Haloperidol: Effect of Long-Term Treatment on Rat Striatal Dopamine Synthesis and Turnover

Abstract. *The short- and long-term effects of neuroleptic drugs differ both clinically and biochemically. Short-term treatment with such a drug causes a kinetic activation of striatal tyrosine hydroxylase. Long-term treatment causes a prompt activation of the enzyme which is followed by a delayed, compensatory deactivation below control levels. Tolerance also develops to the stimulating effect of haloperidol on striatal dopamine turnover.*

The neuroleptic drugs are widely used as antipsychotic agents, and their mechanism of action has been the subject of many investigations (*1*). One characteristic of these drugs is their therapeutic latency. The drugs must be administered to patients for at least 2 to 3 weeks before their specific antipsychotic effects are seen (*2*). Many neurochemical changes are evident with short-term treatment with such drugs, particularly in dopaminergic systems in the brain, but the changes occurring with long-term treatment are probably more relevant to the therapeutic effects of these drugs. The neurochemical effects of long-term treatment with neuroleptic drugs have not been studied extensively. Those studies which have been made reveal striking differences between the biochemical effects of long-term and short-term drug treatment (*3*). We have investigated the effects of long-term treatment with haloperidol on dopamine synthesis and turnover in rat striatum. The effects of short-term treatment with neuroleptic drugs on this dopaminergic brain region have been described (*3*, *4*). We now report that long-term haloperidol treatment induces a series of biochemical changes which combine to give tolerance to the effects of the drug in this system.

The neuroleptic drugs are very effective blocking agents of dopamine receptors in the brain (*5*). Several electrical and chemical changes occur as a result of this receptor blockade. A single dose of a neuroleptic drug to rats causes an increase in the firing rate of nigrostriatal dopaminergic neurons (*6*) and an increase in striatal dopamine turnover (*7*). Although much dopamine is released from nerve endings, the striatal dopamine pool is not depleted because dopamine synthesis is enhanced. The increase in dopamine synthesis is effected through a stimulation of tyrosine hydroxylase (E.C. 1.14.16.2), the enzyme which catalyzes the rate-limiting step in dopamine synthesis (*4*). Tyrosine hydroxylase utilizes three substrates: L-tyrosine, O_2, and a reduced pterin cofactor which serves as electron donor for the reaction. The pterin cofactor is important as a regulatory agent, because the tissue levels of this compound are normally low enough to limit the activity of the enzyme (*8*). When tyrosine hydroxylase is activated by the administration of a neuroleptic, the affinity of the enzyme for its reduced pterin cofactor is increased (*4*). This can be measured as a decrease in the K_m (Michaelis constant) of the enzyme for its pterin cofactor. The decrease in the cofactor K_m of tyrosine hydroxylase and the concomitant increase in the rate of striatal dopamine synthesis are well-defined effects of short-term neuroleptic administration (*4*).

We have studied tyrosine hydroxylase activity in male Sprague-Dawley rats (Zivic-Miller) subjected to long-term treatment with haloperidol. Rats were injected intraperitoneally either with 1 mg of haloperidol (McNeil Laboratories) or with saline at approximately the same time each day. The animals were killed by decapitation either 1 or 23 hours after the last injection. Striatal tyrosine hydroxylase activity was measured by a tritium release assay (*9*). The dopamine content of striata was determined by a spectrophotofluorometric method (*10*). Free and conjugated 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by combined gas chromatography and mass spectrometry (*11*). Total DOPAC or HVA (including both the free and the sulfate-conjugated forms) was determined after enzymatic hydrolysis with glusulase. Protein was determined by the method of Lowry *et al.* (*12*).

Figure 1 shows that 1 hour after a single dose of haloperidol, the K_m for the reduced pterin cofactor, 6-methyltetrahydropterin ($6MPH_4$), is reduced by 75 to 80 percent (Fig. 1A), as shown previously by Zivkovic and co-workers (*4*). Twenty-three hours after a single dose of the drug, control kinetics are restored (Fig. 1B). A similar response is obtained after four daily injections. However, after 8 to 14 daily injections, the enzyme response is different. One change is an increase in the V_{max} (maximum velocity) of the enzyme from drug-treated animals (Fig. 1, C and D, and Table 1). This change suggests that there is an increase

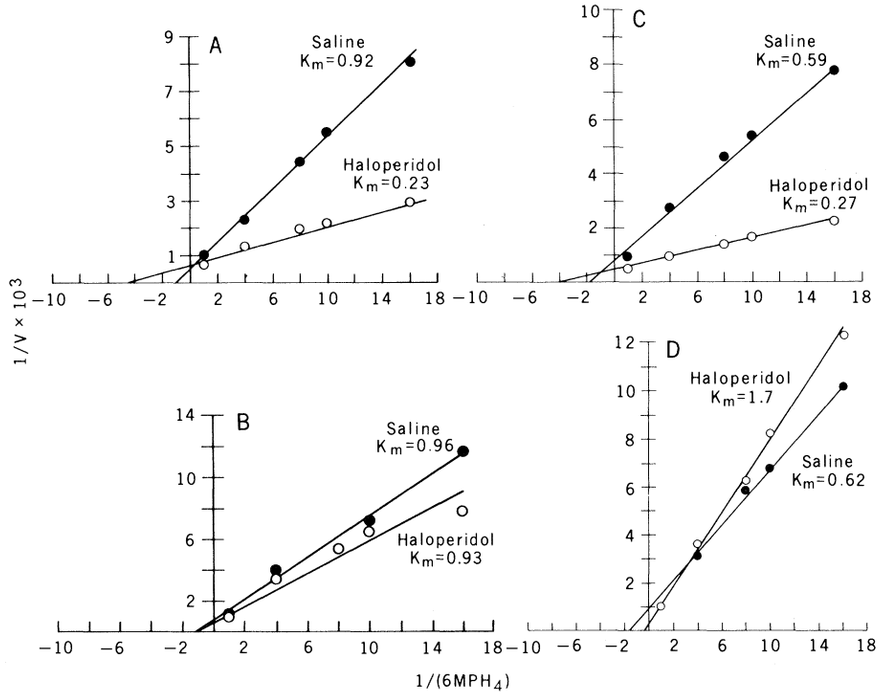

Fig. 1. Tyrosine hydroxylase activity after short- and long-term treatment with haloperidol. (A) Enzyme activity 1 hour after a single injection of the drug. (B) Enzyme activity 23 hours after a single injection of the drug. (C) Enzyme activity 1 hour after the last of ten daily injections of the drug. (D) Enzyme activity 23 hours after the last of ten daily injections of the drug. In each case, V is expressed as picomoles of product released per minute per milligram of protein, and the concentration of $6MPH_4$ is millimolar. Although the observed K_m values differ somewhat from one assay to another, the same relative changes are seen consistently in the enzyme from drug-treated rats.

in the number of tyrosine hydroxylase molecules after prolonged treatment with this neuroleptic. The observed K_m of the enzyme for $6MPH_4$ after long-term drug treatment depends on the time elapsed since the last injection. One hour after the last of ten daily injections, there is a decrease in the K_m for $6MPH_4$ in the drug-treated rats (Fig. 1C). However, 23 hours after the last of ten daily injections, the K_m of the enzyme from drug-treated rats is actually two to four times higher than the control K_m (Fig. 1D and Table 1). This increase in K_m means that the activity of the enzyme from rats receiving haloperidol is deactivated below control levels. Thus long-term haloperidol treatment induces a biphasic response in striatal tyrosine hydroxylase. There is a prompt activation of the existing enzyme molecules, followed by a delayed deactivation below control levels. This study has revealed a novel mechanism for the kinetic regulation of tyrosine hydroxylase—an increase in the K_m for the reduced pterin cofactor to a value higher than the control K_m. The delayed deactivation of the enzyme compensates for the stimulation of the enzyme which occurs rapidly after drug administration.

This study shows two different types of response of tyrosine hydroxylase to long-term haloperidol treatment. First, there is an increase in V_{max}, which suggests an increase in the number of enzyme molecules. It is possible that the initial increase in the firing rate of the dopaminergic neurons is related to the induction of tyrosine hydroxylase synthesis. In other catecholaminergic systems, neuronal activity is known to influence the synthesis of tyrosine hydroxylase (*13*). Second, there are modifications of existing tyrosine hydroxylase molecules which alter their catalytic efficiency. The activation of the enzyme which occurs shortly after drug administration resembles the enzyme activation produced in vitro by protein phosphorylation (*14*). Haloperidol may stimulate tyrosine hydroxylase activity by causing phosphorylation of the enzyme itself or of a regulatory protein. The drug-induced increase in the cofactor K_m, which occurs later in time, has no parallel, as yet, in studies in vitro. This deactivation may represent extensive dephosphorylation of the enzyme or of its regulator. The "control" enzyme may actually be a mixture of the phosphorylated and nonphosphorylated molecules.

The increase in the apparent K_m of tyrosine hydroxylase could be due to either a change in the enzyme itself or an accumulation of a competitive inhibitor. Catechols are known to inhibit tyrosine hydroxylase in a manner competitive with the reduced pterin cofactor (*15*). Catechols in the striatum which could act as competitive inhibitors are dopamine and DOPAC, a dopamine metabolite. A small portion of striatal DOPAC is sulfate-conjugated and would not be expected to function as a competitive inhibitor, since it does not retain the catechol nucleus. We have measured striatal dopamine and free and conjugated DOPAC in order to determine whether there was an accumulation of these compounds after long-term treatment with haloperidol. No such evidence was found. All measurements were obtained 23 hours after the last injection, at the time when the high K_m was observed in drug-treated rats. Dopamine levels were nearly identical in drug-treated and control rats at all times tested (Fig. 2A). Free and total DOPAC were significantly increased in drug-treated rats 23 hours after a single dose of the drug (Fig. 2B). This observation is consistent with the increase in dopamine turnover elicited by short-term neuroleptic treatment (*7*). However, 23 hours after the last of four or more daily injections, DOPAC concentrations tended to be slightly lower in the drug-treated rats (Fig. 2B). This tendency did not reach statistical significance. These experiments indicated that the increase in the apparent K_m of the enzyme could not be explained by an accumulation of either dopamine or DOPAC. In other experiments, extracts from drug-treated and control rats were mixed together, and the enzyme activity was strictly additive. These experiments again indicated that there was no inhibitor present in excess in striatal extracts from drug-treated rats. These data suggest that the deactivation observed in the enzyme from rats given long-term treatment with haloperidol was due to a change in the enzyme itself.

The measurements of DOPAC and HVA, another dopamine metabolite, give an indication of dopamine turnover. Total HVA is significantly increased 23 hours after a single dose of haloperidol (Fig. 2C). This increase reflects enhanced dopamine turnover (*7*). However, 23 hours after 4, 10, or 14 daily injections, both free and total HVA are significantly lower in drug-treated rats than in controls (Fig. 2C). The DOPAC and HVA data indicate a decrease in dopamine turnover with long-term drug treatment. Thus dopamine turnover develops tolerance to repeated doses of haloperidol.

This study has demonstrated several ways in which dopaminergic neurons in the striatum adapt to repeated doses of haloperidol. Our data complement previous reports which describe tolerance in rats to the biochemical and behavioral effects of neuroleptics administered on a long-term basis (*3*). Our results are also consistent with clinical studies on psychiatric patients. Although HVA in cerebrospinal fluid is increased in patients re-

Table 1. Tyrosine hydroxylase activity in rat striatum after long-term treatment of the animals with haloperidol. Tyrosine hydroxylase activity was measured 23 hours after the last of ten daily injections. The kinetic constants were determined from Lineweaver-Burk plots. The values given are means and standard error of the mean for four separate determinations.

Treatment	K_m for $6MPH_4$ (mM)	V_{max} (pmole min^{-1} mg^{-1} protein)
Saline	0.52 ± 0.05	1320 ± 160
Haloperidol	2.00 ± 0.21	2880 ± 330

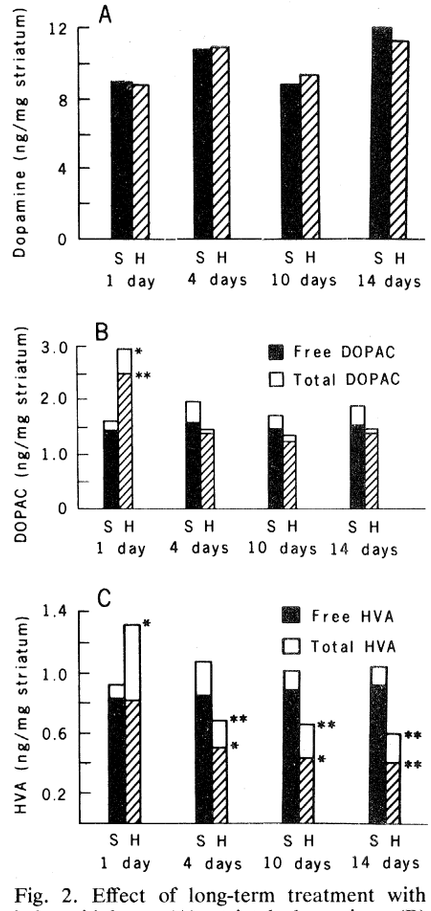

Fig. 2. Effect of long-term treatment with haloperidol on (A) striatal dopamine, (B) striatal DOPAC, and (C) striatal HVA. In each case, the measurements were obtained 23 hours after the last injection of haloperidol. The values shown are means of separate determinations on six individual striata (three rats) for (A) and four pairs of striata (four rats) for (B) and (C). *S*, saline-treated rats; *H*, haloperidol-treated rats. Single asterisks indicate significantly different from control, $P < .05$; double asterisks, $P < .01$.

ceiving short-term treatment with neuroleptics, the amount of this dopamine metabolite decreases after a few weeks of drug treatment (*16*). The clinical effects of the neuroleptics, like the biochemical effects, vary with the duration of drug treatment. Extrapyramidal side effects often occur with short-term drug treatment. After several weeks of drug administration, the extrapyramidal symptoms usually disappear, and the antipsychotic effects become evident. Tolerance to the antipsychotic effects of the drugs does not occur. The adaptive changes in the striatum may be related to the loss of extrapyramidal symptoms or the emergence of the therapeutic effects of neuroleptics, or both. The data presented here provide an example of the remarkable propensity of neurotransmitter systems to return to the baseline state after provocation by pharmacological or environmental stimuli. Such adaptation may represent a tolerance phenomenon or may actually be involved in the onset of the desired therapeutic effects (*17*). Dopaminergic areas in the mesolimbic system and the cortex are also of interest, because these brain regions are known to function as mediators of emotional response. These areas may play an important role in the expression of the antipsychotic effects of the neuroleptics. Our study reemphasizes the complexity of the adaptive mechanisms of neuronal systems and the potential significance of these regulatory changes with regard to the therapeutic efficacy of antipsychotic drugs.

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References and Notes

1. For a recent review, see R. J. Wyatt, *Psychopharmacol. Bull.* **12**, 5 (1976).
2. National Institute of Mental Health—Psychopharmacology Service Center Collaborative Study Group, *Arch. Gen. Psychiatry* **10**, 246 (1964).
3. H. Asper, M. Baggiolini, H. R. Bürki, H. Lauener, W. Ruch, G. Stille, *Eur. J. Pharmacol.* **22**, 287 (1973); M.-J. Besson, A. Cheramy, C. Gauchy, J. Musacchio, *ibid.*, p. 181; M. B. Bowers, Jr., and A. Rozitis, *J. Pharm. Pharmacol.* **26**, 743 (1974); H. R. Bürki, W. Ruch, H. Asper, M. Baggiolini, G. Stille, *Eur. J. Pharmacol.* **27**, 180 (1974); H. R. Bürki, W. Ruch, H. Asper, *Psychopharmacologia* **41**, 27 (1975).
4. B. Zivkovic and A. Guidotti, *Brain Res.* **79**, 505 (1974); _____, E. Costa, *Mol. Pharmacol.* **10**, 727 (1974); B. Zivkovic, A. Guidotti, A. Revuelta, E. Costa, *J. Pharmacol. Exp. Ther.* **194**, 37 (1975).
5. J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972).
6. B. S. Bunney, J. R. Walters, R. H. Roth, G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **185**, 560 (1973).
7. A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.* **20**, 140 (1963); H. Nyback and G. Sedvall, *Eur. J. Pharmacol.* **5**, 245 (1969); N.-E. Andén, S. G. Butcher, H. Corrodi, K. Fuxe, U. Ungerstedt, *ibid.* **11**, 303 (1970); K. G. Lloyd and G. Bartholini, *Adv. Biochem. Psychopharmacol.* **10**, 305 (1974).
8. G. Guroff, C. A. Rhoads, A. Abramowitz, *Anal. Biochem.* **21**, 273 (1967); T. Lloyd and N. Weiner, *Mol. Pharmacol.* **7**, 569 (1971); R. Kettler, G. Bartholini, A. Pletscher, *Nature (London)* **249**, 476 (1974).
9. T. J. Cicero, L. G. Sharpe, E. Robins, S. S. Grote, *J. Neurochem.* **19**, 2241 (1972); W. Lovenberg, E. A. Bruckwick, I. Hanbauer, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2955 (1975). The assay was modified so that ascorbic acid was used as the reductant (P. Lerner, P. Nosé, M. M. Ames, W. Lovenberg, in preparation). Each assay tube contained potassium phosphate, *p*H 6.8 (5 μmole), ascorbic acid (2 μmole), ferrous ammonium sulfate (10 nmole), catalase (2400 units), L-tyrosine (10 nmole), L-[3,5-^{3}H]tyrosine (New England Nuclear; approximately 0.3 μc), $6MPH_4$ (Calbiochem; 6.25 to 100 nmole, as indicated), and tissue extract (20 μl) in a volume of 100 μl.
10. D. Jacobowitz, T. Cooper, N. B. Barner, *Circ. Res.* **20**, 289 (1967); C. W. Nichols, D. Jacobowitz, W. Hottenstein, *Invest. Ophthalmol.* **6**, 642 (1967).
11. E. K. Gordon, S. P. Markey, R. L. Sherman, I. J. Kopin, *Life Sci.* **18**, 1285 (1976).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
13. R. A. Mueller, H. Thoenen, J. Axelrod, *Mol. Pharmacol.* **5**, 463 (1969); H. Thoenen, R. A. Mueller, J. Axelrod, *J. Pharmacol. Exp. Ther.* **169**, 249 (1969); A. Guidotti and E. Costa, *Science* **179**, 902 (1973); I. Hanbauer, I. J. Kopin, E. Costa, *Naunyn-Schmiedebergs Arch. Pharmacol.* **280**, 39 (1973).
14. J. E. Harris, R. J. Baldessarini, V. H. Morgenroth, R. H. Roth, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 789 (1975); T. Lloyd and S. Kaufman, *Biochem. Biophys. Res. Commun.* **66**, 907 (1975); W. Lovenberg, E. A. Bruckwick, I. Hanbauer, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2955 (1975).
15. M. Ikeda, L. A. Fahien, S. Udenfriend, *J. Biol. Chem.* **241**, 4452 (1966).
16. J. Gerlach, K. Thorsen, R. Fog, *Psychopharmacologia* **40**, 341 (1975); R. M. Post and F. K. Goodwin, *Science* **190**, 488 (1975); G. Sedvall, L. Bjerkenstedt, B. Fyrö, C. Harnryd, F. Wiesel, B. Wode-Helgodt, in *Abstracts, Sixth International Congress of Pharmacology*, Helsinki, 1975, p. 597.
17. A. J. Mandell, *Am. Sci.* **61**, 565 (1973).
18. P.L. was supported by the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction, U.S.A. We thank F. K. Goodwin for constructive suggestions.

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Pain Relief by Electrical Stimulation of the Central Gray Matter in Humans and Its Reversal by Naloxone

Abstract. *Relief of intractable pain was produced in six human patients by stimulation of electrodes permanently implanted in the periventricular and periaqueductal gray matter. The level of stimulation sufficient to induce pain relief seems not to alter the acute pain threshold. Indiscriminate repetitive stimulation produced tolerance to both stimulation-produced pain relief and the analgesic action of narcotic medication; this process could be reversed by abstinence from stimulation. Stimulation-produced relief of pain was reversed by naloxone in five out of six patients. These results suggest that satisfactory alleviation of persistent pain in humans may be obtained by electronic stimulation.*

After the initial report by Reynolds (*1*), potent analgesic effects produced by electrical stimulation of discrete areas of medial diencephalon and the brainstem have been demonstrated in the cat (*2*) and monkey (*3*) as well as the rat (*4*). Richardson and Akil (*5*) reported that permanent clinical pain states, in addition to normal pain perception, can be blocked by electrical stimulation of the periventricular and periaqueductal gray matter in humans. We now report the application of the above findings in six human patients suffering from intractable pain (*6*). Electrical stimulation by permanently implanted brain electrodes was chosen as the method of pain alleviation for these patients because of the inability of narcotic analgesics at reasonable dose levels to suppress their constant pain, the widespread and diffuse nature of this pain, and the serious side effects that can occur when conventional neurosurgical lesions in the pain pathways are made as a way to manage such diffused pain.

In all six patients the anatomical target selected for stimulation was at the level of the posterior commissure on the anterior-posterior axis and the ventral-dorsal axis, and 2 to 3 mm lateral from the medial wall of the posterior third ventricle. The electrodes (*7*, *8*) were implanted stereotactically, with the use of anatomical and electrical stimulation-response parameters (*5*). Electrode location was also verified by intraoperative x-ray. Initially, the electrodes were externalized to permit temporary trial stimulation for 1 to 2 weeks. In all patients, self-mediated electrical stimulation began a few days following electrode implantation. Short (0.2 to 0.3 msec) bipolar square pulses, 10 to 20 hertz in frequency, were delivered from a battery-operated stimulator. After successful trial stimulation over a period of 1 to 2 weeks, the electrodes were internalized and connected to a radio-frequency-coupled receiver. The external generator was specifically designed to produce a time-modulated