Time-Dependent Effects of Phenothiazines on Dopamine Turnover in Psychiatric Patients

Abstract. *Psychiatric patients studied early during treatment with chlorpromazine and thioridazine demonstrated elevated probenecid-induced accumulations of homovanillic acid, a major dopamine metabolite, in cerebrospinal fluid. In those studied after longer periods of treatment with phenothiazines, homovanillic acid values were not elevated. This suggests that there are time-dependent effects of phenothiazines on dopamine turnover that may be relevant to the time course of antipsychotic efficacy.*

Patients receiving neuroleptic medication have increased levels of homovanillic acid (HVA), a major dopamine metabolite, in cerebrospinal fluid (CSF) (*1, 2*). These clinical findings are consistent with the more extensive animal data indicating that phenothiazines increase dopamine turnover, possibly as a result of feedback mechanisms secondary to initial dopamine receptor blockade (*3*). We now report that the phenothiazine-induced increases in dopamine turnover may be time-dependent since they are not as evident after more than 3 weeks of drug treatment. It is suggested that the time course of maximal antipsychotic efficacy of the phenothiazines may be related to this return of dopamine turnover toward baseline values.

Homovanillic acid was measured as described (*4, 5*) in the CSF of manic-depressive and schizophrenic patients before and during treatment with chlorpromazine (400 to 1200 mg/day) or thioridazine (400 to 900 mg/day). Anti-Parkinsonian medications were not needed. Before the lumbar puncture, patients were treated with probenecid (100 mg per kilogram of body weight, divided in four doses over 18 hours) by the method of Goodwin *et al.* (*6*). Probenecid blocks transport of HVA out of the CSF, so that accumulation of HVA after probenecid blockade can provide an estimate of central dopamine turnover (*6, 7*).

Evidence that HVA in lumbar CSF is derived from sources of dopamine in the brain, particularly the striatum, includes the following: striatal lesions in animals cause parallel decreases in dopamine in the substantia nigra and in HVA in CSF (*8*); phenothiazine-induced increases in HVA levels in CSF of dogs parallel those observed in caudate tissue (*9*); Parkinsonian patients, who have reduced levels of dopamine in the striatum, also have significantly reduced levels of HVA in CSF (*10*); intravenously injected HVA does not enter CSF to an appreciable degree (*11*); and patients with obstruction of the rostral to caudal flow of CSF have little HVA remaining in lumbar CSF (*12*).

In patients receiving either chlorpromazine or thioridazine for less than 3 weeks (15 to 19 days), HVA accumulations were significantly higher ($P < .001$) than in patients receiving no medication (Fig. 1). However, in patients studied after more than 3 weeks of phenothiazine administration (25 to 77 days), HVA accumulations were no longer higher than the values in drug-free patients. The difference between the short- and long-term treatment groups is significant ($P < .001$) (*13*).

It does not appear that the change in HVA accumulation in the long-term drug treatment group can be accounted for by differences in clinical state or duration of hospitalization, since HVA accumulations in patients after long-term phenothiazine treatment are not significantly different from those in drug-free patients studied while acutely psychotic or after recovery (*14*). Clinical ratings of acute psychosis in the short- and long-term phenothiazine treatment groups were also not significantly different. At the time of lumbar puncture, patients were taking a constant daily dose of phenothiazines; the mean doses in the brief (525 ± 169 mg/day) and prolonged (680 ± 82 mg/day) treatment groups were also significantly different.

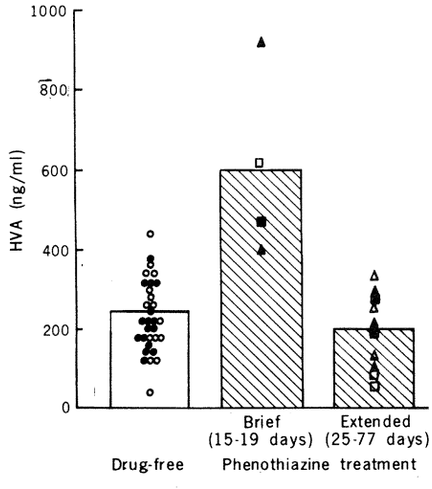

Fig. 1. Duration of phenothiazine treatment: effect on HVA accumulation in CSF. Closed symbols represent values in manic patients while open symbols represent values in schizophrenic patients treated with chlorpromazine (triangles) or thioridazine (squares). The four patients with brief phenothiazine treatment had significantly higher HVA values than did a mixed group of medication-free acutely ill and recovered patients (circles) ($P < .001$, Student's t-test for independent samples) or our independent group of ten patients studied after more extended phenothiazine administration ($P < .001$, Student's t-test for independent samples).

Chlorpromazine and thioridazine both showed the same pattern of an initial increase followed by a decrease in HVA accumulation. In patients treated with phenothiazines, the probenecid-induced accumulations of HVA in manic compared to acute schizophrenic patients also did not differ significantly in this sample.

We are also studying amine metabolite alterations longitudinally during neuroleptic administration in individual patients. Three patients who showed an average 36 percent increase in HVA when studied early in pimozide treatment (day 5 to 12) had only an 8 percent increase when studied after 16 to 26 days of pimozide treatment. Two other patients studied after 3 weeks of pimozide treatment did have 30 percent increases in HVA compared to baseline values, which demonstrates that some patients continue to show elevated HVA values during neuroleptic administration of intermediate length. This is consistent with the recent data of Bowers (*2*) that patients treated with thioridazine had a 48 percent increase in HVA after 18 to 44 days of treatment (although the relationship of HVA values to treatment duration was not reported).

A variety of indirect evidence tends to link the antipsychotic effects of phenothiazines and other neuroleptics to their effects on dopamine metabolism. In apparent relationship to their antipsychotic effectiveness, they produce short-term block of dopamine receptors and increases in dopamine turnover (*3*), enhancement of firing of dopamine neurons in striatal and mesolimbic pathways (*15*), and block of dopamine-mediated increases in adenylate cyclase activity (*16*); and their structures resemble the preferred conformation of dopamine (*17*). Our data suggest that after chronic treatment, the phenothiazines exert less of an effect on dopamine turnover. A report of HVA levels in CSF of monkeys treated with chlorpromazine for 2 to 4 months is consistent with our results in that no statistically significant increases in HVA were observed with this long-term drug administration, despite regular increments in dose (*18*). In other animals, chronic administration of neuroleptics also results in attenuated effects on dopamine turnover (*19, 20*).

To the extent that decreased dopamine function may be related to antipsychotic effects of the phenothiazines, our data suggest a temporal relationship of changes in dopamine turnover to the time course of clinical effectiveness. Although the phenothiazines have an almost immediate effect in many acutely psychotic patients which does not entirely depend on sedation, it is only after longer administration that maximum clinical effectiveness is reached, particularly for the more specific effects on the

primary symptoms of schizophrenia (*21*). The initial, incomplete clinical response to phenothiazines might be explained by the immediate onset of dopamine receptor blockade. However, the decrease in dopamine receptor function may be partially offset by the compensatory increase in presynaptic dopamine synthesis and release, which perhaps stimulates receptors not adequately blocked. When this compensatory increase in dopamine synthesis is terminated by as yet unknown mechanisms (in about 3 weeks), the combined pre- and postsynaptic dopamine function would be optimally reduced, at a time correlating with maximum therapeutic effectiveness of the phenothiazines. This view is consistent with the report that α-methylparatyrosine, an inhibitor of tyrosine hydroxylase, may potentiate neuroleptic efficacy in schizophrenia (*22*). It may do so by preventing the compensatory increase in catecholamine synthesis occurring secondary to receptor blockade with phenothiazines.

An alternate hypothesis suggests that the phase of maximal clinical efficacy may be related to adaptation of the dopamine receptor initially blocked by the phenothiazines, the adaptation being reflected by the return to normal of the presynaptic synthesis and release. The possibility also exists that direct involvement of presynaptic dopaminergic receptors (*23*), coupling mechanisms, or dopamine release (*24*) may mediate the phenothiazine-induced changes in HVA accumulations. Finally, the evidence that tolerance develops to the phenothiazine effect on HVA accumulation could suggest that the long-term antipsychotic effects of the phenothiazines, to which there is no tolerance in the clinical sense, are not mediated by dopaminergic mechanisms. Bowers (*20*), however, reports that tolerance may occur to the neuroleptic effect on HVA only in the striatum (from which most HVA in the CSF is derived) and not in the limbic system, which may be more directly involved in the antipsychotic effects of the neuroleptics.

Our data are as yet inadequate to conclude whether such time-related effects may also occur at noradrenergic synapses (*19*). Moreover, a purposefully simplified "one transmitter" model is discussed here for the sake of clarity, but not with the illusion that dopamine function alone is related to schizophrenic and manic psychoses and their treatment (*25*).

Our findings reemphasize the potential significance of time-related compensatory and regulatory changes in neurotransmitter functions in relation to behavioral change after psychotropic drug administration, as emphasized by Mandell (*26*). It is also possible that such regulatory phenomena and long-term adjustments occur after endogenous biochemical alterations that are not drug-mediated; this would make the time elapsed from the initial biological insult a critical variable in biological psychiatric studies.

Note added in proof: Since submission of this manuscript, there have been two other reports (*27*) of decreased HVA in CSF after long-term compared to short-term neuroleptic administration in psychiatric patients.

ROBERT M. POST
Section on Psychobiology, Adult Psychiatry Branch, National Institute of Mental Health, Bethesda, Maryland 20014
FREDERICK K. GOODWIN
Section on Psychiatry, Laboratory of Clinical Science, National Institute of Mental Health

References and Notes

1. T. Persson and B. E. Roos, *Nature (Lond.)* **217**, 854 (1968); *Br. J. Psychiatry* **115**, 95 (1969); T. N. Chase, J. A. Schur, E. K. Gordon, *Neuropharmacology* **9**, 265 (1970); M. B. Bowers, *Psychopharmacologia* **28**, 309 (1973); *Arch. Gen. Psychiatry* **31**, 50 (1974); B. Fyro, B. Wode-Helgodt, S. Borg, G. Sedvall, *Psychopharmacologia* **35**, 287 (1974).
2. M. B. Bowers, *Clin. Pharmacol. Ther.* **17**, 73 (1975).
3. A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.* **20**, 140 (1963); M. Da Prada and A. Pletscher, *Experientia* **22**, 465 (1966); H. Nyback and G. Sedvall, *J. Pharmacol. Exp. Ther.* **162**, 294 (1968); N. E. Anden, S. G. Butcher, H. Corrodi, K. Fuxe, U. Ungerstedt, *Eur. J. Pharmacol.* **11**, 303 (1970).
4. F. Gerbode and M. B. Bowers, *J. Neurochem.* **15**, 1053 (1968); N. E. Anden, B. E. Roos, B. Werdinius, *Life Sci.* **2**, 448 (1963).
5. Duplicate HVA assays by mass fragmentography correlated well with the spectrofluorometric values [E. K. Gordon, J. Oliver, K. E. Black, I. J. Kopin, *Biochem. Med.* **11**, 32 (1974)].
6. F. K. Goodwin, R. M. Post, D. L. Dunner, E. K. Gordon, *Am. J. Psychiatry* **130**, 73 (1973).
7. N. H. Neff, T. N. Tozer, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **158**, 214 (1967); H. C. Guldberg, G. W. Ashcroft, T. B. B. Crawford, *Life Sci.* **5**, 1571 (1966).
8. R. Papeschi, T. L. Sourkes, L. J. Poirier, R. Boucher, *Brain Res.* **28**, 527 (1971).
9. H. C. Guldberg and C. M. Yates, *Br. J. Pharmacol.* **33**, 457 (1968).
10. R. Olsson and B. E. Roos, *Nature (Lond.)* **219**, 502 (1968); T. N. Chase, *Neurology* **20**, 36 (1970).
11. A. Pletscher, G. Bartholini, R. Tissot, *Brain Res.* **4**, 106 (1967).
12. G. Curzon, E. J. W. Gumpert, D. M. Sharpe, *Nat. New Biol.* **231**, 189 (1971); R. M. Post, F. K. Goodwin, E. Gordon, D. M. Watkins, *Science* **179**, 897 (1973); T. L. Sourkes, *J. Neurol. Transm.* **34**, 153 (1973); E. Garelis and T. L. Sourkes, *J. Neurol. Neurosurg. Psychiatry* **34**, 625 (1973).
13. The lower HVA accumulations after prolonged treatment with phenothiazines do not appear related to differences in probenecid blockade, since a similar trend occurred in our baseline HVA levels without probenecid administration. Values for HVA early in treatment were 46 ± 22 ng/ml (N = 3) which decreased to 22 ± 8 ng/ml (N = 4) later in treatment. Also, J. M. Perel, M. Levitt, and D. L. Dunner [*Psychopharmacologia* **35**, 83 (1974)] showed no significant correlation between HVA accumulation and CSF probenecid levels after the high-dose probenecid technique as employed here. Bowers (*2*) did report lower CSF probenecid levels during thioridazine treatment, but this would not explain differences between short- and long-term phenothiazine treatment; he found HVA increases over baseline values in spite of lower probenecid levels.
14. Patients recovered from acute schizophrenic episodes have lower HVA accumulations than recovered manic patients, however (R. M. Post, E. Fink, W. T. Carpenter, F. K. Goodwin, *Arch. Gen. Psychiatry* **30**, 1063 (1975).
15. B. S. Bunney, J. R. Walters, R. H. Roth, G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **185**, 650 (1973); G. K. Aghajanian and B. S. Bunney, in *Frontiers in Catecholamine Research*, E. Usdin and S. H. Snyder, Eds. (Pergamon, New York, 1974), pp. 643–648.
16. Y. C. Clement-Cormier, J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1113 (19➡ A. S. Horn, A. C. Cuello, R. J. Miller, *J. Neurochem.* **22**, 265 (1974).
17. A. S. Horn and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2323 (1971).
18. F. S. Messiha, *J. Neurol. Sci.* **21**, 39 (1974).
19. R. O'Keefe, D. F. Sharman, M. Vogt, *Br. J. Pharmacol.* **38**, 287 (1970); H. Asper, M. Baggiolini, H. R. Burki, H. Lanener, W. Ruch, G. Stille, *Eur. J. Pharmacol.* **22**, 287 (1973); H. R. Burki, W. Ruch, H. Asper, M. Baggiolini, G. Stille, *ibid.* **27**, 180 (1974); J. Glowinski, in *Association for Research in Nervous and Mental Diseases*, Research Publication No. 64, in press.
20. M. B. Bowers, *J. Pharm. Pharmacol.* **26**, 743 (1974).
21. National Institute of Mental Health–Psychopharmacology Service Center Collaborative Study Group, *Arch. Gen. Psychiatry* **10**, 246 (1964); *Publication Report No. 6* (Public Health Service, Bethesda, Md., April 1966).
22. A. Carlsson, T. Persson, B. E. Roos, J. Walinder, *J. Neurol. Transm.* **33**, 83 (1972).
23. R. H. Roth, J. R. Walters, V. H. Morgenroth, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, E. Usdin, Ed. (Raven, New York, 1974), pp. 369–384.
24. P. Seeman and T. Lee, *J. Pharmacol. Exp. Ther.* **190**, 131 (1974).
25. R. M. Post, *Am. J. Psychiatry* **132**, 225 (1975).
26. A. J. Mandell, *Am. Sci.* **61**, 565 (1973).
27. J. Gerlack, K. Thorsen, R. Fog, *Psychopharmacologia* **40**, 341 (1975); G. Sedval, L. Bjerkenstedt, B. Fryo, C. Harnryd, F. Wiesel, B. Wode-Helgodt, in *Abstracts, Sixth International Congress of Pharmacology, Helsinki, 1975*, p. 597.
28. We thank K. Black, C. Jones, D. Runkle, and A. Fairfax for their expert technical assistance.

25 September 1974; revised 2 February 1975

Identification of Neurons in Cultures

Wahn *et al.* (*1*) claim to have induced "neural differentiation" in cultures of undetermined presumptive epidermis by treatment with adenosine 3′,5′-monophosphate (cyclic AMP) derivatives. The basis of this claim rests on the identification of "neurons" in their explant cultures. This was apparently done by calling any cell which extended a process a "neuron" and then "confirming" the neuronal nature of the cell by formaldehyde-induced fluorescence of biogenic amines. (This latter point was mentioned but no data were illustrated.)

The actual definition of a neuron is hard to specify with precision, especially when cells are no longer seen in their normal surroundings. However, there are a number of generally recognized criteria for neuronal identification which include cell morphology, ability to react with silver stains, ability to generate action potentials, ability to form synaptic connections with other cells, and the ability to synthesize and store specific neurotransmitters. The least satisfactory criterion, especially in tissue culture where cells are growing in a two-dimensional substrate and often assume unusual shapes, is cellular morphology. Similarly, the classification of cells in cul-