Monoamine metabolites and amino acids in serum from schizophrenic patients before and during sulpiride treatment

G. Alfredsson and F.-A. Wiesel
Department of Psychiatry and Psychology, Karolinska Hospital, S-104 01 Stockholm, Sweden

Abstract. Twenty-four acutely ill schizophrenic patients (DSM-III-R), 18–42 years old, were treated for 6 weeks with sulpiride. Sulpiride was administered in three different daily dosages (starting with 400, 800 or 1200 mg) according to a double blind randomized administration schedule. The monoamine metabolites (MAM) homovanillic acid (HVA), 5-hydroxy-indoleacetic acid (5-HIAA), 4-hydroxy-3-methoxy-phenylglycol (HMPG) and the amino acids tyrosine, tryptophan, glutamate and glutamine were measured in serum before treatment and once a week during treatment. There were no significant differences between healthy controls and schizophrenic patients in serum levels of monoamine metabolites and amino acids before treatment. There was no dose-response effect of sulpiride on serum levels of the monoamine metabolites or the amino acids. The results are therefore based on the whole group of patients. During treatment the HMPG levels were reduced at all points in time. The serum level of HVA was significantly reduced after 6 weeks. The 5-HIAA and the amino acid levels were not changed during treatment. There were no significant correlations among the monoamine metabolites before treatment. During treatment, however, significant correlations were found among MAM and amino acids. Since the biochemical findings during the treatment were not related to the dose or the concentration of sulpiride the results may be related to secondary biochemical effects of sulpiride and/or to changes in the clinical state following treatment.

Key words: Sulpiride – Monoamine metabolites – Amino acids – Schizophrenia – Serum

Sulpiride is a selective D₂ dopamine (DA) receptor antagonist that is efficient in the treatment of schizophrenic patients (Toru et al. 1972; Rau et al. 1981; Härnryd et al. 1984a). Using positron emission tomography, it has been shown that sulpiride blocks central D₂-dopamine receptors (Farde et al. 1988). In all probability this blockade explains why sulpiride treatment increases the levels of the main DA metabolite homovanillic acid (HVA) in cerebrospinal fluid (CSF) of schizophrenic patients (Bjerkenstedt et al. 1979; Härnryd et al. 1984b). An interaction with noradrenergic mechanisms in schizophrenic patients was also found, as indicated by a significant decrease of the noradrenaline (NA) metabolite 4-hydroxy-3-methoxyphenyl-glycol (HMPG) in CSF during sulpiride treatment (Härnryd et al. 1984b).

Concentrations of the monoamine metabolites in CSF are supposed to reflect central monoaminergic transmission (Sedvall et al. 1975). However, to follow effects of psychotropic drugs on brain biochemistry one has to perform repeated lumbar punctures in psychiatric patients which may involve great difficulties. A number of reports indicates that plasma HVA may be used to follow clinical and drug effects in schizophrenic patients (Pickar et al. 1984; Harris et al. 1984; Doran et al. 1985; Davidsson and Davis 1988). It is not known if serum 5-HIAA and HMPG levels could be used in a similar way as HVA.

It is also of interest to measure the amount of plasma amino acids in patients since some of them are themselves central transmitters or precursors to transmitters. The importance of peripheral amino acids for the central monoaminergic systems is supported by negative correlations among plasma levels of some of the amino acids transported by the L-system across the blood-brain barrier and CSF levels of HVA and 5-HIAA (Bjerkenstedt et al. 1985).

The aim of this study was to investigate the usefulness of peripheral biochemical measures to characterize schizophrenic patients and to follow drug effects. In a dose-response study of sulpiride in the treatment of schizophrenic patients serum samples were therefore collected for the determination of the monoamine metabolites and their precursor amino acids tyrosine and tryptophan. In a previous study of healthy volunteers significant correlations between HVA and glutamate in CSF and HMPG and glutamate in serum were found (Alfredsson et al. 1988b). The concentrations of the amino acid glutamate and its precursor glutamine were therefore also determined. There has also been a report of deviating concentrations of glutamate in CSF from schizophrenic patients (Kim et al. 1980), although this finding has not been reproduced.

Methods

The protocol of the study was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

Selection of subjects. Patients with an acute psychosis of the schizophrenic type requiring neuroleptic treatment were
selected for the study. The DSM-III-R criteria for schizophrenia had to be fulfilled for inclusion in the study (Spitzer and Williams 1987). Patients with organic brain disorder, major somatic disease, alcohol and drug abuse were excluded. A total of 26 patients consented to participate after having been informed about the purpose of the study. However, two patients were excluded, since it was later found that they did not fulfill the inclusion diagnostic criteria. Of the remaining patients 14 were men and 10 were women. They were between 18 and 42 years of age (mean 28.6 ± 5.2 SD). Physical examinations and routine blood and urine tests indicated that all the patients were physically healthy.

According to anamnestic information obtained from the patients and the relatives, 7 of the patients had never received neuroleptic treatment, 13 patients had not taken oral neuroleptics for at least 1 month, and 4 patients for 2 weeks before entering the study. Depot neuroleptics had not been given during the last 6 months.

**Drug administration.** Sulpiride (Dogmatil forte, 200 mg, Essex AB, Sweden) was administered in a daily dose of 400, 800 or 1200 mg (08 a.m. and 08 p.m. in two equal doses) according to a double blind procedure. After 3 weeks of treatment the dose was increased from 400 mg/day and 800 mg/day to 800 mg/day and 1200 mg/day, respectively, or decreased from 800 mg/day and 1200 mg/day to 400 mg/day and 800 mg/day, respectively, and the treatment of patients was continued for another 3 weeks (Wiesel et al. 1989).

**Sampling of serum.** All patients had fasted for 12 h before the blood samples were taken at 8.00 a.m. before the morning dose. The blood samples were collected by venipuncture and centrifuged within 30 min at 2000 g for 15 min. Serum 2 x 100 μl was mixed with 2 x 300 μl 99% ethanol and centrifuged after 10 min. The supernatants were stored in a temperature of −80°C pending the analysis of glutamate and glutamine. The rest of the serum fraction was also stored at the same temperature pending the analysis of the monoamine metabolites, tryptophan and tyrosine.

**Monoamine metabolites in serum.** Concentrations of HVA and 5-HIAA were determined by gas chromatography–mass spectrometry with selective ion monitoring principally according to the method used for the metabolites in CSF (Swahn et al. 1976), with modifications for HVA in serum (Hunneman 1983) and for 5-HIAA in serum (Alfredsson et al. 1988b).

Serum levels of HMPG were analysed by gas chromatography–mass spectrometry with selective ion monitoring according to Sjöquist et al. (1975) and Swahn et al. (1976), except that 1 ml citrate buffer (pH = 6) was added to the serum sample (1 ml) in order to decrease variation during extraction.

**Glutamate and glutamine in serum.** Concentrations of glutamate and glutamine in serum were determined by high performance liquid chromatography (HPLC) fluorescence detection after derivatization with o-phthalaldehyde (Lindroth and Mopper 1979; Alfredsson et al. 1988b).

**Tryptophan and tyrosine in serum.** Concentrations of total tryptophan in serum were determined by HPLC-fluorescence detection according to Beck and Hesselgren (1980). The same method was used for tyrosine.

**Sulpiride in serum.** Concentrations of sulpiride in serum were determined by HPLC-fluorescence detection according to Alfredsson et al. (1979).

**Statistics.** The product moment correlation coefficient was calculated to study relations among monoamine metabolites and amino acid levels. Student’s t-test was used for the analysis of differences within groups. Two-tailed analysis of covariance was used for comparison of differences among the dose groups.

**Results**

The three dosages of sulpiride did not influence the concentrations of the monoamine metabolites, the amino acids or their changes in concentrations during treatment differently. Therefore, the results are presented only for the whole group of patients (n = 24).

**Plasma versus serum determinations.** Serum samples were used to analyse the monoamine metabolites and the amino acids. To investigate if the serum values were comparable to those in plasma, serum and plasma samples were collected simultaneously from two healthy volunteers. The serum values were in good agreement with those in plasma (Table 1).

**Concentrations of monoamine metabolites in serum.** The mean concentration of HVA in serum before treatment was 62.3 ± 22 (SD) pmol/ml for men and 71.1 ± 22 (SD) pmol/ml for women, which did not differ from the serum levels in healthy volunteers (Table 2) (Alfredsson et al. 1988a). After 6 weeks of sulpiride treatment the HVA level was significantly lower than the pretreatment level (Fig. 1). The pretreatment level of 5-HIAA, 46.5 ± 13 (SD) pmol/ml, was close to that of healthy volunteers (Table 2). The level of 5-HIAA did not change during treatment (Fig. 1). The pretreatment level of HMPG 23.5 ± 9.5 (SD) pmol/ml was not significantly different from the healthy volunteers either (Table 2). The serum level of HMPG was reduced during treatment at all points in time (Fig. 1).

There were no sex differences in any of the metabolites.

**Concentrations of amino acids in serum.** The pretreatment levels of glutamate and glutamine did not differ from those in healthy volunteers (Table 2). There were no changes in the concentrations of any of the amino acids during treatment (Fig. 2).

**Table 1.** Monoamine metabolites and amino acids in plasma and serum in two healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>HVA</th>
<th>5-HIAA</th>
<th>HMPG</th>
<th>Trp</th>
<th>Tyr</th>
<th>Glu</th>
<th>Gln</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>77.8</td>
<td>46.4</td>
<td>12.9</td>
<td>60.5</td>
<td>89.8</td>
<td>8.7</td>
<td>371</td>
</tr>
<tr>
<td>Serum</td>
<td>69.7</td>
<td>42.6</td>
<td>13.3</td>
<td>62.0</td>
<td>95.5</td>
<td>8.9</td>
<td>344</td>
</tr>
<tr>
<td>II</td>
<td>71.5</td>
<td>40.2</td>
<td>14.2</td>
<td>73.1</td>
<td>115.6</td>
<td>26.5</td>
<td>479</td>
</tr>
<tr>
<td>Serum</td>
<td>65.7</td>
<td>36.9</td>
<td>14.7</td>
<td>72.5</td>
<td>101.3</td>
<td>28.7</td>
<td>479</td>
</tr>
</tbody>
</table>

1 pmol/ml, 2 nmol/ml. Each sample was analysed in duplicate.