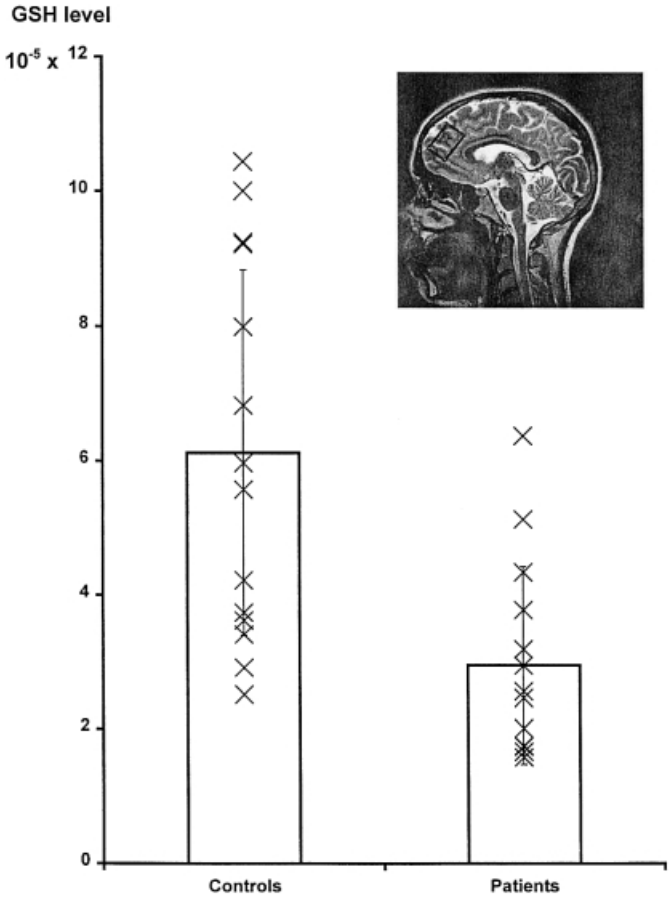

FIG. 3. The GSH level (arbitrary units) as measured by ^{1}H-MRS in the medial prefrontal cortex of schizophrenic patients is 52% ($P = 0.0012$; Mann–Whitney test) lower than in controls. The inset shows the localization of the volume (17.4 mL) measured in the prefrontal cortex.

sufficient to discriminate between patients and controls with a specificity of 96.2% (i.e. predict correctly 25 patients out of 26).

The drug-naive status of most of the patients in the CSF study and of five (out of 14) in the MRS study strongly suggests that the deficit in GSH may underlie the pathophysiology of the schizophrenic disease process and is not a consequence of its treatment. It is unlikely that the GSH deficit reported here is due to some non-specific degenerative process as observed in Parkinson's or Alzheimer's diseases for the following reasons: (i) in CSF study, it was not associated with the duration of illness and age (21 and 53 years); (ii) in MRS study it was already observed in young patients (18–43 years); and (iii) GSH levels in the CSF of patients with Parkinson's and Alzheimer's disease were normal (Konings *et al.*, 2000).

While it is at present difficult to ascertain whether this GSH deficit is primary or secondary to another disorder, data discussed below on reduced activity of the GSH-catalysed enzyme glutathione peroxidase are consistent with an involvement of GSH metabolism. Excess free radical formation has been suggested to occur in patients with schizophrenia (Mahadik & Mukherjee, 1996; Reddy & Yao, 1996). Evidence for this includes an impaired activity of the superoxide dismutase in red blood cells from drug-naive first-episode patients (Mukherjee *et al.*, 1996) and an increase of plasma lipid peroxides, associated with lower red blood cell activity of the glutathione peroxidase at the onset of non-affective psychosis (Mahadik *et al.*, 1998). A correlation has been described between brain atrophy in schizophrenic patients and reduced activity in platelets and erythrocytes of the antioxidant enzyme, glutathione peroxidase, one of the enzymes involved in the metabolism of GSH (Buckman *et al.*, 1987). A decrease in the activity of glutathione peroxidase was also observed in erythrocytes of untreated schizophrenic women (Abdalla *et al.*, 1986). Human plasma glutathione peroxidase was significantly and positively correlated with psychosis rating scores in patients both on and off treatment (Yao *et al.*, 1999). Alternatively, GSH deficit might result from the perturbation of a constellation of enzymes involved in the GSH cycle. Taken together, these observations support the idea that the GSH metabolism is perturbed in schizophrenia, possibly in relation to a polygenic abnormality.

Several lines of evidence implicate the prefrontal cortex as a site of dysfunction in subjects with schizophrenia. These cortical areas are richly innervated with dopamine terminals and play, among other functions, an essential role in working memory, the impairment of which may lead to schizophrenic thought disorder (Goldman-Rakic, 1994). Goldman-Rakic and collaborators (Goldman-Rakic *et al.*, 1989; Krimer *et al.*, 1997) have identified a specialized synaptic architecture in which the dendritic spine of a pyramidal neuron was the target of both a dopamine-positive and an excitatory, probably glutamate, boutons. This synaptic organization is important for the considerations below. We propose a hypothesis that is based on a central role of GSH in the pathophysiology of schizophrenia and that integrates many established biological aspects of this disorder.

GSH plays a fundamental role in protecting cells from damage by hydrogen peroxide, quinones and reactive oxygen species (Cohen, 1983; Meister & Anderson, 1983), which are generated, among others, by the metabolism of dopamine (Graham *et al.*, 1978). Indeed, intrastriatal injection of dopamine causes an increase in reactive metabolites, a decrease in endogenous glutathione content and a degeneration of tyrosine hydroxylase-containing terminals. Moreover, these effects were potentiated if GSH synthesis is compromised and can be attenuated by coadministration of GSH (Rabinovic & Hastings, 1998). The protective effect of GSH (Offen *et al.*, 1996) against catecholamine-induced apoptosis may involve a reductive conjugation of the *o*-quinones by the glutathione transferase M2-2 (Baez *et al.*, 1997). Moreover, dopamine can be oxidized to generate semiquinones/quinones which can then form conjugates with GSH in cell-independent reactions (Spencer *et al.*, 1995). This process may lead to decreased GSH levels. In schizophrenia, a deficit in GSH would result in a decreased protection of cells from damage by oxidation leading to degenerative processes in the surrounding of dopaminergic terminals. The toxicity of the dopamine metabolites would be restricted to the microenvironment of the terminals of the dopamine fibres innervating the cortex (Goldman-Rakic *et al.*, 1989), leading to the degeneration of spines and dendrites rather than of the entire cell bodies. This is consistent with *post mortem* histological analysis of the frontal cortex of schizophrenic patients, which reveals an increased density of neurons, indicating a decrease in neuropile (Selemon *et al.*, 1995, 1998). Indeed, a decrease in the numbers of spines on pyramidal neurons in temporal and frontal cortex of schizophrenics has been observed (Garey *et al.*, 1998; Harrison, 1999). This hypothesis is also consistent with the decreased D1 receptor binding observed in the prefrontal cortex of schizophrenic patients (Okubo *et al.*, 1997), with an increase in plasma lipid peroxides (Mahadik *et al.*, 1998) and with alterations in membrane phospholipids as revealed by an MRS study (Pettegrew *et al.*, 1991). In addition, a reduction in the number of cells in the medial-dorsal nucleus of the thalamus of schizophrenics has been reported (Pakkenberg, 1990; Glantz & Lewis, 1996), possibly resulting from

retrograde degeneration of neurons projecting to areas affected in schizophrenia, the prefrontal and cingulate cortex. As the dopamine and serotonin metabolite CSF levels in drug-naive schizophrenics did not differ from control (Do *et al*., 1995), a dysfunction of the dopamine system would not seem to be at the origin of the trouble. However, the neurotoxic consequences of the GSH deficit may be particularly expressed in regions richly innervated by dopamine in the cortex of the anterior parts of the telencephalon. Finally, the proposed hypothesis is compatible with the fact that neuroleptics not only block dopamine receptors, but also reduce the dopamine level; this effect takes a few days to be achieved and is consistent with the delay needed for treatment to be effective.

A deficit of GSH and/or GSH-related enzymes during early development could constitute a major risk factor in schizophrenia. Combined with environmental factors, e.g. stress situations which would induce important dopamine release and increase in reactive oxygen species, it would have drastic consequences leading to spines degeneration and abnormal connectivity, as proposed in the neurodevelopmental hypothesis (Murray & Lewis, 1987; Weinberger, 1987; Benes, 1991; Bloom, 1993; Parnas *et al*., 1996). This may be responsible for part of the symptoms of schizophrenia, those involving cognitive and perceptive functions. At least indirectly, our negative findings regarding the effects of age and duration of both the illness and the current episode on GSH concentrations are in line with the assumption of a developmental rather than an acute illness-related process. Interestingly, depletion in rats of GSH with buthionine sulphoximine followed by dopamine treatment produced deficits in psychomotor behaviour (Shukitt-Hale *et al*., 1997).

In addition, GSH is known to potentiate the NMDA receptors response to glutamate (Kohr *et al*., 1994) either by acting at redox modulatory site(s) (Sullivan *et al*., 1994) or by blocking high-affinity Zn^{2+} inhibition through Zn^{2+} chelation (Paoletti *et al*., 1997). We have shown that GSH is released into the extracellular space, predominantly in the cortex (Zangerle *et al*., 1992), and GSH has been proposed to play a neuromodulator/neurotransmitter role (Janaky *et al*., 1999). At a low level of GSH, the potentiation of the NMDA receptor might be deficient. Such an inadequate activation of the NMDA receptors could be related to some of the symptoms of schizophrenia as the phencyclidines, NMDA receptor antagonists, induce psychotic-like symptoms.

Intraorgan and interorgan cycles of GSH transport and metabolism are well documented, especially for liver and kidney, but relatively little is known about such cycles in the brain (Cooper, 1997). Intercellular trafficking of GSH and its metabolites has been proposed between neurons and glial cells. Dringen *et al*. (1999) pointed to an important role of astrocytes to supply CysGly, generated by γ-glutamyl transpeptidase from GSH released from astrocytes, as precursor for neuronal GSH synthesis. The astroglial ectoenzyme γ-glutamyl transpeptidase, which transfers the γ-glutamyl moiety of GSH to an amino acid acceptor, has a particularly high affinity for glutamine (Tate & Meister, 1974), leading to the generation of γ-Glu-Gln. One possible mechanism for a reduced level of GSH could be an increased activity of the γ-glutamyl transpeptidase. However, the observed decrease in γ-Glu-Gln level (Do *et al*., 1995) does not support this interpretation.

In conclusion, a deficit in GSH and GSH-related enzymes might play an essential role in the pathophysiology and might constitute a major risk factor of schizophrenia, or some forms of schizophrenia. The proposed hypothesis brings together some elements known about the disease. If it proves to be correct, it could lead to new approaches to its treatment.

Acknowledgements

We thank U. Gutteck-Amsler and B. Benz for expert technical assistance, Dr M. Zollinger for counselling in mass spectrometry, and Drs Dieter Meier and Corinne Duc for technical support and helpful discussions on MRS. This work was supported by grants from the Swiss National Foundation to K.Q.D. (31-55924.98) and P.B. (31-52173.97).

Abbreviations

CF-FAB-MS, continuous flow-fast atom bombardment-mass spectrometry; CSF, cerebrospinal fluid; DF, discriminant function; DQC, double quantum coherence; FMOC, *N*-9-fluorenylmethyloxycarbonyl; GABA, γ-aminobutyric acid; GSH, glutathione; γ-Glu-Gln, γ-glutamylglutamine; 5-HIAA, 5-hydroxyindoleacetic acid; ^{1}H-MRS, proton magnetic resonance spectroscopy; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; TFA, trifluoroacetic acid; VOI, volume of interest.

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