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Serum amino acid profiles and dopamine in schizophrenic patients and healthy subjects: Window to the brain?*

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Summary. Altered dopamine turnover has been postulated as underlying cause for schizophrenia. This is partially inferred from pharmacological studies and from changes in serum dopamine and dopamine metabolite levels. It is not clear whether the serum amino acid precursors' availability and neurotransmitter-mediated hormonal release could be indicative of the neurotransmitter turnover. We speculate in this context that the profile of serum amino acids and neurotransmitters reflects differences of neurotransmitter activity in the central nervous system and may be considered in a broad sense "window to the brain".

We analyzed basal serum amino acids (including monoamine precursors), and monoamines in schizophrenic patients after a drug holiday of 3 or more days, and in healthy subjects.

Asparagine, phenylalanine, and cystine were higher and tyrosine, tryptophan, and the ratio of tryptophan to competing amino acids lower in schizophrenic patients than in healthy subjects ($P < 0.05$). Dopamine was increased in schizophrenic patients compared to healthy subjects.

We speculate that these results sustain the notion for dopamine overactivity in schizophrenia, which might be caused by altered amino acid precursor availability.

Keywords: Amino acids – Schizophrenia – Neuroleptic drug response – Serum amino acids – Central monoamines

Introduction

In schizophrenia changes in dopaminergic activity have been postulated as the primary neurotransmitter abnormality; this hypothesis has been advanced with respect to the involvement of other central monoamines such as norepinephrine and serotonin (van Kammen and Antelman, 1984; Bleich et al., 1988; Meltzer,

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1987; Reynolds, 1989). Central monoamines' metabolism is related to their amino acid precursors' availability, i.e. serum concentrations of tyrosine, tryptophan, and histidine are linked with the catecholamines, indolamines and histamine synthesis (Richardson, 1990; Huether, 1988). Whether serum concentrations of amino acid precursors and catecholamines are altered in schizophrenia has not been shown conclusively. This could be due to the heterogeneity of schizophrenias, to previous neuroleptic treatment, to the time of sampling or to the methods employed. We speculate in this context that the profile of serum amino acids and neurotransmitters reflects differences of neurotransmitter activity in the central nervous system and may be considered in a broad sense "window to the brain".

In order to address to these problems we determined in unmedicated schizophrenic patients and in healthy subjects basal serum amino acids and monoamines.

Subjects and methods

Participants were healthy subjects (medical and technical personell, and students from the University of Bonn), and schizophrenic inpatients from the Psychiatric Clinic of the University. Healthy subjects (49 men and 41 women, 25 ± 5 years old, mean ± 1 SD), drug-free patients (37 men and 40 women, 34 ± 11 years) filled in a questionnaire comprising previous health history, personal data and current activities to assure that participants with excessive smoking, drinking, exercise, and overweight, as well as healthy subjects with neurological and psychiatric diseases in first degree relatives and those not eating the standard diet were excluded from the evaluation. Patients and subjects received a standard hospital diet which consisted of 80 ± 5 g protein, 100 ± 5 g fat, and 220 ± 20 g carbohydrate per day; 50% of the caloric intake was at noon. Psychiatric patients and healthy subjects did not show clinical signs of endocrinopathies or other diseases and their weight and height was in the normal range.

The diagnosis of schizophrenia and the ratings were performed independently by three psychiatrists who were blind to the findings of the laboratory. Patients were diagnosed according to the criteria of K. Schneider (1980); all patients met the criteria for schizophrenia.

We drew blood from bed-rested patients and healthy subjects by venipuncture between 7 and 8 a.m. after a 12 hour fast; 2 ml blood was taken into tubes containing EDTA for the determination of serotonin. The remaining blood was kept for 30 min and was centrifuged for 10 min at 3000 g. The serum was divided and stored separately to permit repeated determinations without repeated freezing and thawing.

For the analysis of amino acids, serum was diluted 1:1 with 5% sulfosalicylic acid, swirled for 30 sec and centrifuged for 2 min at 12000 g. The supernatant was frozen at −28°C. One part of the serum was stabilized by the addition of EGTA (final concentration 6.1 mmol/l) and gluthathione (4.8 mmol/l) and frozen at −82°C for the catecholamine determination. From internal quality controls which were run with each assay we inferred that the amino acids, central monoamines, and hormones were stable for more than 12 months; samples were usually analyzed within 6 months.

We determined the amino acids (Table 1), by ion exchange chromatography on a DTC-2710 resin (LC 5000, Biotronik, Maintal, Germany) according to a modified method by Moore and Stein (1951). The evaluation of the chromatograms was carried out with a Shimadzu integrator. The intra- and interassay coeffients of variation for tryptophan and other amino acids were 4 and 8%, respectively. The lower limit of detection as inferred from standard curves for 5 key amino acids (i.e., tyrosine, phenylalanine, tryptophan, taurine, and glutamate) was 3 μmol/l (for methodological details and quality control refer to Rao and Fels, 1987).

Table 1. Serum amino acid levels of healthy subjects and schizophrenic patients

Amino Acid (μmol/l)		Healthy Subjects Mean	(SD)	n	Patients Mean	(SD)	n
Taurine		112	(44)	90	109	(38)	87
Aspartate		11	(5)	81	11	(6)	51
Threonine		150	(31)	90	146	(29)	86
Asparagine		58	(12)	90	55	(12)	86
Glutamate		71	(60)	88	88	(50)	82
Glycine		261	(61)	90	266	(66)	87
Alanine		383	(113)	90	370	(94)	87
Citrulline		36	(9)	88	37	(12)	59
Tyrosine		82	(18)	90	74	(18)*	87
Phenylalanine		74	(12)	90	82	(19)*	87
Ornithine		88	(12)	90	90	(21)	87
1-Methylhistidine		26	(16)	66	25	(15)	43
Tryptophan		85	(26)	90	70	(24)*	87
Histidine		103	(16)	85	101	(16)	87
3-Methylhistidine		13	(7)	90	11	(9)	82
Arginine		107	(02)	90	116	(24)*	86
Cystine	men	62	(10)	49	73	(19)*	47
	women	57	(7)	41	69	(10)*	40
Methionine	men	34	(7)	49	32	(7)	47
	women	31	(87)	41	28	(9)	40
Serine	men	128	(23)	49	133	(29)	45
	women	141	(28)	41	150	(31)	40
Glutamine	men	680	(157)	49	558	(128)*	47
	women	605	(81)	41	559	(113)	40
Proline,	men	267	(92)	47	245	(64)	34
	women	220	(77)	34	213	(39)	26
Valine	men	201	(40)	49	225	(43)	47
	women	233	(36)	41	233	(39)	40
Isoleucine	men	81	(14)	49	86	(15)	47
	women	71	(17)	41	74	(14)	40
Leucine	men	157	(25)	49	142	(40)	47
	women	136	(26)	41	115	(39)	41
Lysine	men	202	(34)	49	211	(39)	47
	women	189	(35)	41	189	(24)	40
Tyr/competing amino acids							
	men	0.090	(0.009)	49	0.082	(0.016)	46
	women	0.089	(0.018)	41	0.083	(0.014)	40
TRP/competing amino acids							
	men and women						
		0.092	(0.025)	90	0.077	(0.025)	86*

*$P < 0.05$

We analyzed the catecholamines by a modification (Rao, Mager 1987) of the radioenzymatic assay of Peuler and Johnson (1977). Pretreatment of rats for the isolation of catechol-o-methyltransferase according to Brown and Jenner (1981) improved the sensitivity of the assay to 65, 59 and 54 pmol/l for dopamine, norepinephrine, and epinephrine, respectively, when 0.05 ml serum was analyzed . The intra-assay coefficients of variation of dopamine, norepinephrine and epinephrine were 5, 4 and 3% ($n = 10$), respectively. The

respective inter-assay coefficients were 13, 8 and 10%. Influence on stability and posture of patients on serum catecholamines were given by Rao and Mager (1987).

Serotonin was determined with a sensitive high-performance liquid chromatography and electrochemical detection (Rao and Fels, 1987); the intra- and inter-assay coefficients of variation were 3.6 and 5%. The lower limit of sensitivity was 0.05 μmol/l.

Serum neuroleptic levels were determined to assure that the participants were drug-free (<0.3 ng haloperidol/ml). Serum neuroleptic activity was assayed by a modification (Rao, 1986) of the radioreceptor assay of Creese and Snyder (1977).

Statistics

Assays were carried out in duplicate. Data were grouped when no difference was observed with the Mann-Whitney U-test in serum levels between men and women. Linear regression analysis was performed to test for correlation. Serum concentrations were tested for normal distribution with the Kolmogoroff-Smirnoff Test. Group differences were tested with a multiple analysis of variance (Manova) and the Bonferroni-Test. The values given are means $\pm$ 1 S.D.; the level of difference is 5%.

Results

In healthy subjects most variables were normally distributed. When men and women's serum levels differed they were analyzed for both sexes separately (Table 1). The schizophrenic patients were older than the healthy subjects ($P < 0.05$). In healthy subjects between age 18 to 60 years no correlation was observed between age and serum concentrations of the variables.

We observed previously no differences in the concentrations of serum amino acid and monoamine levels between patients that had never taken neuroleptic drugs (drug-naive) and those that were drug-free for 3 or more days. We combined the data of both cohorts and compared them with those of healthy subjects.

Tyrosine, phenylalanine, tryptophan, arginine, cystine, glutamine and the ratio of tryptophan to competing amino acids differed between patients and healthy subjects (Table 1). Serum dopamine and serum norepinephrine levels were higher in patients than in healthy subjects and serotonin was similar in both cohorts (Table 2).

Discussion

The results of this study point to a putative involvement of aromatic acid precursors for neurotransmitter availability in schizophrenic patients.

Our patients were older than the healthy subjects, since we were unable to recruit a similar proportion of matched subjects over 40–50 years who were healthy and refrained from medication and substance use. Inspite of this we feel it appropriate to compare both patients' and healthy subjects' variables since we could not show age dependency of these in the latter cohort.

We observed serum phenylalanine to be higher, and tryptophan and tyrosine, the large neutral amino acid precursors for central monoamine synthesis, to be

Table 2. Serum catecholamine and blood serotonin concentrations in health subjects and schizophrenic patients

Parmeter	Healthy subjects Mean	(SD)	n	Patients Mean	(SD)	n
Dopamine (nmol/l)	0.20	(0.11)	89	0.29	(0.24)*	87
Norepinephrine (nmol/l)						
men	3.4	(1.2)	48	3.1	(2.1)	46
women	3.0	(1.0)	41	4.3	(2.1)*	39
Epinephrine (nmol/l)						
men	0.36	(0.24)	48	0.31	(0.18)	46
women	0.23	(0.09)	41	0.31	(0.20)	40
Serotonin (μmol/l)	0.89	(0.25)	67	1.01	(0.45)	45

*$P < 0.05$

lower in schizophrenic patients than in healthy subjects; the ratio between tryptophan and its competing amino acids, was also lower in patients. These findings suggest lowered CNS availability for tryptophan and tyrosine. Bjerkenstedt et al. (1985) observed that the large neutral amino acids, valine, isoleucine, leucine, and phenylalanine were higher in schizophrenic patients than in controls and thus competed for the entry of tryptophan and tyrosine into the CNS. In the context of phenylalanine as a precursor of tyrosine it has been shown in experimental animals that increasing phenylalanine levels decrease the central tyrosine/phenylalanine ratio, and inhibit the rate limiting step in central monoamine synthesis, the tyrosine-hydroxylase, thus decreasing tyrosine's turnover for central monoamine synthesis (Maher, 1988). However, in our study we did not see an increase in the serum tyrosine/competing amino acid ratio, thus still leaving the influence of amino acid availability for dopamine synthesis in schizophrenia open for discussion. Roth et al. (1988) speculated that low amino acid precursor availability might be related to an enhanced rate of midbrain dopaminergic neurons' activity since it could promote up-regulation of dopaminergic receptors, and increased firing, as may be speculated to take place during a psychotic phase; thus increasing dopamine's release. However, it might be noted, that it is also still a matter of debate whether central dopamine receptors are increased in schizophrenia since results of PET studies in schizophrenics are equivocal in that no change (Farde et al., 1987) and an increase in dopamine receptors (Wong et al., 1986) have been observed.

Changes in serum phenylalanine/competing amino acid ratio were observed in schizophrenic patients and were positively associated with tardive dyskinesia (Richardson et al., 1989); a link was made in this context to phenylketonuria since mentally retarded phenylketonuric patients are particularly vulnerable to tardive dyskinesia (Richardson et al., 1986). In our study we do not find a change in the phenylalanine/competing amino acid ratio in the schizophrenic patients compared to healthy subjects. We did not screen our patients specifically for tardive dyskinesia to be able to analyze this group separately.

None of the serum amino acids that function as excitatory (glutamate and aspartate) or inhibitory (glycine and taurine) neurotransmitters were altered in

drug-free or neuroleptic-treated patients compared to healthy subjects as has been observed by Gershon et al., (1969).

We and others (Perry and Hansen, 1985) observed, that amino acids that participate in transmethylation processes such as serine and glycine, whose disturbed metabolism might be related to a faulty transmitter metabolite production, were normal. Increased serum concentrations of glycine, and serine have been observed previously (Bruinvels et al, 1980; Macciardi et al., 1990); in our patient cohort we observed also increased levels, but these were not significant. This difference might be due to the fact that Bruinfels et al. (1980) investigated schizophrenic patients with altered visual perception.

Plasma dopamine and its metabolite, homovanilic acid have been assessed in humans and in the rat to reflect central dopaminergic activity (Bowers, and Swigar, 1987; Davidson and Davis, 1988, Rao et al., 1984; Bacopoulos et al., 1979). Generally serum dopamine concentrations are low and thus measurements are prone to pitfalls. Therefore we analyzed the serum dopamine concentrations with a radioenzymatic assay, for which we pretreated the rats for the preparation of catechol-o-methyltransferase to lower the blank and increase the sensitivity. Basal dopamine concentrations were higher in schizophrenic patients compared to healthy controls.

In addition to increased dopaminergic activity in schizophrenic patients, hyperarousal, and disturbances in attention and information processing have been attributed to increased noradrenergic activity (Hornykiewicz, 1982, van Kammen and Gelernter, 1987); increased CSF norepinephrine concentrations have been observed in schizophrenic patients (Jeste et al., 1984; Kemali et al., 1982; Barbeito et al., 1984) as well as increased plasma norepinephrine levels (Dajas et al., 1983; van Kammen and Antelman, 1984); this is supported by our schizophrenic women's higher serum norepinephrine and a tendency for higher epinephrine concentration when compared to healthy subjects.

Blood serotonin concentrations were somewhat, albeit not significantly elevated in our patients; however, the standard deviation was twice as high in patients than in controls. We have previously seen large intra-individual fluctuations in blood serotonin that could be traced to the individual patients' psychopathology (Rao and Bräunig, 1989). Elevated blood serotonin levels have been associated with auditory hallucination, lack of insight and disorganization, lowered serotonin with depressive mood and suicidality (Bleich et al., 1988; Asberg and Nordström, 1988). The present study did not include suicidal patients. Delisi et al.'s (1981) finding of an increase in blood serotonin in schizophrenics might be due to the inclusion of a differerent patient population compared to ours.

These results suggest: 1. Drug-free schizophrenic patients showed changes in the amino acid precursor availability for monoamine synthesis, that might be involved in catechol amine and serotonin receptor upregulation. 2. The schizophrenic patients' serum dopamine was increased when compared to that of healthy subjects. 3. However, our study shows a discrepancy as regards the decreased availability of tryptophan and no change in the blood serotonin level and thus raises the question as regards peripheral variable availability and their significance as "window to the brain".

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