

A META-ANALYSIS OF HOMOVANILLIC ACID CONCENTRATIONS IN SCHIZOPHRENIA

HENRY C. TUCKWELL

*Institute of Advanced Studies, Australian National University, GPO Box 4,
Canberra, ACT, Australia 2601*

and

JAMES A. KOZIOL

*Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Road, La Jolla,
CA 92037, U.S.A.*

3

(Received June 21, 1992)

The results of several experiments in which homovanillic acid (HVA) concentrations were measured, mainly in the cerebrospinal fluid, of schizophrenics are examined using Fisher's combination procedure. It is found that the data does not support the claim that the level of this dopamine catabolite is raised whereas some evidence strongly supports the claim that it is actually lowered. This finding is discussed in relation to the hypothesis of dopaminergic neuronal hyperactivity in schizophrenia.

Keywords: Schizophrenia, dopamine hypothesis, homovanillic acid, meta-analysis.

The hypothesis that some populations of dopaminergic neurons are hyperactive in various parts of the brains of schizophrenics has been the subject of many investigations. The associated theory and many of the experiments which have been performed in the expectation of testing that hypothesis have been comprehensively reviewed, as for example in Meltzer and Stahl (1976) and Heritch (1990). Despite this large body of data, it has been difficult to test the hypothesis because evidence which purports to do this is usually gathered indirectly. The most direct approach available has been to perform post-mortem examinations of the brains of known schizophrenics. The results of such examinations have not yielded conclusive results. Rather, increases in dopamine levels have sometimes been found in some parts of the brain and not at all in other parts, as summarized in Heritch (1990).

Generally, however, evidence is sought by estimating the concentrations of the end-products of dopamine metabolism. The underlying assumption is that higher than usual levels of activity of dopaminergic neurons will lead to increased synthesis, turnover, release and hence an increase in dopamine metabolites. The most important of such end-products is homovanillic acid (HVA).

Many quite different experimental studies have been performed to examine HVA concentrations in body fluids, particularly cerebrospinal fluid (CSF), urine and blood plasma. A succinct summary was given in the above mentioned article of Heritch. The results were quite disparate so we decided to examine them in a combined (meta) analysis to see if there was overall support for the hypothesis of increased HVA levels in schizophrenia and hence, possibly, an inferred increase in the activity of certain dopaminergic neurons.

METHOD

There are 19 works to be considered as in Table 1 of Heritch's article. It was decided that if results were to be pooled then they must have come from studies which were similar in as many respects as possible. Hence the 19 studies were examined to see which of the following applied or not:

- (i) were the patients in acute or chronic condition?
- (ii) was there a drug free period before the analysis was performed?
- (iii) was a comparison made between a group of schizophrenics and a control group of normals?
- (iv) if the body fluid examined was CSF, was probenecid applied (to increase the retention of HVA) before the results were obtained?
- (v) were age and sex variables taken into account?

The studies of Bowers (1974), Bowers and Study (1979), Bowers and Van Woert (1972), Nyback et al. (1983), Post et al. (1975), Rimon et al. (1971), Sedvall et al. (1974) and Van Praag (1977) involved patients with acute schizophrenia; whereas those of Berger et al. (1980), Davidson and Davis (1988), Davis et al. (1985), Gerner et al. (1984), Gottfries et al. (1971), Karoum et al. (1987), Kirstein et al. (1976), Lindstrom (1985), Van Kammen et al. (1983) and Van Kammen et al. (1986) involved groups composed either of all or some chronic patients. The remaining one of the 19 studies did not state whether the patients were acutely or chronically afflicted and was not considered further.

After further considering the details of each of these 18 studies, it was decided to use combination procedures on the findings of HVA levels in the following 9 broad categories.

Test 1A. All studies in which chronic patients were compared with normals.

Test 1B. All studies in which chronic patients were compared with normals and where probenecid was administered.

Test 1C. All studies in which chronic patients were compared with normals and where there was no probenecid administered.

Tests 2A, 2B and 2C. As in 1A, 1B and 1C, but all studies in which acute schizophrenics were compared with normals.

Tests 3A, 3B and 3C. As in 1A, 1B and 1C but including all studies in which either acute or chronic schizophrenics were compared with normals.

The combination of results from different experiments which purport to measure the same variable or effect is called meta-analysis. There are several approaches which can be adopted, as described in Cox and Hinkley (1974) and more recently by Hedges and Olkin (1985). A simple method is Fisher's combination procedure, known also as the inverse chi-square method. This relies firstly on the fact that if P is a random variable representing the significance probability of an experimentally obtained test statistic, then if the null hypothesis is true, P is uniformly distributed on $(0,1)$. Considering n independent experiments, with significance probabilities P_1, P_2, \dots, P_n , one calculates the value of the combined statistic

$$C = -2 \sum_{k=1}^n \log(P_k)$$

Under the null hypothesis, C has a chi-square distribution with $2n$ degrees of freedom. Large values of chi-square are evidence against the null hypothesis.

RESULTS AND DISCUSSION

The use of the above combination procedure to execute the above nine tests is described in the following. However, in some cases there was only one study under a given test regime so that a combined analysis is degenerate.

For Test 1A, the results of Davidson and Davis (1988), Gottfries et al. (1971) and Lindstrom (1985) gave significance probabilities of .02, .01 and .03 respectively, for a 1-sided test in which the null hypothesis is that there is no difference between HVA levels in normals and chronic schizophrenics and the alternative hypothesis is that HVA levels are lower in chronic schizophrenics. That is, these experiments supported quite strongly the hypothesis that HVA was in fact higher in normals. On examination of the data of Lindstrom (1985), the given P -value was corrected to .017. The articles of Berger et al. (1980), Gerner et al. (1984) and Karoum et al. (1987), did not report P -values. It was nevertheless possible to calculate them from the given results. This yielded P -values of .53 and .80 for the pre- and post-probenecid results of Berger et al. and .71 and .07 respectively for the results of Gerner et al. and of Karoum et al.

The seven P -values are combined as described above to give a value 32.903 for a chi-square variable with 14 degrees of freedom. Overall this means that the hypothesis of no difference in HVA levels is rejected in favour of that of lowered HVA in chronic schizophrenics. Note that even though some experiments reject the null hypothesis and others do not, the overall result is a very strong rejection of the null hypothesis ($p = .003$).

Only one experiment falls in the domain of test 1B, namely that of Berger et al. (1980) who analyzed CSF levels of HVA before and after the administration of probenecid. The combination procedure is thus degenerate. From Berger et al.'s results, we calculated a P -value of .08, which implies that there is insufficient evidence to reject the null hypothesis.

For Test 1C all of the results in Test 1A except that of Berger et al. obtained with probenecid are relevant for this test. The individual p -values are relevant for this test. The individual p -values of .53, .02, .71, .01, .07, and .017 give a value for C of 32.46. There are 12 degrees of freedom for this chi-square variable, leading one to reject H_0 ($p \approx .0005$). Thus the data support the claim that HVA levels are less in chronic schizophrenics after the administration of probenecid.

We now turn our attention to tests involving acute rather than chronic schizophrenics. In relation to test 2A, the articles of Bowers and Van Woert (1972), Nyback et al. (1983) and Post et al. (1975) gave a comparison of results of HVA levels in acute schizophrenics with non-afflicted individuals. The P -values for one-sided tests as described above are .91, .23 and .38, respectively, where that latter two values are calculated from the data. Combining these P -values gives a value for C of 5.06. The resulting significance probability is .54 (chi-square with 6 degrees of freedom) with the conclusion of insufficient evidence to reject H_0 .

There are just two studies to consider for Test 2B, namely those of Bowers and Van Woert and of Post et al. which consider acute schizophrenics treated with probenecid, give $C = 5.77$. For a chi-square test with 4 degrees of freedom, the result is $p = .21$. That is, the hypothesis of a difference in HVA levels is not supported by the data.

In the category 2C, only the work of Nyback et al. considered acute schizophrenics without the application of probenecid. With $p = .23$, there is insufficient evidence to reject H_0 .

Finally, we consider the combination of results for experiments in which patients were either acute or chronic schizophrenics. For Test 3A, we employ P -values from studies of both types of schizophrenics, regardless of whether probenecid was administered or not. There are thus 10 P -values (those in Tests 1A and 2A) which when combined give a value for C of 37.97. The corresponding P -value is .009 (chi-square, 20 degrees of freedom). The evidence thus strongly supports the hypothesis of reduced HVA.

In Test 3B, the results employed in tests 1B and 2B are pooled; that is, we consider chronic or acute patients treated with probenecid before the measurement of HVA levels. The value of C is 2.57 and for chi-square with 6 degrees of freedom, the P -value is .86. The evidence does not support rejection of the hypothesis of no difference.

Finally, in Test 3C, we compare all results on HVA for chronic or acute patients with normals without the prior application of probenecid. With P -values of .53, .71, .01, .07, .017 and .23, the resulting C -value is 27.57. For chi-square with 12 degrees of freedom the corresponding P -value is .006. Thus there is in this test strong evidence of reduced HVA in schizophrenics.

It can be seen that of the 9 tests executed, none indicated that there was sufficient evidence to reject the null hypothesis in favour of the alternative that HVA concentration is higher in schizophrenics, whether these are in acute or chronic phases. The results of the tests with the largest numbers of combined experiments, tests 1A (7 data sets) and test 3A (10 data sets) both strongly rejected the null hypothesis with overall P -values of .003 and .009, respectively. Of the remaining seven tests, five indicated that there was insufficient evidence to reject H_0 , whereas the results of the other two strongly supported the claim that HVA was reduced in schizophrenics versus normals. It seems therefore that the results obtained so far lead one to suspect that HVA levels in schizophrenics are equal or perhaps less than those in controls.

Of course this conclusion is based on the application of a single (Fisher's) combination procedure. However, it is apparent that the application of other methods, such as the inverse normal method (see Hedges and Olkin, 1985, p39) would doubtless lead to the same conclusions because the results for all of the nine above tests were never marginal or borderline. Another possible criticism is that the sample sizes in the various experiments were different. However, the numbers of patients in the various groups were always of the same order of magnitude (20 or so) and sample size is taken account when obtaining the P -values of the individual tests which were combined to calculate the test statistic C .

The drawing of conclusions from such results is however complicated, especially in relation to the dopamine hypothesis. Examples of complications include the effect of antipsychotic drugs and the appearance of extra-pyramidal dysfunction. Chase et al. (1970) studied a group consisting mainly of chronic schizophrenics and found that those who had not been treated with antipsychotic drugs had similar HVA levels to those found in normals by Persson and Roos (1969). However, of the patients who had received antipsychotic drugs, one group who showed no signs of extra-pyramidal dysfunction tended to have elevated HVA, whereas another group without the manifestations of such dysfunction, had HVA levels which were apparently not so elevated.

The complications in interpreting HVA measurements have been elucidated in a timely recent review by Amin et al. (1992). Most of the body pool of HVA is evidently generated by noradrenergic activity and most brain HVA enters the blood directly without appearing in the CSF, which might receive about 3.5 per cent. Furthermore, large accumulations of HVA occur in the brain after the administration of

probenecid. Many drugs, including haloperidol and chlorpromazine, inhibit the egress of HVA into the blood. These claims cast doubt on the usefulness of many of the experiments analyzed in our study. However, if there is HVA in the CSF it has almost certainly originated from the striatum adjacent to the lateral ventricles, but there is a very steep gradient in HVA (80 per cent drop) within the CSF from ventricles to spinal cord where measurements are made.

Since many other factors apart from brain DA neuronal activity influence HVA levels in body fluids, Amin et al. (1992) expressed no surprise that the results considered by Heritch (1990) and further analyzed above show variability. Measurements at multiple time points were advocated as well as simultaneous analysis of more than one body fluid. Nevertheless, despite the many complications, measurement of HVA was still considered the best method of detecting changes in the activities of brain DA neurons.

In part, Heritch (1990) tried to interpret the variability of the results on HVA levels in schizophrenics in terms of type of symptomatology. Acute patients perhaps have high DA turnover whereas chronic patients refractory to treatment possess low turnover. An explanation was sought in terms of an hypothesis of MacKay (1980): schizophrenia involves a reduction in DA release which causes postsynaptic receptor supersensitivity. If a subsequent increase in DA release rate occurs it gives rise to hyperactivity due to the oversensitive receptors and a concomitant acute phase of the disorder. The results of our analysis seem to lend support to these ideas, but in accordance with Amin et al. (1992) and Heritch (1990), a more detailed and broader set of experiments must be performed before definite conclusions can be drawn.

REFERENCES

- Amin, F., Davidson, M. & Davis, K. L. (1992). Homovanillic acid measurement in clinical research: a review of methodology. *Schizophrenia Bulletin*, 18, 123-141.
- Berger, P. A., Faull, K. F., Kilkowski, J., Anderson, P. J., Kramer, H., Davis, K. J., & Barchas, J. D. (1980). CSF monoamine metabolites in depression and schizophrenia. *American Journal of Psychiatry*, 137, 174-180.
- Bowers, M. B. (1974). Central dopamine turnover in schizophrenic syndromes. *Archives of General Psychiatry*, 31, 50-54.
- Bowers, M. B. & Study, R. E. (1979). Cerebrospinal fluid cyclic AMP and acid monoamine metabolites following probenecid: studies in psychiatric patients. *Psychopharmacology*, 62, 7-22.
- Bowers, M. B. & Van Woert, M. H. (1972). 6-hydroxydopamine, noradrenergic reward and schizophrenia. *Science*, 175, 920-921.
- Chase, T. N., Schnur, J. A. & Gordon, E. K. (1970). Cerebrospinal fluid monoamine catabolites in drug-induced extrapyramidal disorders. *Neuropharmacology*, 9, 265-268.
- Cox, D. R. & Hinkley, D. V. (1974). *Theoretical Statistics*. London: Chapman and Hall.
- Davidson, M. & Davis, K. L. (1988). A comparison of plasma homovanillic acid concentrations in schizophrenic patients and normal controls. *Archives of General Psychiatry*, 45, 561-563.
- Davis, K. L., Davidson, M., Mohs, R. C., Kendler, K. S., Davis, B. M., Johns, C. A., DeNigris, Y. & Horvath, T. B. (1985). Plasma homovanillic acid concentration and the severity of schizophrenic illness. *Science*, 227, 1601-1602.
- Gerner, R. H., Fairbanks, L., Anderson, G. M., Young, J. G., Scheinin, M., Linnoila, M., Cohen, D. J., Hare, T. A. & Schaywitz, B. A. (1984). CSF neurochemistry in depressed manic and schizophrenic patients, compared with that of normal controls. *American Journal of Psychiatry*, 141, 1533-1540.
- Gottfries, C. G., Gottfries, I., Johansson, B., Olson, R., Persson, T., Roos, B.-E. & Sjöström, R. (1971). Acid monoamine metabolites in human cerebrospinal fluid and their relations to age and sex. *Neuropharmacology*, 10, 665-672.
- Hedges, L. V. & Olkin, I. (1985). *Statistical Methods for Meta-Analysis*. New York: Academic Press.
- Heritch, A. J. (1990). Evidence for reduced and dysregulated turnover of dopamine in Schizophrenia. *Schizophrenia Bulletin*, 16, 605-615.

- Karoum, F., Karson, C. N., Bigelow, L. B., Lawson, W. B. & Wyatt, R. J. (1987). Preliminary evidence of reduced combined output of dopamine and its metabolites in chronic schizophrenia. *Archives of General Psychiatry*, 44, 604-607.
- Kirstein, L., Bowers, M. B. & Heninger, G. (1976). CSF amine metabolites, clinical symptoms and body movement in psychiatric patients. *Biological Psychiatry*, 11, 421-434.
- Lindstrom, L. H. (1985). Low HVA and normal 5-HIAA CSF levels in drug-free schizophrenic patients compared to healthy volunteers: correlations to symptomatology and family history. *Psychiatry Research*, 14, 265-273.
- MacKay, A. V. P. (1980). Positive and negative schizophrenic symptoms and the role of dopamine. *British Journal of Psychiatry*, 137, 379-383.
- Nyback, H., Berggen, B. M., Hindmarsh, T., Sedvall, G. & Wiesel, F. A. (1983). Cerebroventricular size and cerebrospinal fluid monoamine metabolites in schizophrenic patients and healthy volunteers. *Psychiatry Research*, 9, 301-308.
- Persson, T. & Roos, B.-E. (1969). Acid metabolites from monoamine in cerebrospinal fluid of chronic schizophrenics. *British Journal of Psychiatry*, 115, 95-98.
- Post, R. M., Fink, E., Carpenter, W. T. & Goodwin, F. K. (1975). Cerebrospinal fluid amine metabolites in acute schizophrenia. *Archives of General Psychiatry*, 32, 1063-1069.
- Rimon, R., Roos, B. E., Rakkolainen, V. & Alanen, Y. (1971). The content of 5-HIAA and HVA in the CSF of patients with acute schizophrenia. *Journal of Psychosomatic Research*, 15, 375-378.
- Sedvall, G., Pyro, B., Nyback, H., Wiesel, F. & Wodo-Helodt, B. (1974). Mass fragmentometric determination of homovanillic acid in lumbar cerebrospinal fluid of schizophrenic patients during treatment with antipsychotic drugs. *Journal of Psychiatric Research*, 11, 75-80.
- Van Kammen, D. P., Mann, L. S., Sternberg, D. E., Scheinin, M., Ninan, P. T., Marder, S. R., Van Kammen, W. B., Rieder, R. O. & Linoila, M. (1983). Dopamine- β -hydroxylase activity and homovanillic acid in spinal fluid of patients with brain atrophy. *Science*, 220, 974-977.
- Van Kammen, D. P., Van Kammen, W. B., Mann, L. S., Seppala, T. & Linoila, M. (1986). Dopamine metabolism in the cerebrospinal fluid of drug-free schizophrenic patients with and without cortical atrophy. *Archives of General Psychiatry*, 43, 978-983.
- Van Praag, H. M. (1977). The significance of dopamine for the mode of action of neuroleptics and the pathogenesis of schizophrenia. *British Journal of Psychiatry*, 130, 463-474.