Biological Aspects of Schizophrenia in Arab Culture

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Abstract

Plasma amino acids, cortisol, prolactin, oestrogen, progesterone, thyroxine, thyrotropin, folate, vitamin B12, urinary biopterins (B) and neopterins (N) were measured in 16 male and 12 female patients with DSM-III-R schizophrenia. The results were compared with those obtained in 32 male and 38 female normal controls. Male patients had significantly higher levels of plasma methionine, phenylalanine, arginine, phenylalanine/tyrosine ratio and had significantly lower plasma levels of cysteine, ornithine, estrogen and vitamin B12 than normal controls. Female patients, however, had significantly higher levels of tryptophan, tyrosine, alanine, leucine, isoleucine, aspartic acid, arginine and prolactin and significantly lower levels of N:B ratio, folate and phenylalanine/tyrosine ratio than normal controls. The results are consistent with those obtained in western populations and support the notion of a universal biological substrate for schizophrenia.

Introduction

There is a marked paucity of studies of the biology of schizophrenia outside the developed world with a general conception that whilst there is cultural variation in the phenomenology and outcome of this illness, its biology is universal. We have recently studied the phenomenology and the interrelationships between a number of definitions of schizophrenia in a large series studied in the United Arab Emirates and reported similar patterns to results obtained in studies in Western Europe and North America (Daradkeh et al, 1997). We have concurrently undertaken a preliminary investigation of a number of metabolic variables in schizophrenic patients in comparison with results obtained in a group of normal controls. These variables comprized plasma amino acids, hormones, folate and vitamin B12 and urinary pterins.

Amino acids

One approach to the validation of the dopamine hypothesis for schizophrenia has been the investigation of plasma amino acids particularly tyrosine as a precursor to dopamine. Bjerkenstedt et al (1990) reported higher plasma concentrations of thorine, alanine, valine, methionine, isoleucine, lysine, phenylalanine and leucine and lower concentrations of glutamine and histidine. Plasma levels of the large neutral amino acids (valine, methionine, isoleucine, lysine, phenylalanine) were significantly negatively correlated with CSF levels of homovanillic acid,. amino acids that compete with tyrosine for transport through the blood brain barrier. Moreover, tyrosine transport into fibroblasts from schizophrenic patients was significantly lower than its transport in healthy controls. Reveley et al (1978) reported raized CSF levels of alanine, glycine, lysine and phenylalanine in schizophrenia in association with enlarged ventricles in these patients.

Pterins

Tetrahydrobiopterin (BH4) is the essential cofactor for the conversion of phenylalanine to tyrosine and the hydroxylation of tyrosine, the rate limiting step in the formation of dopamine. Total neopterins (dihydroneopterin and neopterin) and biopterins (BH4, dihydrobiopterin and biopte-

rin) can be reliably measured in human urine by high-performance liquid chromatography (HPLC) (Nichol et al, 1985) and the neopterin:biopterin (N:B) ratio used as an index of the synthesis of BH4. A raised ratio implies a failure to convert neopterin to biopterin with consequent failure to produce BH4 (Barford et al, 1984) and has been used as a method of detecting abnormalities of biopterin synthesis in childhood (Dhondt, 1984). We have previously reported raized N:B in depressed patients in comparison with healthy control subjects (Anderson et al, 1992; Abou-Saleh et al, 1995). We have also shown that raized N:B distinguished patients who responded to electroconvulsive therapy who also showed an increased conversion of phenylalanine to tyrosine after treatment which suggested that enhanced BH4 dependent hydroxylation accompanies recovery. However, this was not found in non-responders (Anderson et al, 1994). A connection between depression BH4 and folate, has been suggested by Coppen et al (1989) and elaborated for a modified amine hypothesis (Anderson & Abou-Saleh, 1995). Studies of pterins in schizophrenia, however, have been negative in relation to urinary neopterins and biopterins and CSF biopterins. (Duch et al, 1984; Garbutt et al, 1982; Garbutt et al, 1985) although an early report by Leeming et al (1976) found raized serum biopterin levels but normal urinary values in schizophrenic patients.

Folate and Vitamin B12

Folate and Vitamin B12 has been studied in schizophrenic patients and were reported to be lower than normal suggesting a contribution to the aetiology of this condition (Abou-Saleh & Copen, 1986).

Methods

Sixteen male and 12 female patients with schizophrenia who were admitted to the Psychiatric Department of Al Ain Hospital were studied. Patients were diagnosed in accordance with DSM-IIIR and had a mean age of 31.5, SD 10.4 years. All patients were receiving antipsychotic medication which was not stopped for ethical reasons. A control group of normal subjects who were hospital employees was also studied comprizing 32 male and 38 female subjects within a mean age of 35.5, SD 10.1 years. The control group was ascertained to be free of psychiatric disorder by a brief interview. Blood and urine samples were obtained between 9 and 10 am for assay.

The blood was drawn from median cubital vein using Vacutainers (Beckton-Dickinson) of volume 5 ml with clot activator for serum samples and 7 ml with sodium EDTA for plasma ones. The collected blood was transferred on ice to the laboratory and centrifuged at 2,000 g at 4∞ C for 15 minutes using desk-top centrifuge (Jouan). The plasma and serum samples were separated into aliquots and stored at -85∞ C until analyzed not more than 6 months later. The urine samples were collected into standard urine beakers and stored at -85∞ C until analysis.

Levels of total thyroxine (T4), progesterone, cortisol and estradiol in plasma were determined using Radio Immuno Assay (RIA) kits from ICN Flow and DPC for estradiol. Thyroid Stimulating Hormone (TSH) and prolactin levels in serum were analyzed using Immuno Radio-Metric Assay (IRMA) kits from ICN Flow. Vitamin B12 and folic acid in serum were measured by Dual-Label kit from ICN Flow.

Total biopterins and neopterins

Urine samples were spun down at standard bench-top centrifuge at 14,000 rpm for 5 minutes to settle all insoluble particles and clear supernatant was taken for further oxidation. Oxidation was proceeded as follows: 300 ml of cleared urine was mixed with 50 ml of iodine solution (1% iodine dissolved in 2 KJ in 1N HC1), vortexed thoroughly and kept in the dark for 45 min-

utes. Then 50 ml of 1% ascorbic acid solution and 50 mkl of 1N NaOH were added to neutralize excess of not-reduced iodine and hydrochloric acid respectively. Treated that way samples were assessed by HPLC with following conditions:

50 ml of sample was injected into a ChromSpher 250x4.5 mm, 5 mm column (ChromPak Netherlands) using 1090M (Hewlett Packard) automatic HPLC system. Biopterin and neopterin were eluted at flow rate 1 ml/min by linear gradient (from 0 to 4% in 10 minutes) of acetonitrile (HPLC grade. Sigma-Aldrich) in 0.03 M phosphate buffer with pH 3.0. Eluted profile was recorded by 1060 (Hewlett Packard) fluorescent detector with excitation at 350nm and emission at 440 nm. Concentration of pterins was calculated using calibration curve for external standard. All the measurements were normalized to creatinine concentration in the sample. The level of creatinine in urine was determined using colorimetric kit from Sigma.

Plasma amino acids

Amino acids measured were isoleucine, leucine, methionine, phenylalanine, tryptophan, and tyrosine. 0.8 ml of venous blood was collected in a lithium heparinised bottle and centrifuged immediately for five minutes at 2000 rpm, the plasma was deproteinised by adding 20ul of 50% sulphosalicyclic acid, and centrifuged at 10,000 rpm for 10 minutes at -4∞ C, the supernatant stored at - 20∞C until analysis. The supernatant was diluted 1:1 with Li-s Beckman buffer and analysis is carried out for individual free amino acid according to Spackman et al (1958) ion exchange chromatography techniques on Beckman 6300 automatic amino acid analyser by Beckman method: a three Li-buffer system used with a cation exchange resin column 10 x 0.4 (I.D) cms at around 1200 psi and at 20 ml/hr. buffer flow. The method is based on the post column derivatisation by ninhydrin colouring reagent at 135∞ C, derivatised amino acids were detected at 570 nm, while proline and hydroxyproline at 440 nm. The ratios of tryptophan and tyrosine to large neutral amino acids (LNAA) (valine, isoleucine, leucine, phenylalanine) were calculated.

Statistical methods

Student t-tests were calculated to compare the results of all variables between patients and normal controls. Factor analysis of all variables were calculated to identify principle components. Significant differences were considered at the 5% level. All statistical analyses were carried out by using SPSS for Windows.

Results

Male patients had significantly higher levels of plasma methionine, phenylalanine, arginine, phenylalanine/tyrosine ratio and had significantly lower plasma levels of cysteine, ornithine, and estrogen and Vitamin B12 than normal controls. Female patients, however, had significantly higher levels of tryptophan, tyrosine, alanine, leucine, isoleucine, aspartic acid, arginine and prolactin and significantly lower levels of NB, folate and phenylalanine/tyrosine ratio than normal controls (Table 1). Factor analysis with varimax rotation of all biological variables and age generated a number of meaningful factors: a first factor for amino acids with high negative loading for cortisol and folate (41% of variance), a second factor for age, vitamin B12, folate, tyrosine, tyrosine/LNAA with negative loading for N:B and phenylalanine/tyrosine (30%), a third factor for tryptophan, tryptophan/LNAA, ornithine, cysteine, prolactin, TSH and negative loading for T4(20%) and a fourth factor for glycine, histidine, N:B, and negative loading for oestrogen, progesterone and cortisol (10%).

Table (1)
Comparison of results of biological variables between schizophrenic patients and normal controls. Only statistically significant differences are shown. Results expressed as mean ± SD.

	Groups		
Males	**Normal Controls n=32**	**Schizophrenic Patients n=16**	**P**
Age (Yrs)	34.78±11.32	29.0±12.44	0.14
Arginine (Um/L)	82.8±30.50	105.8±47.18	0.05
Cysteine (Um/L)	60.76±25.68	39.72±16.93	0.005
Glycine (Um/L)	202.42±41.82	238.11±75.95	0.04
Methionine (Um/L)	25.36±7.11	35.99±10.07	0.000
Ornithine (Um/L)	83.62±25.40	71.58±14.52	0.04
Phenylalanine (Um/L)	60.06±10.95	70.52±11.65	0.004
Phenylalanine/ Tyrosine	0.78±0.10	0.93±0.19	0.001
Oestrogen (pmol/L)	115.41±35.15	50.75±55.28	0.000
Vitamin B12 (pg/ml)	613.93±277.26	346.6±189.52	0.002
Females	**n=38**	**n=12**	
Age (Yrs)	36.16±9.09	33.08±7.16	0.29
Alanine (Um/L)	422.31±83.97	488.36±100.4	0.04
Arginine (Um/L)	63.31±22.32	93.18±19.56	0.000
Aspartic Acid (Um/L)	5.95±1.93	7.87±2.42	0.02
Isoleucine (Um/L)	76.52±24.48	94.74±20.81	0.04
Leucine (Um/L)	142.1±38.58	173.15±43.05	0.03
Phenylalanine/ Tyrosine	0.87±0.19	0.68±0.10	0.006
Prolactin (Mgl/L)	12.59±7.09	122.67±67.21	0.000
Tryptophan (Um/L)	48.38±10.78	66.66±17.28	0.004
Tyrosine (Um/L)	82.37±25.71	103.9±31.04	0.03
Neopterin:Biopterin	0.64±0.32	0.42±0.12	0.04
Folate (ng/ml)	8.0±6.23	5.09±1.22	0.04

Discussion

These results confirm in part findings of previous investigations concerning differences in plasma amino acids of alanine, methionine, phenylalanine, leucine and isoleucine (Bjerkenstedt et al, 1985), who also showed a negative correlation between LNAA and CSF homovanillic acid which may be mediated by decreased tyrosine availability for the synthesis of dopamine.

Reveley et al (1978) reported a similar finding of reduced tyrosine transport into fibroblasts from schizophrenic patients. We found, however, higher plasma levels of tyrosine but not higher tyrosine/LNAA in female schizophrenic patients which is consistent with lower levels of phenylalanine/tyrosine probably reflecting increased hydroxylation of phenylalanine to tyrosine, findings that are consistent with the dopamine hypothesis for schizophrenia. Male patients, however, had higher phenylalanine/tyrosine ratio than normal subjects.

Factor analysis of the data identified a factor for tyrosine, tyrosine/LNAA, vitamin B12 with negative loading for N:B and phenylalanine/tyrosine accounting for 30% of the variance. These associations support the dopamine hypothesis: increased availability of tyrosine in association with increased hydroxylation of phenylalanine into tyrosine also reflected by high N:B ratio and high vitamin B12 and folate (Anderson & Abou-Saleh, 1995).

In relation to our findings, Wei et al (1995) reported low plasma tyrosine and tyrosine/phenylalanine ratio in early onset schizophrenic patients and Rao et al (1993) reported a phase advance in phenylalanine/LNAA in schizophrenic patients.

Interestingly, Lucca et al (1995) reported that tryptophan/LNAA and not tyrosine/LNAA distinguished schizophrenic from depressed patients and Serres et al (1995) reported an association between decreased tryptophan transport into erythrocytes and loss of impulse control in schizophrenic patients.

In relation to N:B, female patients had lower N:B ratio which is consistent with increased availability of tyrosine. Previous studies have not reported differences in pterins between schizophrenic patients and normal controls (Duch et al, 1984; Garbutt et al, 1982; Garbutt et al, 1985; Dunbar et al, 1992) although an early report by Leeming et al (1976) found raized serum biopterin levels but normal urinary values in schizophrenic patients.

Low vitamin B12 and folate have been reported previously (Abou-Saleh & Coppen, 1986).Hormonal changes were only obtained for oestrogen which was higher in male patients and for prolactin which was higher in female patients than normal counterparts.

Although it is expected that patients had higher plasma prolactin levels since they were receiving neuroleptic medication only female patients showed a significant difference which is consistent with the finding that females have greater prolactin response to neuroleptics than males (Kuruvilla et al, 1993).

Previous studies showed increased basal plasma cortisol and progesterone levels and increased progesterone but not cortisol response to hypoglycemia in schizophrenic patients (Breier & Buchnan, 1992) and decreased TSH but normal T4 and cortisol concentration (Rao et al, 1995).

In conclusion, this is a first study of a number of putative biological factors in an Arab series of schizophrenic patients which yielded positive findings, some of which are consistent with the results obtained in a Western culture and support the notion of a universal biological substrate for schizophrenia.

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دراسة النواحى البيولوجية
لمرضى الفصام فى الثقافة العربية

تم قياس الاحماض الامينية ببلازما الدم و البول فى (٢٨) مريضابمرض الفصامنهم (١٦) ذكور و (١٢) اناث حسب التصنيف الامريكى الثالث المعدل.

تم مقارنة نتائج هذه الدراسة بالنتائج التى حصلنا عليها من العينة الضابطة والتى تكونت من (٧٠) مريضا منهم (٣٢) ذكور و (٣٨) اناث، وجد ان المرضى الذكور لديهم زيادة ملحوظة فى نسب بعض الاحماض الامينية و كذلك فى الاناث.

وجد ان النتائج متوافقة مع مثيلاتها من هذه الدراسات فى الابحاث الغربية، مما يؤكد تناسب المتغيرات البيولوجية فى مرض الفصام فى جميع الثقافات.