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METHYLATION IN SCHIZOPHRENICS: A PHARMACOGENETIC STUDY

J. PRICE*

Nuffield Unit of Medical Genetics and the Department of Psychiatry, University of Liverpool, England

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INTRODUCTION

THE HYPOTHESIS that there might be faulty transmethylation in schizophrenics, originated by OSMOND and SMYTHIES,[1] has attracted considerable interest over the last few years and has stimulated much research. Evidence relating to this hypothesis has been discussed in some detail by KETY[2] and by SMYTHIES.[3] It would be repetitious to go over this in detail here, but a brief outline would not, perhaps, be out of place. Thus, anyone arguing in support of the hypothesis could cite the following (to much of which objection can be raised):

1. That there is a resemblance between schizophrenia and the psychoses induced by various methylated hallucinogens. SMYTHIES[3] in this connection wrote: "... it is surely significant that the hallucinogens are all either O-methyl or N-methyl derivatives of the cerebral neurohumors noradrenaline, dopamine and 5-hydroxytryptamine".
2. That methyl donors (when given in combination with a monoamine oxidase inhibitor) are capable of exacerbating the specific psychopathology of schizophrenia.
3. That abnormal methionine metabolism has been reported in schizophrenia[4], and that schizophrenia has been noted to co-exist in a number of pedigrees with a known disorder of methionine metabolism: homocystinuria.[3, 5]
4. That methyl acceptors (e.g. nicotinamide) can prove beneficial in schizophrenia, particularly in the more acutely disturbed patient.[6]
5. That the administration of a methylating enzyme (catechol-O-methyl transferase, COMT) can have an adverse effect on schizophrenia.[7]
6. That methionine sulphoximine (which may act as a methionine antagonist) can benefit chronic schizophrenics while having an adverse effect on non-schizophrenic individuals, namely the induction of a toxic psychosis.[8]
7. That two methylated amines, bufotenin and 3,4-dimethoxy-phenylethylamine (DMPE) occur in the urine of schizophrenics but not in that of control subjects.

* Author's present address: St. Giles' Hospital, Suva, Fiji.

It must be stated again that a great deal of what is set out above is subject to dispute (e.g. the amine studies mentioned in paragraph) 7[3, 9, 10] or lacks satisfactory corroboration (e.g. the efficacy of nicotinamide)[11] or can be explained without invoking a faulty transmethylation hypothesis. For example, although there is a fair amount of agreement that a methionine/monoamine oxidase inhibitor (MAOI) combination is deleterious in schizophrenia,[12] this combination has been shown to elevate urinary amine levels more than does MAOI alone even when the amine involved is not methylated.[13, 14]

Many would, however, not disagree with KETY[2] who wrote—"That hypothesis has by no means been proved nor is it likely to be proved very easily. It remains, however, a remarkably parsimonious explanation of a mass of otherwise disparate information and for that reason appears to be worth entertaining and testing."

There would now appear to be general agreement that schizophrenia is in part genetically determined.[15] Accepting this, one would infer the presence of a biochemical lesion, and while it is true that this may be confined to the brain, there can be no certainty that this will prove to be the case. There would seem to be no logical reason in our present state of knowledge, either of schizophrenia on the one hand or of biochemical genetics on the other, to limit research into this or any other biochemical hypothesis of schizophrenia to studies of the metabolism of brain only.

The pharmacogenetic approach

The term 'pharmacogenetics', first introduced by VOGEL,[16] can be defined as the study of genetically determined variation in animal species which are revealed by the effects of drugs. For example, it is known that the drug isoniazid is acetylated in the body and that two genetically determined acetylator types are to be found in a Caucasian population, the rapid and the slow.[17] This situation constitutes an example of genetic polymorphism and the hypothesis has been put forward[18] (and attacked)[19] that schizophrenia constitutes another example of this phenomenon.

In the present study, pharmacogenetic technique has been applied to the problem of measuring methylation *in vivo*. A substance was administered which undergoes O-methylation as an initial metabolic transformation, namely 3,4-dihydroxybenzoic acid (protocatechuic acid, PCA). O-methylation of PCA takes place chiefly in the 3–O position (leading to the formation of vanillic acid, VA), but 4–O methylation (leading to the production of isovanillic acid, IVA) also occurs,[20] a process of particular interest because the majority of one ring compounds possessing a mescaline effect contain a 4–O methyl group.[21, 22] After O-methylation, glycine conjugation of both vanillic and isovanillic acid occurs[10, 23] (see Fig. 1).

PCA

PCA can safely be consumed in gram doses[24], a circumstance which not only facilitates the measurement of its metabolites but also allows the enzyme systems to be subjected to a greater load than is generally possible in amine studies (which must frequently be done under tracer conditions). Here (dosage being proportional to the body weight of each subject) a 63·5 kg individual received 1 g of PCA.

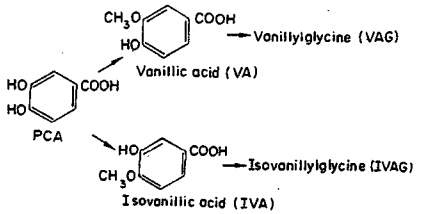

FIG. 1. The metabolism of protocatechuic acid (PCA) in man.

Subjects

The control subjects consisted of university undergraduates, junior laboratory technicians and nurses, all in good physical health. In general older subjects than these proved more difficult to recruit and were found more likely to default.

The schizophrenic subjects were chosen from those admissions or readmissions to hospital in whom the diagnosis was clearly schizophrenia and who had taken no psychotropic drugs for several weeks (at least) prior to testing. All the patients were tested shortly after admission at a time when they were considerably disturbed.

Methods

The test. On the day before the test a number of dietary restrictions were imposed in order to reduce interference from extraneous phenolic acids, chiefly the omission of coffee and all fruit. The remaining interference was estimated in urine passed immediately prior to testing (in 6 controls subjects and all but one of the schizophrenics), shown to be of very low order and ignored.

On the day of the test no breakfast was permitted. Subjects emptied the bladder prior to the consumption of PCA which was presented as a standard solution in water, adjusted with alkali to pH 6 and sweetened with glucose which, being a reducing agent, prevented the oxidation of PCA. The recovery of PCA from this standard solution was estimated by previously described methods[23, 25] and shown to be 100 per cent. Subjects then fasted for 1½ hr (to allow time for the PCA to be absorbed) and then took fluid freely. All urine passed in the three hours after PCA had been consumed was collected (into 0·2 ml 5 per cent wt. vol. sodium azide) and at the end of 3 hr exactly, all subjects were asked to empty the bladder and thus complete the urine collection. It was at this stage that the cooperation of the schizophrenics was most difficult to obtain but that it was obtained is suggested by the experimental results, there being close agreement for total recovery of metabolites between all groups tested.[26] An aliquot of urine was put aside for pH estimation and 5 ml 3*N* HCl added to the remainder, this then being stored at −20°C until analysis was carried out (using methods which have been described elsewhere.)[23, 25, 27]

The following measurements were made on all urine specimens: volume, pH, creatinine content (using the picric acid method),[28] PCA content, total VA content and total IVA content (total = free plus glycine-conjugated). In addition, on 30 control and all schizophrenic urines, the amount of unconjugated vanillic acid was estimated. Subsequently it

was found that the ratio of conjugated vanillic acid (vanillylglycine, VAG) to free vanillic acid (VA) was not normally distributed; this situation was remedied by taking logarithms to base 10.

RESULTS

These are given in the Tables 1 and 2.

TABLE 1. RESULTS FOR MALES: MEANS ± STANDARD DEVIATION

	42 Controls	11 schizophrenics	p*
Age (yr)	21·0 ± 3·4	28·7 ± 10·7	<0.001
Body wt. (lb)	154·9 ± 20·0	136·0 ± 29·1	$0.02>p>0.01$
Urine vol. (ml)	414·2 ± 252·1	484·4 ± 319·4	NS†
Urine pH	6·11 ± 0·60	6·57 ± 0·71	$0.05>p>0.02$
VA (total)/PCA (%)**	57·5 ± 13·1	52·6 ± 14·0	NS
IVA (total)/PCA (%)**	4·70 ± 1·36	4·66 ± 1·81	NS
VA (total)/IVA (total)**	12·7 ± 2·0	12·4 ± 2·3	NS
	24 controls	11 schizophrenics	p
Log_{10}VAG/VA (free)**	0·919 ± 0·213	0·902 ± 0·270	NS

*p = the value of the probability coefficient for Student's t test.
†NS indicates $p>0.05$.

TABLE 2. RESULTS FOR FEMALES: MEANS ± STANDARD DEVIATION

	13 controls	10 schizophrenics	p
Age (yr)	23·8 ± 5·5	33·5 ± 12·5	<0.001
Body wt. (lb)	135·2 ± 13·7	116·1 ± 21·7	$0.02>p>0.01$
Urine vol. (ml)	446·8 ± 225·2	482·3 ± 337·9	NS
Urine pH	5·98 ± 0·70	6·50 ± 0·76	NS
VA (total)/PCA (%)**	51·4 ± 11·94	55·4 ± 20·29	NS
IVA (total)/PCA (%)**	4·17 ± 1·31	4·93 ± 2·14	NS
VA (total)/IVA (total)**	12·8 ± 2·5	11·7 ± 2·3	NS
	6 controls	10 schizophrenics	p
Log_{10}VAG/VA (free) **	0·921 ± 0·239	1·017 ± 0·339	NS

Comments on the results shown in Tables 1 and 2

1. The schizophrenic groups were significantly older than the control groups, a reflection of the difficulty experienced in recruiting older control subjects. In no group did age correlate significantly with any other measurement made.

2. Mean body weight was greater in the control subjects than in the schizophrenics, consistent with the findings of REES[29] (in men) and BETZ[30] (in women). When the values obtained for VA (total)/PCA, IVA (total)/PCA, VA (total)/IVA (total) and $\log_{10}$ VAG/VA (free) were corrected for the effect of body weight, t-tests still showed that group means did not differ significantly.

3. Urinary pH was significantly higher in the schizophrenic males than in the control males, a finding which remains unexplained.

4. There were no statistically significant differences in the methylation of PCA between schizophrenic and normal subjects, either in the 'rate of methylation' (as judged by $\frac{\text{VA (total)}}{\text{PCA}}$ or in the ratio of 3-O to 4-O methylation.

Further results

(a) In a study on 12 control subjects who were given PCA on two separate occasions, it was found that there was significantly more variation in test result between one subject and another than between repeated tests on the same subject (for VA (total)/PCA the F ratio was 3·71, $p < 0{\cdot}05$ and for IVA (total)/PCA the F ratio was 4·17, $p < 0{\cdot}025$), indicating the test to be satisfactorily repeatable.

(b) It will be appreciated from Tables 1 and 2 that within the results for each group of subjects it is possible to correlate many pairs of variables. The total number of such pairs is large: over 200. It was decided at the outset to study particularly the interrelationships between those variables relating directly to the metabolic transformation of PCA. These variables are marked with double asterisks in Tables 1 and 2.

A highly significant correlation was found between VA (total)/PCA and IVA (total)/PCA in all four groups of subjects ($p < 0{\cdot}001$ throughout). The explanation for this would seem likely to be that the body handled VA and IVA in a very similar manner (e.g. with respect to tissue distribution, glycine conjugation and excretion) and that factors tending to prevent correlation (the only obvious one is the precise way in which methylation took place) proved insufficient to prevent highly significant correlation occurring.

A positive correlation between IVA (total)/VA (total) and VA (total)/PCA was noted in the male schizophrenic group only ($p < 0{\cdot}01$). Examination of this correlation in the other three groups of subjects showed positive but non-significant correlations in each case. However, an analysis of covariance on data from all four groups (using the method of SNEDECOR)[31] showed that the four regression lines did not differ significantly from each other but that their mean gradient did differ significantly from zero (although only at a low order of significance: $0{\cdot}02 < p < 0{\cdot}05$).

Putting this into words, one can say that when methylation may be judged to have proceeded relatively rapidly (as shown by high values for VA (total)/PCA), relatively more of the less common of the two methylated isomers, namely IVA, was formed. Perhaps both VA (total)/PCA and IVA (total)/VA (total) are related to the total availability of methyl groups or perhaps two (or more) enzymes with differing kinetics are operating. In this connection it might be relevant that ANDERSON and D'IORIO[32] found that rat liver catechol-O-methyl-transferase can be separated by electrophoresis into three distinct bands.

(c) A 14 year old homocystinuric boy (who failed to show biochemical improvement on Vitamin B6) and both his parents gave PCA test results within the normal ranges. In this homocystinuric (as is typically the case) the serum level of the methyl donor methionine was elevated above normal. However this was not associated with any increase in the rate at which he methylated PCA.

DISCUSSION

The present study differs from previous studies of methylation in several important respects.

(a) It utilises a substrate for which the major excretion pathway is via O-methylation and which undergoes only a small number of metabolic transformations. It may be noted that dopamine used by FRIEDHOFF and VAN WINKLE[33] and FAURBYE and PIND[34] for *in vivo*

studies has a metabolism which is very complex. Thus, GOODALL and ALTON[35] reported that there are at least 18 metabolites of dopamine in man.

(b) In none of the studies mentioned above, nor in the *in vitro* studies of KUEHL[36] was a separate measurement of 4-O-methylation made. SHULGIN *et al.*[21], in a study of one ring psychomimetic compounds, have suggested that 3-O-methylation generally lessens the potency of such compounds whereas 4-O-methylation enhances their potency. Hence a study of 4-O-methylation becomes more relevant to the hypothesis that the body may construct a hallucinogen than does a study of 3,4-di-O-methylation.

(c) The present study has the disadvantage that it is concerned with whole body methylation rather than with methylation in brain (examined separately by KUEHL).[36]

As far as the process of 4-O-methylation of PCA is concerned, the mean ratio of the amount of 3-O-methylated metabolites to that of 4-O-methylated metabolites (12·5:1) was of the same order as that obtained by DALY *et al.*[37] in a study of the metabolism of a variety of catechols both *in vivo* and *in vitro*. The value obtained by KUEHL[36] for this ratio (using dopamine as substrate in a rat liver experiment) was, however, somewhat lower (4:1).

SUMMARY

An account is given of a pharmacogenetic study of O-methylation (with particular reference to 4-O-methylation) by 21 schizophrenics who were in the acute phase of illness and 55 control subjects. The method employed was to measure the urinary metabolites of a compound which is extensively methylated in man and which can be administered under loading conditions: protocatechuic acid. No significant differences in the methylation process were detected between normal subjects and schizophrenics.

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