MOLECULAR PHARMACOLOGY, 10, 727–735

Effects of Neuroleptics on Striatal Tyrosine Hydroxylase: Changes in Affinity for the Pteridine Cofactor

B. ZIVKOVIC, A. GUIDOTTI, AND E. COSTA

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D. C. 20032

(Received April 24, 1974)

SUMMARY

ZIVKOVIC, B., GUIDOTTI, A., AND COSTA, E.: Effects of neuroleptics on striatal tyrosine hydroxylase: changes in affinity for the pteridine cofactor. *Mol. Pharmacol.* **10**, 727–735 (1974).

We assayed tyrosine hydroxylase activity in various brain regions and in adrenal glands of rats receiving reserpine or other neuroleptics which are chemically related to dibenzothiepines (methiothepin), butyrophenones (haloperidol), or diphenylbutylamines (pimozide). These drugs reduced the interaction of brain dopamine with specific postsynaptic receptors by various mechanisms. Intraperitonal administration of haloperidol (24 μmoles/kg), methiothepin (10 μmoles/kg), pimozide (11 μmoles/kg), or reserpine (8 μmoles/kg) increased the affinity of striatal tyrosine hydroxylase for the pteridine cofactors 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine (DMPH) and 2-amino-4-hydroxy-6-methyltetrahydropteridine (6MPH$_4$) within 30 min. When reserpine or the other neuroleptics were added to striatal enzyme preparations they changed neither the K_m for DMPH$_4$ nor that for 6MPH$_4$. A dose of cycloheximide (70 μmoles/kg intraperitoneally) that reduced the rate of brain protein synthesis by about 90% failed to reduce the increased affinity of tyrosine hydroxylase for pteridines elicited by injections of reserpine or methiothepin. Intraperitoneal doses of apomorphine (32 μmoles/kg) or trivastal (70 μmoles/kg) that stimulate brain dopaminergic postjunctional receptors reduced the increased affinity of striatal tyrosine hydroxylase for pteridines produced by injection of methiothepin or reserpine. The injection of haloperidol reduced the K_m of the striatal enzyme for DMPH$_4$ from 0.69 to 0.13 mM but failed to change the K_m for tyrosine (0.054 mM). The injection of reserpine also reduced the K_m of striatal tyrosine hydroxylase for 6MPH$_4$ from 0.5 to 0.13 mM. In the same animals the properties of tyrosine hydroxylase from hypothalamus, brain stem, and adrenals were unchanged. Addition of dopamine to striatal tyrosine hydroxylase preparations from rats receiving 0.9% NaCl exhibited sigmoidal kinetics in addition to competitive inhibition of 6MPH$_4$ binding. The addition of dopamine to preparations of striatal tyrosine hydroxylase from rats receiving reserpine yielded simple competitive inhibition. Since the blockade of dopaminergic receptors by neuroleptics increases the firing rate of dopaminergic neurons, these results suggest that the affinity of striatal tyrosine hydroxylase for the cofactor is enhanced when the activity of dopaminergic neurons is increased.

INTRODUCTION

The biosynthesis of striatal dopamine *in vivo* is regulated by three mechanisms: product inhibition on tyrosine hydroxylase (1–3), recurrent inhibition of dopaminergic neu-

A partial account of the data contained in this paper was presented at the Annual Meeting of the American College of Neuropsychopharmacology, Palm Springs, Cal., December 1973.

ronal activity by a polysynaptic neuronal loop (4, 5), and activation of specific dopaminergic receptors located in the plasma membranes of dopaminergic axons (6). The present results may help to clarify how drugs can change dopamine synthesis by acting on product inhibition of tyrosine hydroxylase and neuronal feedback inhibition (7, 8).

The injection of neuroleptics can increase the turnover rate of striatal dopamine (4, 5, 9–11) and accelerate the activity of dopaminergic neurons (12). These drugs reduce the effect of dopamine on the postjunctional receptors (13). Hence the acceleration of the firing rate of dopaminergic neurons and the increase of striatal dopamine turnover rate elicited by haloperidol and other neuroleptics has been attributed to their antagonism against the dopamine action on postjunctional receptors. Thus blockade by neuroleptics of postjunctional dopaminergic receptors appears to activate the recurrent polysynaptic neuronal loop, resulting in an increase of the activity of dopaminergic neurons.

The present report shows that methiothepin (1-[10,11-dihydro-8-(methylthio)dibenzo(*b*,*f*])thiepin-10-yl]-4-methylpiperazine), pimozide [1-(1-[4,4-bis(*p*-fluorophenyl)butyl]-4-piperidyl)-2-benzimidazolinone], and haloperidol (4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone) injected into rats cause an immediate increase in the affinity of striatal tyrosine hydroxylase for pteridine cofactors. Reserpine produces a functional blockade of dopaminergic receptors by depleting catecholamines from the axon terminals and decreases the K_m of striatal tyrosine hydroxylase for the pteridine cofactors. Thus these drugs change the properties of pre-existing enzymes molecules. The characteristics of tyrosine hydroxylase present in brain areas containing a considerable number of noradrenergic axons remains unchanged after the injection of these drugs.

MATERIALS AND METHODS

[1-^{14}C]L-Tyrosine (specific activity, 54 mCi/mmole) and [3,5-^{3}H]L-tyrosine (specific activity, 50 Ci/mmole) were purchased from New England Nuclear Corporation. 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine, pyridoxal 5-phosphate, and NADPH (enzymatically reduced) were obtained from Calbiochem. Reserpine and apomorphine were purchased from Sigma Chemical Company and S. B. Penick Company, resepctively. The following drugs were kindly donated by the producers: haloperidol and pimozide (Janssen Pharmaceutica Research Laboratoria, Beerse, Belgium), methiothepin maleate (Hoffmann–La Roche, Nutley, N. J.), and trivastal (Servier Laboratories, Ltd., Harrow, England). All other reagents were obtained commercially in the purest form available.

Sheep liver pteridine reductase was purified through the second ammonium sulfate precipitation step according to Kaufman (14). Hog kidney L-aromatic amino acid decarboxylase was prepared as reported by Waymire *et al.* (15). The other cofactor, 2-amino-4-hydroxy-6-methyltetrahydropteridine, was synthesized according to Storm *et al.* (16); the product was reduced according to Pohland *et al.* (17). The concentration of reduced 6MPH$_4$[1] was determined by measuring ultraviolet absorption at 265 nm in 0.1 N HCl.

Male Sprague-Dawley rats (Zivic Miller, Allison Park, Pa.) weighing 125–150 g were housed in our quarters for at least 5 days at 24°; each light period of 14 hr being followed by 10 hr of darkness. The animals were allowed free access to water and standard laboratory chow. All drugs were injected intraperitoneally, and the animals were killed by decapitation. Striatum, brain stem, hypothalamus, and adrenal glands were rapidly dissected (18) and immediately frozen on Dry Ice. Tyrosine hydroxylase activity was measured within 2 hr. We found that the activity and kinetic properties of the striatal enzyme were the same when tissues were either homogenized immediately after dissection or were kept frozen for 2 hr before homogenization. Striata or other brain parts were weighed and then homogenized in 5

[1] The abbreviations used are: 6MPH$_4$, 2-amino-4-hydroxy-6-methyltetrahydropteridine; DMPH$_4$, 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine.

volumes of 0.05 M Tris-acetate buffer, pH 6, containing 0.2% Triton X-100. Homogenates were centrifuged for 10 min at 9000 × *g* (4°), and the tyrosine hydroxylase activity of the supernatant fraction was measured. A pair of adrenals were homogenized in 0.5 ml of 0.05 M Tris-acetate buffer, pH 6, containing 0.2% Triton X-100, and the homogenate was dialyzed for 4 hr against the same buffer. The tyrosine hydroxylase activity of adrenal glands was determined in an aliquot of the crude dialyzed homogenate.

Tyrosine hydroxylase activity was measured according to Waymire *et al.* (15). The standard assay mixture (110 μl) contained 20 mM Tris-acetate buffer (pH 6), 90 mM potassium phosphate buffer (pH 6.2), 10 mM sodium phosphate buffer (pH 7), 0.5 mM $DMPH_4$, 0.2 mM NADPH, 0.25 mM pyridoxal 5-phosphate, 7 units of hog kidney L-aromatic amino acid decarboxylase, 1000 units of catalase, 10 μl of sheep liver pteridine reductase (125 μg of protein), and 50 μl of the tissue preparation containing 0.15–0.4 mg of protein. In some experiments $6MPH_4$ (0.16 mM) was substituted for $DMPH_4$. The pH at 37° of the assay mixture containing the tissue homogenates of 0.9% NaCl- or drug-treated rats was 6.2. The enzymatic catalysis was initiated by addition of 10 μl of [1-^{14}C]L-tyrosine (specific activity, 12 mCi/mmole) to reach a final substrate concentration of 0.1 mM.

The incubation was carried out at 37° for 30 min. Under these assay conditions the enzymatic reaction was linear with time up to 45 min and proportional to the amount of protein between 0.05 and 0.5 mg. To ascertain that the results obtained were due to an interaction of the drug tested with L-aromatic amino acid decarboxylase, the experiments were replicated using the assay procedure described by Nagatsu *et al.* (19), which does not require the addition of this enzyme. In these experiments the standard assay mixture was identical with that described above except that [1-^{14}C]L-tyrosine was substituted for [3,5-^{3}H]L-tyrosine and pyridoxal 5-phosphate and L-aromatic amino acid decarboxylase were omitted. The tissue concentration of dopamine was assayed by the method of Neff *et al.* (20); proteins were measured according to Lowry *et al.* (21). The kinetic properties of striatal tyrosine hydroxylase with respect to tyrosine or the pteridine cofactors ($DMPH_4$ or $6MPH_4$) were analyzed statistically by the method of Cleland (22) and Worcel *et al.* (23). The statistical significance of the results was determined by Student's *t*-test (two-tailed). Unbiased estimates of the standard errors of the means were computed, and the means were compared as unpaired observations with equal variances.

RESULTS

Tyrosine hydroxylase activity measured in the presence of 0.5 mM $DMPH_4$ was dramatically increased in striata of rats 1 hr after an intraperitoneal injection of methiothepin (10 μmoles/kg), haloperidol (24 μmoles/kg), or pimozide (11 μmoles/kg) (Table 1). A similar increase of activity was also observed 2 hr after an intraperitoneal injection of reserpine (8 μmoles/kg). In rats receiving these drugs the tyrosine hydroxylase activity of brain stem, hypothalamus, and adrenal gland (Table 1) remained unchanged. Results similar to those reported in Table 1 were obtained when the enzymatic activity of various tissues was assayed according to Nagatsu *et al.* (19).

The activity of striatal tyrosine hydroxylase, measured in the presence of 0.5 mM $DMPH_4$, increased 30 min after the injection of methiothepin (10 μmoles/kg). This increase persisted for at least 4 hr but was no longer evident at 12 hr after the injection (Fig. 1). In rats receiving reserpine (8 μmoles/kg) the striatal tyrosine hydroxylase activity increased for more than 24 hr and approached the basal value at 72 hr (Fig. 1). In rats receiving methiothepin (10 μmoles/kg) or reserpine (8 μmoles/kg), the time courses of the increases in striatal tyrosine hydroxylase activity and of ptosis are similar. Methiothepin and reserpine (1 nM–10 μM) failed to change the enzyme activity when added to homogenates of rat striatum. When various volumes of striatal homogenates from NaCl-, reserpine-, or methiothepin-treated rats were assayed separately or combined, the resulting enzymatic activity was always additive. Moreover, the enzyme activity of striatal homogenates

TABLE 1

Tyrosine hydroxylase activities of various brain parts and adrenal glands of rats receiving neuroleptics and reserpine

Methiothepin, haloperidol, or pimozide was injected 1 hr and reserpine 2 hr before the rats were killed. Tyrosine hydroxylase activity was measured using 0.5 mM $DMPH_4$ as a cofactor with the standard assay system (15). Each point represents the mean ± standard error of four animals. Similar results were obtained using the methods of Nagatsu *et al.* (19).

Treatment	Dose	Tyrosine hydroxylase activity			
		Striatum	Brain stem	Hypothalamus	Adrenal gland
	μmoles/kg, ip	*nmoles CO_2 formed/hr/mg protein*			*nmoles CO_2 formed/hr/gland*
NaCl		2.2 ± 0.11	0.67 ± 0.051	0.93 ± 0.060	4.2 ± 0.29
Methiothepin	10	6.0 ± 0.56[a]	0.76 ± 0.077	1.1 ± 0.10	5.2 ± 0.50
Haloperidol	24	6.1 ± 0.44[a]			4.8 ± 0.35
Pimozide	11	3.6 ± 0.22[a]			4.2 ± 0.17
Reserpine	8	6.3 ± 0.73[a]	0.65 ± 0.085	1.2 ± 0.13	4.3 ± 0.71

[a] $p < 0.01$ when compared with NaCl-treated rats.

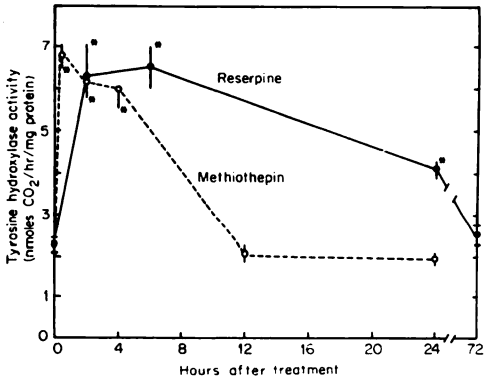

FIG. 1. *Activity of striatal tyrosine hydroxylase in rats receiving reserpine (8 μmoles/kg) or methiothepin (10 μmoles/kg)*

Each point represents the mean of four animals. Vertical bars indicate standard errors. The tyrosine hydroxylase activity of striatum of rats receiving the drug vehicle did not differ from that of untreated rats.

* $p < 0.01$ when compared with untreated rats.

from NaCl- and drug-treated rats declined linearly with successive dilutions.

The kinetic properties of striatal, brain stem, and hypothalamic tyrosine hydroxylase of rats receiving NaCl or haloperidol (24 μmoles/kg intraperitoneally) are compared in Table 2. The haloperidol injection decreased the apparent K_m of the striatal enzyme for $DMPH_4$ by 5-fold but failed to change the apparent K_m for $DMPH_4$ of brain stem and hypothalamic homogenates. The apparent V_{max} with respect to $DMPH_4$ remained unchanged in homogenates of tissues from rats receiving haloperidol. Haloperidol did not alter the K_m for tyrosine but increased the apparent V_{max} of striatal tyrosine hydroxylase when this kinetic parameter was measured in the presence of 0.5 mM $DMPH_4$; the apparent V_{max} of the enzyme in brain stem and hypothalamic homogenates was not changed by these doses of haloperidol. A dose of cycloheximide (70 μmoles/kg intraperitoneally) which inhibits the incorporation of labeled amino acid into brain proteins by about 90% (24) failed to reduce the increase in striatal tyrosine hydroxylase affinity for the cofactor elicited by reserpine or methiothepin.

Double-reciprocal plots were obtained at various concentrations of tyrosine (Fig. 2A) or $6MPH_4$ (Fig. 2B) against the initial velocity of dopa formation in striatal homogenates of rats receiving NaCl or reserpine (8 μmoles/kg intraperitoneally). Reserpine did not change the apparent K_m for tyrosine, but when the measurements were performed with 0.16 mM $6MPH_4$ it increased the apparent V_{max} of the enzyme from 8 ± 0.3 to 12 ± 0.6 nmoles of dopa per hour per milligram of protein ($p < 0.01$). In addition, reserpine reduced the apparent K_m for $6MPH_4$ about 4-fold. The V_{max} of the enzyme with respect to $6MPH_4$ was similar

TABLE 2

Kinetic properties of striatal, brain stem, and hypothalamic tyrosine hydroxylase in rats receiving NaCl or haloperidol

Haloperidol (25 μmoles/kg intraperitoneally) or NaCl was injected 1 hr before the enzyme assay. Kinetic constants with respect to tyrosine were determined using tyrosine concentrations of 0.006–0.2 mM at a $DMPH_4$ concentration of 0.5 mM. Kinetic constants with respect to $DMPH_4$ were determined using concentrations of $DMPH_4$ of 0.1–2 mM at a tyrosine concentration of 0.1 mM.

Substrate and treatment	K_m			V_{max}		
	Striatum	Brain stem	Hypothalamus	Striatum	Brain stem	Hypothalamus
	mM	*mM*	*mM*	*nmoles CO_2/hr/mg protein*		
$DMPH_4$						
NaCl	0.69 ± 0.16	0.47 ± 0.05	0.58 ± 0.06	7.5 ± 0.8	1.1 ± 0.1	2.5 ± 0.4
Haloperidol	0.13 ± 0.05[a]	0.40 ± 0.05	0.46 ± 0.07	6.1 ± 0.7	1.0 ± 0.2	2.4 ± 0.2
Tyrosine						
NaCl	0.054 ± 0.01	0.065 ± 0.01	0.061 ± 0.008	5.5 ± 0.6	1.0 ± 0.2	2.3 ± 0.2
Haloperidol	0.045 ± 0.01	0.065 ± 0.008	0.054 ± 0.01	9.2 ± 0.6[a]	1.1 ± 0.09	2.5 ± 0.3

[a] $p < 0.05$.

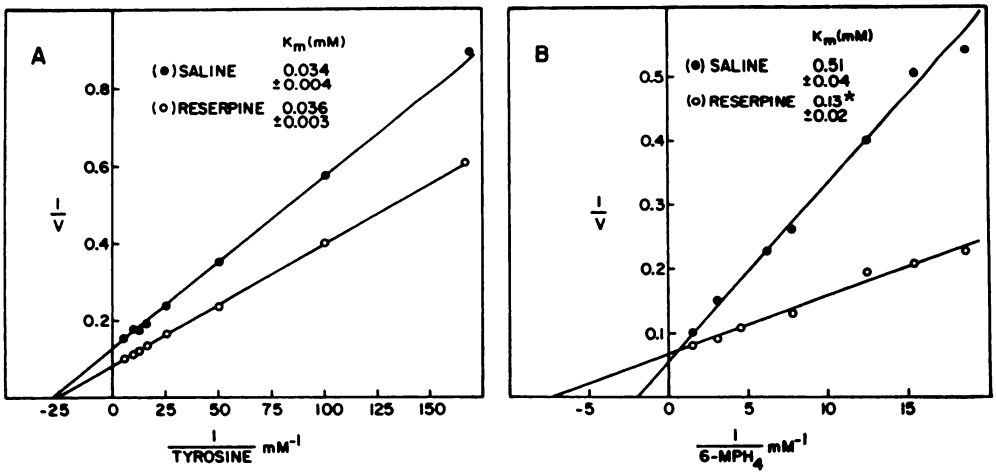

FIG. 2. *Effect of reserpine on the kinetics of striatal tyrosine hydroxylase.* A. Double-reciprocal plot of initial velocity of tyrosine hydroxylase against various tyrosine concentrations in the presence of $6MPH_4$ (0.16 mM). B. Double-reciprocal plot of initial velocity of tyrosine hydroxylase against various concentrations of $6MPH_4$ in the presence of tyrosine (0.1 mM). The kinetic properties of striatal homogenates of rats receiving NaCl (5 ml/kg intraperitoneally, are compared with those of rats receiving reserpine (8 μmoles/kg intraperitoneally) 2 hr before decapitation. Enzyme velocity is expressed as nanomoles of CO_2 formed per hour per milligram of protein.

* $p < 0.01$ when compared with NaCl-treated rats.

in striatal homogenates from animals treated with NaCl (18 ± 1 nmoles dopa/hr/mg of protein) or reserpine (16.5 ± 0.8 nmoles dopa/hr/mg of protein).

The effects of different concentrations of dopamine on the initial velocity of dopa formation catalyzed by striatal homogenates incubated with various concentrations of $6MPH_4$ are reported in Figs. 3 and 4. The data plotted in Fig 3 refer to striata of rats receiving NaCl, and those in Fig. 4, to striata from rats treated with reserpine (8 μmoles/kg intraperitoneally). The addition of various concentrations of dopamine to tyrosine

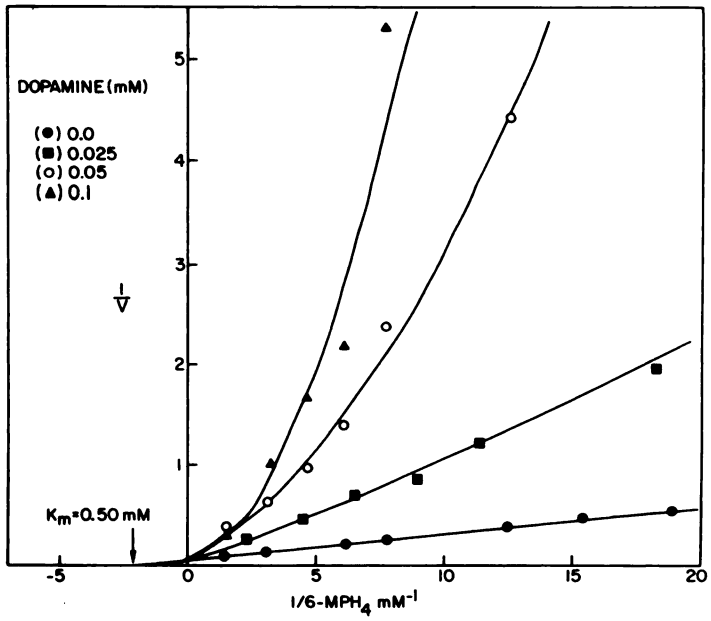

FIG. 3. *Effect of three concentrations of dopamine added to striatal tyrosine hydroxylase preparations from NaCl-treated rats on double-reciprocal plot of initial velocities vs. various concentrations of 6MPH$_4$*

The concentration of tyrosine in the assay was 0.1 mM. Velocity is expressed in nanomoles of CO_2 per hour per milligram of protein.

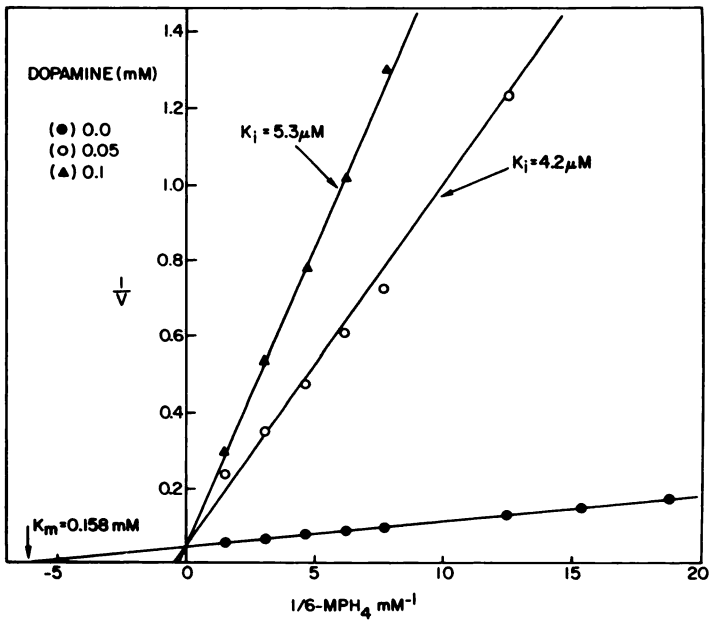

FIG. 4. *Effect of two concentrations of dopamine added to striatal tyrosine hydroxylase preparations from reserpine-treated rats on double-reciprocal plot of initial velocities vs. various concentrations of 6MPH$_4$*

Reserpine (8 μmoles/kg intraperitoneally) was injected 2 hr prior to death. The concentration of tyrosine in the assay was 0.1 mM. Enzyme velocity is expressed as nanomoles of CO_2 per hour per milligram of protein.

hydroxylase preparation of striata from NaCl-treated rats suggests that this amine competitively interferes with the $6MPH_4$. In the presence of various dopamine concentrations the double-reciprocal plots of the enzyme velocities vs. $6MPH_4$ concentration yielded parabolic lines (Fig. 3). Therefore the K_i of dopamine cannot be readily estimated. Hill plots of the data (25) yieled n_H values of 1.6 and 1.9 for dopamine concentrations of 0.05 mM and 0.1 mM, respectively. When various concentrations of dopamine were added to hydroxylase preparations from striata of reserpine-treated animals, this amine competitively inhibited $6MPH_4$. In the presence of various concentrations of dopamine the double-reciprocal plots of enzyme velocity vs. different concentrations of $6MPH_4$ followed classic Michaelis-Menten kinetics. Thus the K_i for dopamine can be calculated to be about 5 μM (Fig. 4).

The effect of apomorphine (32 μmoles/kg intraperitoneally) on striatal tyrosine hydroxylase activity of rats treated with NaCl, reserpine, or methiothepin is shown in Fig. 5. The enzyme activity was measured in the presence of an unsaturating concentration (0.5 mM) of $DMPH_4$. Apomorphine injected 90 min after reserpine (8 μmoles/kg intraperitoneally) significantly reduced enzyme activity. When apomorphine (32 μmoles/kg) was injected 5 min before methiothepin (10 μmoles/kg) the increase in enzyme activity elicited by the neuroleptic was prevented. Fig. 5 also shows that the injection of apomorphine neither changed the dopamine concentration in striatum nor altered its hydroxylase activity. Similar results were obtained with trivastal (70 μmoles/kg intraperitoneally), another dopamine receptor stimulant (26, 27).

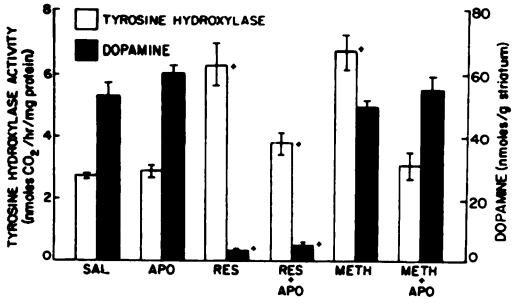

FIG. 5. *Striatal tyrosine hydroxylase and concentrations of striatal dopamine in rats receiving intraperitoneal doses of apomorphine (APO) (32 μmoles/kg) 90 min after reserpine (RES) (8 μmoles/kg) or NaCl (SAL) (5 ml/kg) and 5 min before methiothepin (METH) (10 μmoles/kg)*

The rats were killed 30 min after the injection of apomorphine. Each column refers to the mean of four animals. Brackets indicate standard errors.

* $p < 0.01$ when compared with NaCl-treated rats.

DISCUSSION

The turnover rate of striatal dopamine increases when the firing rate of dopaminergic neurons is accelerated (28, 29). Perhaps this prompt change of dopamine turnover rate is possible because product inhibition continually depresses tyrosine hydroxylase activity. This depression is reduced when the utilization of the transmitter is increased (1–3). Another modulation of catecholamine synthesis depends on the trans-synaptic control of tyrosine hydroxylase activity (30), which regulates the synthesis of enzyme molecules (31). This long-term regulation is operative in adrenal medulla and in noradrenergic axons but has not yet been described in central dopaminergic axons.

The experiments reported in this paper suggest regulation of striatal tyrosine hydroxylase by changes in the affinity of the enzyme for the pteridine cofactor. A single intraperitoneal injection of methiothepin, haloperidol, pimozide, or reserpine produced a decrease in the K_m of striatal tyrosine hydroxylase for the pteridine cofactor in about 30 min. These drugs failed to change the apparent V_{max} of the enzyme. Thus when the enzyme was tested *in vitro* in the presence of unsaturating concentrations of pteridine cofactors its activity was increased. This increase was observed in striatum but not in brain stem, hypothalamus, or adrenal glands of rats treated with the neuroleptics mentioned above. A similar increase in the affinity of tyrosine hydroxylase for pteridines was obtained *in vitro* by the addition of heparin to the soluble form of the brain enzyme (32, 33). Since soluble tyrosine hydroxylase in the presence of heparin exhibited the same kinetic properties as the membrane-bound enzyme (32–34), it was postulated that changes of the enzyme binding to membrane components may regulate its activity *in vivo* (32, 34). An identity be-

tween the enzymatic changes produced *in vitro* by heparin and those produced *in vivo* by the drugs discussed in the present report cannot be established, because in our experiments the measurement was performed on enzyme solubilized by Triton X-100.

Since end product inhibition may regulate tyrosine hydroxylase activity *in vivo* (1–3), we studied the kinetics of dopamine inhibition with the enzyme prepared from rats treated with neuroleptics. The addition of various dopamine concentrations to enzyme preparation from striata of NaCl-treated rats exhibited competitive inhibition against $6MPH_4$, but the double-reciprocal plots of the initial velocity of dopa formation against the concentrations of $6MPH_4$ yielded parabolic curves. However, reserpine injections changed the inhibitory patterns of dopamine with respect to $6MPH_4$ from sigmoidal to classical hyperbolic kinetics. Our observation that reserpine injections changed the kinetics of inhibition of striatal tyrosine hydroxylase by dopamine is similar to a report by Kuczenski and Mandell (32) concerning the effects of heparin on the inhibition of the soluble enzyme by dopamine. Our results suggest that the inhibitory effects of dopamine are not decreased in conjunction with the affinity change of enzyme for the cofactor. Thus neuroleptics increased the synthesis of dopamine but did not impair its control by product inhibition. It is tempting to speculate that the increased affinity of tyrosine hydroxylase for the cofactor is related to the increased firing rate of dopaminergic neurons elicited by the drugs we tested. This possibility is suggested by our observation that the increased affinity of the enzyme for the cofactor induced by neuroleptics was reversed by injections of apomorphine and trivastal. These drugs reduced the increased firing rates of dopaminergic neurons elicited by neuroleptics (35).

REFERENCES

1. Neff, N. H. & Costa, E. (1966) *Life Sci.*, **5**, 951–959.
2. Spector, S., Gordon, R., Sjoerdsma, A. & Udenfriend, S. (1967) *Mol. Pharmacol.*, **3**, 549–555.
3. Neff, N. H. & Costa, E. (1968) *J. Pharmacol. Exp. Ther.*, **160**, 40–47.
4. Carlsson, A. & Lindqvist, M. (1963) *Acta Pharmacol. Toxicol.*, **20**, 140–144.
5. Neff, N. H. & Costa, E. (1967) *Proc. Int. Symp. Antidepressant Drugs*, **122**, 28–34.
6. Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T. & Atack, C. (1972) *J. Pharm. Pharmacol.*, **24**, 744–747.
7. Bloom, F. E., Costa, E. & Salmoiraghi, G. C. (1965) *J. Pharmacol. Exp. Ther.*, **150**, 244–252.
8. Aghajanian, G. K. & Bunney, B. S. (1973) in *Frontiers in Catecholamine Research* (Usdin, E. & Snyder, S., eds.), pp. 643–648, Pergamon Press, London.
9. Anden, N.-E., Butcher, S. G., Corrodi, H., Fuxe, K. & Ungerstedt, U. (1970) *Eur. J. Pharmacol.*, **11**, 303–314.
10. Nyback, H. & Sedvall, G. (1969) *Eur. J. Pharmacol.*, **5**, 245–252.
11. Lloyd, K. G. & Bartholini, G. (1974) *Adv. Biochem. Psychopharmacol.*, **10**, 305–310.
12. Bunney, B. S., Walters, J. R., Roth, R. H. & Aghajanian, G. K. (1973) *J. Pharmacol. Exp. Ther.*, **185**, 560–571.
13. Kebabian, J. W., Petzold, G. L. & Greengard, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 2145–2149.
14. Kaufman, S. (1962) *Methods Enzymol.*, **5**, 809–819.
15. Waymire, J. C., Bjur, R. & Weiner, N. (1971) *Anal. Biochem.*, **43**, 588–600.
16. Storm, C. B., Shiman, R. & Kaufman, S. (1971) *J. Org. Chem.*, **36**, 3925–3927.
17. Pohland, A., Flynn, E. H., Jones, R. G. & Shive, W. (1951) *J. Am. Chem. Soc.*, **73**, 3247–3252.
18. Glowinski, J. & Iversen, L. L. (1966) *J. Neurochem.*, **13**, 655–669.
19. Nagatsu, T., Levitt, M. & Udenfriend, S. (1964) *Anal. Biochem.*, **9**, 122–126.
20. Neff, N. H., Spano, P. F., Groppetti, A., Wang, C. T. & Costa, E. (1971) *J. Pharmacol. Exp. Ther.*, **176**, 701–710.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
22. Cleland, W. W. (1963) *Nature*, **198**, 463–465.
23. Worcel, A., Goldman, D. S. & Cleland, W. W. (1965) *J. Biol. Chem.*, **240**, 3399–3407.
24. Grahame-Smith, D. G. (1972) *J. Neurochem.*, **19**, 2409–2422.
25. Atkinson, D. E., Hathaway, J. A. & Smith, E. C. (1965) *J. Biol. Chem.*, **240**, 2682–2690.
26. Corrodi, H., Fuxe, K. & Ungerstedt, U. (1971) *J. Pharm. Pharmacol.*, **23**, 989–991.
27. Goldstein, M., Anagnoste, B. & Shirron, C. (1973) *J. Pharm. Pharmacol.*, **25**, 348–351.

28. Roth, R. H., Walters, J. R. & Aghajanian, G. K. (1973) in *Frontiers in Catecholamine Research* (Usdin, E. & Snyder, S., eds.), pp. 567–574, Pergamon Press, London.
29. Costa, E. & Neff, N. H. (1970) in *Handbook of Neurochemistry*, Vol. 4 (Lajtha, A., ed.), pp. 45–80, Plemum Press, New York.
30. Thoenen, H., Mueller, R. A. & Axelrod, J. (1969) *J. Pharmacol. Exp. Ther.*, **169**, 249–254.
31. Joh, T. H., Geghman, C. & Reis, D. J. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, **70**, 2767–2771.
32. Kuczenski, R. T. & Mandell, A. J. (1972) *J. Biol. Chem.*, **247**, 3114–3122.
33. Kuczenski, R. T. & Mandell, A. J. (1972) *J. Neurochem.*, **19**, 131–137.
34. Kuczenski, R. (1973) *Life Sci.*, **13**, 247–255.
35. Bunney, B. S., Aghajanian, G. K. & Roth, R. H. (1973) *Nat. New Biol.*, **245**, 123–125.