

Relationship of symptomatology, gender, and antipsychotic drug treatment with plasma homovanillic acid in schizophrenia¹

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KEY WORDS schizophrenia; homovanillic acid; antipsychotic agents; sex

ABSTRACT

AIM: To study the role of dopamine neurotransmission in schizophrenia and its drug treatment by assessing the relationship of plasma homovanillic acid (pHVA), a major central dopamine metabolite to various clinical parameters in schizophrenic patients. **METHODS:** pHVA was measured by high-performance liquid chromatography with electrochemical detection in a large cohort of both medicated and unmedicated DSM-IV schizophrenic patients. Prior to the measurement of pHVA, the patients were rated on the schedule for the assessment of positive and negative symptoms (PANSS). **RESULTS:** (1) pHVA in 46 patients receiving antipsychotic drugs was decreased, and in 58 drug-free patients increased, (7.4 ± 2.7) $\mu\text{g/L}$ and (10 ± 4) $\mu\text{g/L}$ compared with a matched control group ($9 \mu\text{g/L} \pm 3 \mu\text{g/L}$, $n = 62$) (ANOVA $F = 8.57$, $df = 2$, $P < 0.01$), respectively. Within the drug-free group, pHVA was higher in the patients with a more negative symptom profile. (2) No significant correlation of pHVA with overall SAPS or SANS scores was apparent in the drug-free patients, although within the SANS subscales, a significant relationships to anhedonia-asociality ($r = 0.32$, $P < 0.05$) was apparent. The male drug-free patients showed a positive correlation of pHVA with negative symptoms ($r = 0.42$, $P < 0.05$) while females showed no significant relationship with any PANSS subscales. **CONCLUSION:** The results suggest that an increased

dopaminergic metabolism is apparent in (male) schizophrenic patients with predominantly negative symptoms, supporting reports that this change in neuronal activity may be related to the neuropathological abnormalities seen in the disease, which may differ between males and females. Such neuronal deficits of developmental origin may thus result in an elevation/disinhibition of central dopamine metabolism in schizophrenia.

INTRODUCTION

The dopamine hypothesis of schizophrenia has provided the major impetus to neurochemical study of this disease in the past 30 years. The observations in support of this hypothesis are essentially circumstantial and there remains little direct evidence for a primary abnormality of dopamine function in the brain in schizophrenics^[1,2]. One approach to determine central dopamine function has been the measurement of the major dopamine metabolite, homovanillic acid (HVA), in blood. Approximately 30 % of HVA concentration in plasma (pHVA) are considered to be of central neuronal system (CNS) origin, and animal experiments show it to correlate with striatal HVA concentrations^[3]. Previous studies have demonstrated that pHVA can be used as a potential index to assess changes of dopamine metabolism caused by antipsychotic treatment in schizophrenics, as well as a reflection of treatment response and the improvement in symptoms^[3]. More recently, a significant relationship between pHVA and clinical symptoms has been reported. However, the nature of this relationship is not fully understood^[3,4]. Thus, the measurement of pHVA may be regarded as a practical and accessible tool for assessing central dopaminergic function in schizophrenia and for studying the pathophysiological mechanisms underlying clinical symptoms and the pharmacological action of antipsychotic drugs. In this study, we have measured pHVA in a large cohort of both chronic medicated and un-

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medicated schizophrenic patients and a matched normal control group to determine the relationship of pHVA to various parameters including clinical symptoms, gender, and antipsychotic treatment.

MATERIALS AND METHODS

Subjects The schizophrenic patients [drug-free group; age, (33 ± 10) a, male:female = 28:30; drug-treated group; age, (38 ± 11) a, male:female = 28:18] were referred to the Department of Psychiatry, University of Baroda, India. Diagnosis was confirmed by research psychiatrist (SS) using DSM IV. The patients were also rated using the schedule for the assessment of positive and negative symptoms (PANSS). The controls [age; (35 ± 12) a, male:female = 32:30] were all healthy volunteers with no history of mental illness who were unrelated to the patients but shared the same demographic, social and dietary characteristics. They all gave written informed consent to the study approved by the local ethical committee. In all subjects, whole blood samples were taken between 7:30 AM and 8:00 AM following overnight fasting. After centrifugation at $2500 \times g$ at 4°C for 5 min, plasma supernatant were collected and immediately stored at -80°C prior to assay. All chemicals used in the subjects were purchased from Sigma and/or BDH companies, England.

pHVA determination The pHVA levels were measured using a high-performance liquid chromatography (HPLC) (Millipore; Waters™ 717 Autosampler; KNAUER; HPLC PumpK501) system with electrochemical detection (ECD) (ESA; Coulochem II). The assay method employed was a modified version of that reported by Reynolds^[5]. Perchloric acid (PCA) 0.5 mol/L was added to each sample to precipitate the protein. The samples were kept at -20°C for 20 min, then thoroughly mixed and centrifuged for 10 min at $12\,000 \times g$. The clear supernatants were transferred to clean vials and 50 μL of the sample (in duplicate for each case) was directly injected into the HPLC-ECD system. Isocratic separation was performed on an reversed-phase column (Spherisorb ODS-25 μm , 250 mm \times 4.6 mm ID) at 40°C , with a mobile phase flowing at 1 mL/min and consisting of sodium dihydrogen orthophosphate (NaH_2PO_4) 0.1 mol/L, octane sulphonate 2.5 mmol/L, edetic acid 0.5 mmol/L, 10.25 % glacial acetic acid and 12 % (φ , volume percentage) methanol. ECD employed a coulometric detector (Coulochem II, ESA) with the first electrode set at +350 mV and the second (work-

ing) electrode set at +500 mV. The retention time of the HVA peak was about 14 min. A linear relationship was observed from HVA 2.5 $\mu\text{g/L}$ up to 50 $\mu\text{g/L}$. The recoveries of the spiked samples for the standard of HVA 10 $\mu\text{g/L}$ averaged 98.7 %. The coefficients of variation (CV) for within-day ($n = 4$) and between day ($n = 3$) analysis of pHVA were on average 1.2 % and 7.8 %, respectively.

Statistical analysis Statistical analysis was performed using Window SPSS 9.0. Testing for normality was accomplished by normality plots and K-S (Lilliefors) tests. All pHVA data showed normal distribution. Analysis of variance (ANOVA) was used to compare pHVA between groups with age and gender as the group factor, and the differences between the sample means of pHVA were further analyzed by a *post hoc* multiple comparison test (Tukey-HSD test). A 95 % ($P < 0.05$) confidence level was used to determine statistical significance. Subgroup means were compared by non-paired *t*-test for independent samples. Results were expressed as $\bar{x} \pm s$. One-way ANOVA was used to examine mean age of the subjects between groups. The Mann-Whitney U-test was used to compare SAPS or SANS scores. Chi-square test was used to compare gender proportion between groups. Bivariate comparisons were conducted using Spearman rank correlation.

RESULTS

(1) The subjects were well matched for age, [(33 \pm 10), (38 \pm 11), (35 \pm 12) a; $F = 1.6$, $df = 2$, $P > 0.05$], gender (Male:Female = 28:28, 32:30, 18:30; $\chi^2 = 2.07$, $df = 2$, $P > 0.05$) between three groups. Tab 1 shows the demographic features of schizophrenic patients. Drug-treated patients primarily took chlorpro-

Tab 1. Clinical demographic features of schizophrenic patients. ^a $P > 0.05$, ^b $P < 0.05$ vs drug-treated group.

Features	Drug-free group ($n = 58$)	Drug-treated group ($n = 46$)
Age at onset/a	28 \pm 8 ^a	27 \pm 9
Illness duration/a	5 \pm 5 ^b	11 \pm 9
Months off drugs	31 \pm 37 (1 - 120)	-
Years on treated/a	-	12 \pm 10
CPZ doses/mg \cdot d ⁻¹	-	378 \pm 135
Clinical picture (P:M:N)	8:29:21	17:17:11
Mean SAPS scale	22 \pm 15 (2 - 90) ^a	24 \pm 16 (1 - 69)
Mean SANS scale	31 \pm 17 (2 - 81) ^b	23 \pm 14 (2 - 53)

mazine (CPZ). The drug-free patients consisted of 26 (45 %) previously medicated and 32 (55 %) drug-naive patients. Patients were categorised into three clinical types, defined as having predominantly positive, negative or mixed profiles according to Andreasen and Olsen^[6]. An inverse correlation between total SANS and SAPS scales was observed in the drug-free patients ($r = -0.53$, $P < 0.01$). No gender difference was apparent in the overall SANS score in the patient groups.

(2) The results of pHVA determination in the subjects are shown in Tab 2. ANOVA indicated the main effect of diagnostic group ($F = 8.57$, $df = 2$, $P < 0.01$) on pHVA. Although there was no significant main effect of gender ($F = 3.08$, $df = 1$, $P > 0.05$) or diagnosis by gender interaction ($F = 0.38$, $df = 2$, $P > 0.05$) with pHVA, the male drug-free patients revealed a marked elevation in pHVA as compared to the male controls and the male drug-treated patients. However, difference in pHVA between the female drug free patients and the female control groups was not significant. Within the drug-free group, there was higher pHVA in the patients having a more negative symptom profile as compared to the patients with predominantly positive symptoms.

(3) Tab 3 shows the correlation between pHVA and SAPS and SANS scores in the patients with schizophrenia. There were no correlation between pHVA and either total SAPS scores or total SANS scores both in drug-free and drug-treated patients. However, there was a significant positive correlation between pHVA and the subscales of SANS in the patients with schizophrenia.

Interestingly, a positive correlation was found between pHVA and total SANS scores in male drug-free patients, while females showed no significant relationship with SANS or any SANS subscales. In contrast, pHVA was correlated negatively with two subscales of SAPS in

Tab 2. Plasma homovanillic acid concentration in schizophrenic patients and normal controls. $\bar{x} \pm s$. ^a $P < 0.05$ vs controls. ^{*} $P < 0.05$, [†] $P < 0.01$ vs drug-treated group. ^b $P < 0.05$ vs the patients with predominantly positive symptoms.

Subjects	Number of samples	Plasma HVA levels / $\mu\text{g} \cdot \text{L}^{-1}$
Normal controls	62	9 ± 3
Male	32	8.0 ± 2.3
Female	30	10 ± 4
Drug-free patients	58	10 ± 4 ^{bc}
Male	28	10 ± 4 ^{bf}
Female	30	10 ± 4 ^c
Positive picture	8	8.4 ± 2.3
Negative picture	21	11 ± 5 ^b
Mixed picture	29	10 ± 3
Drug-treated	46	7.4 ± 2.7 ^b
Male	28	7.2 ± 2.6
Female	18	8 ± 3

male drug-free patients (pHVA with global delusions scale $r = -0.541$, $P < 0.01$, $n = 28$; with global thought disorder $r = -0.495$, $P < 0.01$, $n = 28$). No marked effect of the rest of the clinical parameters was found on pHVA in the subjects (data not shown). However, there was a borderline-significant negative correlation between pHVA and age of onset in male drug-free patients ($r = -0.373$, $P = 0.051$, $n = 28$).

DISCUSSION

In this large cohort of schizophrenic subjects and matched controls, we have identified an marked elevation in pHVA in chronic unmedicated patients, more than half of whom were drug-naive, relative both to control subjects and to drug-treated patients. This elevation appears

Tab 3. Correlation of pHVA with SAPS and SANS scores in schizophrenic patients. Data are Spearman rank correlation coefficients. ^b $P < 0.05$ (SAPS, SANS, and subscales of SANS vs pHVA).

Subjects	SAPS total	SANS total	Affective Flattening	Subscales of SANS			
				Alogia	Avolition Apathy	Anhedonia-Asociality	Attention
Drug-treated (46)	-0.18	0.21	0.14	0.38 ^b	0.14	0.28	0.18
Males (28)	-0.22	0.31	0.11	0.30	0.21	0.32	0.34
Females (18)	-0.20	0.05	0.12	0.60 ^b	-0.09	0.12	-0.23
Drug-free (58)	-0.08	0.19	0.07	0.07	0.10	0.32 ^b	0.21
Male (28)	-0.11	0.42 ^b	0.42 ^b	0.37	0.40 ^b	0.37	0.28
Female (30)	-0.03	0.03	-0.20	-0.17	-0.06	0.31	0.14

to occur in the patients with predominantly negative symptoms. The results are consistent with some reports^[7], but not all. Such discrepancies may be due to a combination of individual variation and small cohort size, as well as the effects of antipsychotic drugs, since in some studies, there may have been an inadequate period of withdrawal from treatment.

The relationship of the results with gender is notable. The elevation in drug-free cases is substantially greater (+21%) in the males than the non-significant effect in the females (+8%), conceivably related to the influence of the relationship between negative symptomatology and pHVA, suggesting that higher pHVA is correlated with more severe negative features in male patients. Nibuya *et al.*^[4] found an increase in pHVA in patients with deficit syndrome; this was a study of mainly male subjects (17 of 20 in each group) and thus is certainly consistent with our findings. They also showed ventricular enlargement in the deficit patients; this is found to be greater in male patients in whom negative features are generally more severe. Combining with other previous studies^[8,9], such results indicated that the neuronal deficits reflected by ventricular enlargement, and presumably related to the cognitive/negative features of deficit patients, may also result in elevation of pHVA. In the respect, the tendency may have a more severe neuropathology.

The pHVA deficit associated with drug-treated patients seems likely to be a direct effect of drug treatment itself, considering the known dopaminergic activity and CNS actions of the antipsychotic drugs. That the deficit is a correlate of effective treatment has been suggested by various studies. Those studies demonstrate a decrease in pHVA with symptom improvement following drug treatment, although there is some discrepancy between the findings mentioned above demonstrating improvements primarily in negative symptoms^[10] and those describing a correlation with the improvement in psychotic/positive symptoms^[11]. Whether the activity of dopaminergic systems reflected by pHVA relates in any way to negative symptomatology is unproven. However, we may speculate that the brain deficits which correlate with symptoms may be responsible for a loss of inhibitory control of brain, perhaps striatal, dopaminergic activity and that this is normalized by antipsychotic drug treatment.

In conclusion, the present study describes both deficits of pHVA in drug-treated schizophrenia and elevations in drug-free schizophrenic patients which are sex-specific and related to the severity of certain negative features. In addition, our findings provide further evidence for differences between male and female subjects in terms of the neurobiological basis of schizophrenia and its effect on dopaminergic function.

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精神分裂症患者临床症状、性别和抗精神病药物治疗与血浆高香草酸的关系¹

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关键词 精神分裂症; 高香草酸; 抗精神病剂; 性别

目的: 通过分析精神分裂症患者中枢多巴胺代谢产物血浆高香草酸浓度(pHVA)与临床症状的关系, 进一步探讨多巴胺神经递质及其药物在精神分裂症治

疗中的作用. **方法:** 在 46 例长期药物治疗、58 例未治疗精神分裂症患者血浆中, 采用高压液相色谱连接电化学分析仪测定 pHVA; 测前评定阳性症状量表(SAPS)和阴性症状量表(SANS). **结果:** (1) 与 62 例健康对照比, 治疗组 pHVA 显著降低; 未治疗组显著增高, 以阴性症状组为主. (2) 未治疗患者 pHVA 与 SAPS 或 SANS 总分无显著相关, 但与 SANS 因子兴致/社交缺乏显著正相关($r = 0.32, P < 0.05$); 男性未治疗患者 pHVA 与 SANS 总分显著正相关($r = 0.42, P < 0.05$). **结论:** 一些主要伴有阴性症状的精神分裂症患者(男性)中枢多巴胺转化增强, 提示多巴胺能神经元活性的改变可能与该病神经发育异常导致的神经病理改变有关, 且存在性别差异.

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