

## HETEROGENEITY IN PLASMA HOMOVANILLIC ACID LEVELS IN SCHIZOPHRENIFORM DISORDER

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*Plasma homovanillic acid (pHVA) levels were estimated in 20 cases of schizophreniform disorder, 14 cases of schizophrenia 'on medication' and 17 cases of schizophrenia 'off medication'. A bimodal distribution of pHVA was seen in schizophreniform disorder subjects, suggesting heterogenous groups in terms of dopaminergic function. No significant difference in the pHVA values was seen in the 3 groups, nor was there a relationship between the severity of the illness and the pHVA values; these results suggest plasticity of the dopaminergic system to neuroleptics.*

The dopamine hypothesis of schizophrenia is founded on the observation that antipsychotics are potent dopamine (DA) antagonists and that clinical potency of these drugs parallels their affinity for dopamine receptors. The quantification of the DA metabolite, homovanillic acid (HVA), in body fluids is one of the most commonly used methods for studying DA transmission in the brain (Degrell and Nagy, 1990). Of late, there is a renewed interest in the study of DA metabolism using levels of plasma HVA (pHVA) as an index of central DA activity (Davila, 1989; Chang *et al.*, 1990).

Various findings have led to the interpretation that about 30-50% of pHVA is of central origin (Bacopoulos *et al.*, 1979; Mass *et al.*, 1980; Stenberg, 1983). Both animal and human 'data suggest that pHVA levels quantitatively reflect brain DA metabolism (Bacopoulos *et al.*, 1979, Stenberg *et al.*, 1983, Kendler *et al.*, 1982). However, studies of pHVA concentrations in schizophrenic patients have produced inconsistent results (Pickar *et al.*, 1984, 1986; Davidson *et al.*, 1987; Davidson and Davis, 1988; Chang *et al.*, 1988, Davila, 1989, Chang *et al.*, 1990).

The present study addressed the pHVA levels in three sub-populations of schizophrenia (DSM III): drug-naive schizophreniform disorder, schizophrenia on neuroleptic treatment and schizophrenia off neuroleptics for more

than 3 months. The objectives of the study were to analyse the distribution of pHVA and the relationship of pHVA to degree of psychopathology in these three groups.

### MATERIAL AND METHODS

The sample comprised all adult males (20-40 years of age) diagnosed on DSM III as schizophreniform disorder (drug-naive) or schizophrenia (on or off medication), presenting over a 6 month period at the Department of Psychiatry at a general hospital in the city. For schizophrenia patients, operationalization of 'on medication' status was daily neuroleptic use for at least the preceding 6 months, while operationalization of 'off medication' status was non-ingestion of oral neuroleptics for at least the preceding 3 weeks, or non-use of parenteral neuroleptics for at least the preceding 3 months.

After confirming suitability for entry into the study, and after obtaining informed consent, the subjects were admitted to the hospital. The study was conducted while they remained as inpatients. Within 72 hours, the subjects underwent detailed medical and psychiatric evaluation including a confirmation of the diagnosis. They were placed on a low catecholamine diet, prepared at the hospital, for 3 days. They were kept fasting and at complete bed rest for at least

14 hours immediately prior to collection of a blood sample. 5 ml of venous blood was collected in a heparinised tube at 9 a.m. Within 10 minutes, the plasma was separated by centrifugation and stored at  $-70^{\circ}\text{C}$  until assay.

The plasma homovanillic acid concentration was determined by the extraction procedure described by Chang *et al.* (1983) followed by high pressure liquid chromatography (HPLC) with electrochemical detection (Pradhan *et al.*, 1990).

The subjects were rated on the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962). The rating was subscaled into schizophrenic thought disorder symptoms (subscale-1), schizophrenic non-thought disorder symptoms (subscale-2) and non-schizophrenic symptoms or arousal symptoms (subscale-3) as described by Yorkston *et al.* (1974). The raters were blind to the diagnostic status of the subjects. The rating was done at a fixed time - 10.30 a.m. on the day prior to blood sampling.

During the study, the 'on medication' schizophrenic group continued their drugs in the dose prescribed to them by the treating psychiatrist. The drug-naive schizophreniform disorder and the 'off medication' schizophrenic patients did not receive any neuroleptics other than diazepam in an oral dose of 10mg S.O.S.

## RESULTS

The original sample comprised 60 subjects; 3 of these were dropped because of haemolysis of their blood samples and 4 more were dropped because of failure to comply with the requirements of the study protocol. Of the remaining 53, 2 subjects, one from group A and one from group C, were excluded from the analysis because of outlying pHVA values (the values were 4-5 times greater than the mean of

their respective groups). Thus, the final sample comprised 20 in group A (drug-naive schizophreniform disorder), 14 in group B (schizophrenia 'on medication') and 17 in group C (schizophrenia 'off medication').

The mean (M),  $\pm$  standard deviation (SD) age in Groups A, B and C were  $26.5 \pm 5.9$ ,  $31.0 \pm 6.5$  and  $33.0 \pm 5.9$  respectively; Group A was significantly younger ( $p < 0.05$ ) than Groups B and C (one way ANOVA with Student-Newman-Keuls multiple comparison test). The BPRS full scale and subscale scores were presented in Table-1. The group A subjects were significantly more ill than the group B or group C subjects on the total BPRS scale and on subscales 1 and 2, but there was no significant difference between groups B and C (one way ANOVA with Student-Newman-Keuls' multiple comparison test).

D'Agostino's test of normality, a powerful test to detect non-normal distributions (Zar, 1984), was applied to the pHVA values in each of the 3 groups. Non-normal distribution was identified in Group A ( $D = 0.2516$ ,  $p$ ) but not in groups B ( $D = 0.275$ ,  $p > 0.2$ ) and C ( $D = 0.2751$ ,  $p > 0.2$ ).

There was no correlation between pHVA values and BPRS full scale or subscale scores in the 3 groups (Table-2). To test the association of higher pHVA values (in the absence of one to one correspondence) with greater degree of psychopathology, the pHVA values were divided by a median split; the  $M \pm SD$  full scale and subscale BPRS scores in 'high HVA' and 'low HVA' categories in the 3 groups were compared. No significant association emerged (Table-3).

The pHVA values were compared in groups A ( $57.8 \pm 34.9$ ), B ( $67.8 \pm 41.9$ ) and C ( $55.1 \pm 32.9$ ); there was no significant difference (one way ANOVA).

## DISCUSSION

Two of the subjects who completed the study protocol had to be dropped from analysis because of outlying pHVA values. It is difficult to speculate on the reasons responsible for levels so far removed from the rest of the group, but such outlying values are not uncommon in biological research. These values if retained in the analysis, could potentially cause a skewed distribution and prejudice the statistical inferences.

Although the 3 groups differed in age, this was not considered as a separate indepen-

dent variable as there is no evidence that pHVA varies with age in young adults, especially within a narrow span (difference between extreme means = 6.5 years).

The schizophreniform disorder subjects were more severely ill than the schizophrenics (Table-1). This could be expected, as these subjects were preselected for being drug-naive, and were actively psychotic, while the schizophrenics 'on medication, were largely in remission, and the schizophrenics 'off medication' were mostly asymptomatic (having the lowest scores on BPRS). However, the scores did not differ significantly between groups B and C.

**Table-1 : Mean  $\pm$  Standard Deviation Brief Psychiatric Rating Scale (BPRS) and subscale scores in groups A, B and C**

BPRS Scores	Group A (n = 20)	Group B (n = 14)	Group C (n = 17)	Significance*
BPRS Total	49.8 $\pm$ 8.7	36.8 $\pm$ 14.1	28.0 $\pm$ 11.3	F (2,48) = 17.62, p < 0.001, AB; AC
Subscale 1	15.0 $\pm$ 3.4	7.0 $\pm$ 5.2	4.5 $\pm$ 3.6	F (2,48) = 33.91, p < 0.001, AB; AC
Subscale 2	18.8 $\pm$ 5.1	13.4 $\pm$ 3.8	10.9 $\pm$ 4.7	F (2,48) = 14.16, p < 0.001, AB; AC
Subscale 3	16.0 $\pm$ 3.9	16.4 $\pm$ 7.2	12.5 $\pm$ 4.3	N.S.

\*One way ANOVA with Student-Newman-Keuls' multiple comparison test

A non-normal distribution of pHVA was observed in group A. An examination of the raw data revealed 2 clusters, one between 20-60 ng/ml, and other between 100-140 ng/ml. The difference in the severity of the illness did not account for this bimodal distribution (Table-2 and 3). It is speculated that at least two dopaminergic subtypes of schizophreniform disorders may exist, one with high and the other with low dopaminergic activity. Interestingly, a similar hypothesis has been proposed by Chang *et al.* (1990) in their studies with DSM III diagnosed schizophrenia, based on the baseline pHVA, response to haloperidol challenge and treatment response. However, in this study there was no non-normal distribution or dichot

**Table-2 : Pearson's product-moment correlation coefficients (r) for pHVA Values and corresponding BPRS Scores In group A, group B and group C.**

BPRS	Group A (n = 20)	Group B (n = 14)	Group C (n = 17)
BPRS Total	0.18	-0.32	-0.17
Subscale 1	0.21	-0.34	-0.22
Subscale 2	0.00	-0.14	0.17
Subscale 3	0.21	-0.30	-0.08

(All correlation coefficients non-significant)

omy in the baseline values in schizophrenia 'on medication' or in schizophrenia 'off medication' groups. This study hence suggests that the DSM III diagnosis of schizophreniform disorder may represent a heterogeneous group biochemically, while DSM III schizophrenia may truly represent a biologically homogenous group in terms of central dopaminergic activity.

The absence of significant correlations between pHVA values and BPRS scores (Table-2) indicates an absence of a one to one relationship between pHVA and symptomatology. Since these results do not preclude the possibility of association of high pHVA with more severe symptoms, further analysis was

conducted (Table-3.) but again with negative results. These findings are not in consonance with the dopaminergic hypothesis of schizophrenia, and are in sharp contrast to certain other studies (Davis *et al.*, 1985; Davidson and Davis, 1988; Mass *et al.*, 1988). It has been remarked that controversies and contradictions are two common features of pHVA studies due to variations in the study designs (Davila, 1989). In this study, the role of dopaminergic activity in severity of schizophrenic symptomatology could not be established. The same conclusion has been derived from the inter-group comparison of pHVA values (Table-1). Despite differing degrees of psychopathology, pHVA levels did not differ significantly between the 3 groups.

**Table-3 : Mean  $\pm$  Sd BPRS Scores in higher and lower pHVA categories In group A, group B and group C.**

BPRS Scores	Group A		Group B		Group C	
	Median (n = 10)	Median (n = 10)	Median (n = 7)	Median (n = 7)	Median (n = 8)	Median (n = 9)
BPRS Total	50.7 4.1	48.9 12.0	50.7 4.1	48.9 12.0	50.7 4.1	48.9 12.0
Subscale 1	15.1 3.5	14.9 3.6	15.1 3.5	14.9 3.6	15.1 3.5	14.9 3.6
Subscale 2	18.9 3.5	18.7 6.4	18.9 3.5	18.7 6.4	18.9 3.5	18.7 6.4
Subscale 3	16.7 3.5	15.3 4.3	16.7 3.5	15.3 4.3	16.7 3.5	15.3 4.3

*All t test comparisons (with modified degrees of freedom wherever indicated, to correct for heterogeneity of variances), between above median and below median categories within each of the three groups, were non-significant.*

The pHVA values in group B were not significantly different from those in groups A and C. The expected rise of pHVA values with neuroleptic therapy (indicating increased dopamine turnover in a homeostatic effort to overcome to dopaminergic blockade) was not seen in this group. The finding, however, could be understood in the light of development of either tolerance in dopaminergic neurons to chronic D2 receptor blockade or supersensitivity of D2 autoreceptors (Davila, 1989, Langer and Lehmann, 1988).

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