MOLECULAR PHARMACOLOGY, 21:114–120

The Autoreceptor Control of Dopamine Synthesis

An *in Vitro* and *in Vivo* Comparison of Dopamine Agonists

D. R. HAUBRICH[1] AND A. B. PFLUEGER

Neuropsychopharmacology Section, Department of Pharmacology, Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486

Received July 20, 1981; Accepted September 15, 1981

SUMMARY

Regulation of the rate of dopamine synthesis was studied using dopamine receptor agonists in two biochemical models of the dopamine autoreceptor. An *in vivo* model was used to measure the ability of dopamine receptor agonists to antagonize the increase in striatal tyrosine hydroxylase activity that occurs when rats are treated with γ-butyrolactone (GBL) to diminish the rate of dopamine release. The following compounds were active in this model (in order of potency): *N-n*-propylnorapomorphine > pergolide > apomorphine > lergotrile> 3,3′-[(propylimino)di-2,1-ethanediyl]bis[phenol] (RU-24926) > bromocriptine > 9,10-didehydro-6-methyl-3β-(2-pyridylthiomethyl)ergoline (CF 25-397) > *N-n*-propyl-3-(hydroxyphenyl)-piperidine (3-PPP). The duration of activity of these compounds ranged from less than 4 hr for the aporphines and 3-PPP to up to 8 hr for some of the ergots. The ability of all eight compounds to reverse the GBL-induced stimulation of tyrosine hydroxylase activity was antagonized by pretreatment with haloperidol. Administration of the dopamine agonists, 3,4-dihydroxyphenylamino-2-imidazoline or 2-amino-6,7-dibenzoyloxy-1,2,3,4-tetrahydronaphthalene (ADTN ester), had little or no effect in the GBL model of the dopamine autoreceptor. An *in vitro* model was used to assess the ability of dopamine agonists to inhibit the rate of hydroxylation of [^{3}H] tyrosine by synaptosomes from the corpus striatum of rat brain. In this synaptosomal model, the rate of hydroxylation of tyrosine was inhibited by low concentrations (IC_{50} values < 1 μM) of the following dopamine receptor agonists (in order of potency): 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) > RU-24926 ≃ *N-n*-propylnorapomorphine > apomorphine. Haloperidol reversed the inhibitory effect *in vitro* of apomorphine and *N-n*-propylnorapomorphine, but failed to antagonize the inhibitory actions of ADTN or RU-24926, suggesting that the latter two compounds inhibit synaptosomal tyrosine hydroxylase activity by a mechanism that is not linked to a dopamine receptor located upon the nerve membrane. 3-PPP and the ergoline-type dopamine receptor agonists did not appreciably inhibit the rate of synaptosomal tyrosine hydroxylation. Furthermore, the ergolines failed to reverse the inhibitory effect of apomorphine, indicating that such compounds lack affinity for the receptor that controls the rate of dopamine synthesis in synaptosomes. These results suggest that more than one type of autoreceptor regulates the rate of synthesis of dopamine.

INTRODUCTION

The existence of receptors for dopamine and dopaminergic compounds located presynaptically ("autoreceptors") is now widely accepted (for reviews, see refs. 1 and 2), and can explain the paradoxical findings that dopaminergic agonists (a) inhibit the firing rate of nigrostriatal neurons (3), (b) cause a decrease in motor activity of animals (4), (c) improve the symptoms of schizophrenia in humans (5), and (d) inhibit the rate of release of dopamine *in vitro* (6).

The rate of synthesis of dopamine is also controlled by dopamine receptors. For example, dopamine and dopamine receptor agonists have been shown to inhibit the rate of conversion of [^{3}H]tyrosine to [^{3}H]dopamine in synaptosomes or slices prepared from the corpus striatum (7–9). Inhibition of the rate of dopamine synthesis also occurs *in vivo* when rats are treated with compounds that stimulate dopamine receptors (10). Although this inhibition *in vivo* of the dopamine synthesis rate could result from a postsynaptic action mediated via neuronal feedback mechanisms, studies have shown that administration of dopamine agonists counteracts the increases in dopamine synthesis rate that occur when impulse flow

[1] Present address, Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

0026-895X/82/010114-07$02.00/0

through the pathway is blocked either pharmacologically (e.g., by administration of GBL[2]) or by placement of a lesion in the nigrostriatal pathway (7, 11). Because the neuronal feedback process presumably cannot operate under conditions of reduced impulse flow, Roth and coworkers (11–13) have proposed that dopamine agonists counteract the increase in the dopamine synthesis rate induced by these procedures through activation of dopamine receptors located presynaptically.

The objective of the present study was to evaluate the pharmacology of the dopamine receptor(s) that control the rate of dopamine synthesis. Compounds representing several chemical classes, but all of which are known to have dopamine agonist activity in various other systems, were evaluated in the *in vivo* model of the dopamine autoreceptor using GBL to inhibit nerve impulse flow, and in the presumably analogous *in vitro* model using synaptosomes that synthesize [^{3}H]dopamine from [^{3}H] tyrosine.

METHODS

Male Sprague-Dawley rats weighing 180–250 g were used. In the *in vivo* experiments, rats were treated with GBL (750 mg/kg i.p.; Sigma Chemical Company, St. Louis, Mo.). Five minutes later, NSD-1015 (Sigma Chemical Company) was administered i.p. to inhibit aromatic L-amino acid decarboxylase (EC 4.1.1.28). The rate of accumulation of dopa was used as an index of the activity of tyrosine hydroxylase. Dopa was measured in paired corpora striata from a single rat using the fluorometric method of Kehr *et al.* (7). All treatments and assays were performed using a random design protocol.

For the *in vitro* experiments, a crude synaptosomal fraction (P_2) was prepared at 4° by homogenization of the corpus striatum in 9 volumes of 0.32 M sucrose. The homogenate was centrifuged at 1,000 × *g* (10 min) and the supernatant fluid was decanted and centrifuged again at 27,000 × *g* (20 min) to obtain the P_2 fraction, which was resuspended in oxygenated Krebs bicarbonate buffer. Synaptosomal tyrosine hydroxylase activity was measured essentially as described by Iversen *et al. (8).* L-[*ring*-2,6-^{3}H]Tyrosine (specific activity 37.4 Ci/mmole) was incubated with the tissue in 200 μl of Krebs bicarbonate buffer. Total radioactive products (dopa and dopamine) were isolated by ion exchange chromatography from unreacted [^{3}H]tyrosine and quantitated by liquid scintillation spectrometry. Average blank and control values were 2.1×10^4 and 3.1×10^5 dpm/mg of protein, respectively.

Uptake of [^{3}H]tyrosine or [^{3}H]dopamine (3,4-[*ethyl*-1-^{3}H(*N*)]; specific activity 31.8 Ci/mmole) was assessed by addition of the isotopes (10^{-7} M final concentration) to the P_2 suspension, incubation for 5 min, and harvesting of the pellets by centrifugation. Radioactivity taken up into the pellet was measured by liquid scintillation spectrometry (38% counting efficiency) in the presence of a tissue solubilizer and used as an index of uptake.

To measure soluble tyrosine hydroxylase activity, the corpora striata were homogenized in 0.2% Triton X-100 and centrifuged, then incubated with L-[*ring*-2,6-^{3}H]tyrosine (specific activity 37.4 Ci/mmole) as described by Nagatsu *et al.* (14). Dopa was isolated by means of alumina columns and measured by liquid scintillation spectrometry.

All isotopes were purchased from New England Nuclear Corporation (Boston, Mass.), and D,L-α-methyltyrosine from Aldrich Chemical Company (Milwaukee, Wisc.). The following compounds were generously donated by the companies indicated: *N-n*-propylnorapomorphine (Sterling-Winthrop, New York, N. Y.), pergolide and lergotrile (Eli Lilly and Company, Indianapolis, Ind.), RU-24926 (Roussell-Uclaf, Romainville, France), CF 25-397 and bromocriptine (Sandoz Pharmaceuticals, East Hanover, N. J.), DPI (Boehringer-Ingelheim, Ingelheim, Federal Republic of Germany), sulpiride (Laboratoire Delagrange, Paris, France), metoclopramide (Lederle Laboratories, Wayne, N. J.), chlorprothixene (Hoffmann-La Roche, Nutley, N. J.) clozapine (Wander, Bern, Switzerland), nomifensin (Hoechst-Roussel Pharmaceuticals, Somerville, N. J.), and chlorpromazine (Smith Kline & French Laboratories, Philadelphia, Pa.). The following compounds were synthesized in the Medicinal Chemistry Department, Merck Sharp & Dohme Laboratories (Rahway, N. J.): ADTN, ADTN ester, 3-PPP, apomorphine, and tiapride. For *in vitro* experiments, the water-insoluble compounds were dissolved in DMSO to give a final concentration no higher than 0.05% DMSO, and values were compared with samples that contained the vehicle. A concentration of 0.05% DMSO had no effect on the assay. For *in vivo* experiments, water-insoluble compounds were suspended in 1% methylcellulose and rats were treated with the vehicle.

RESULTS

In vivo assay of dopamine autoreceptors (GBL technique). The optimal dose of NSD-1015 and the optimal time for measurement of dopa accumulation were determined. As shown in Fig. 1, maximal elevation of striatal dopa occurred when a dose of NSD-1015 between 100 and 200 mg/kg i.p. was administered, and the peak increase occurred 30 min after treatment. No significant fluorescence could be detected in brains of rats not treated with NSD-1015. In all subsequent experiments, rats were given 100 mg/kg of NSD-1015 and were killed 30 min later.

Administration of GBL (750 mg/kg i.p.) to rats 5 min before NSD-1015 accelerated the rate of accumulation of dopa by more than 3-fold in the corpus striatum. In 33 experiments performed over a 10-month period, the average rates of accumulation of dopa were 12.6 ± 2.4 (SD) nmoles/g·hr (N = 122) in control rats and 42.0 ± 7.4 (SD) nmoles/g·hr (N = 177) in rats treated with GBL.

The increase in the rate of synthesis of dopamine induced by GBL was inhibited by pretreatment with dopamine agonists. Figure 2 shows the duration of action

[2] The abbreviations used are: GBL, γ-butyrolactone; NSD-1015, *m*-hydroxybenzylhydrazine dihydrochloride; dopa, 3,4-dihydroxyphenylalanine; RU-24926, 3,3′-[(propylimino)di-2,1-ethanediyl]bis[phenol]; CF 25-397, 9,10-didehydro-6-methyl-8β-(2-pyridylthiomethyl) ergoline; DPI, 3,4-dihydroxyphenylamino-2-imidazoline; ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; ADTN ester, 2-amino-6,7-dibenzoyloxy-1,2,3,4-tetrahydronaphthalene; 3-PPP, *N-n*-propyl-3-(hydroxyphenyl)piperidine; DMSO, dimethyl sulfoxide; HVA, homovanillic acid.

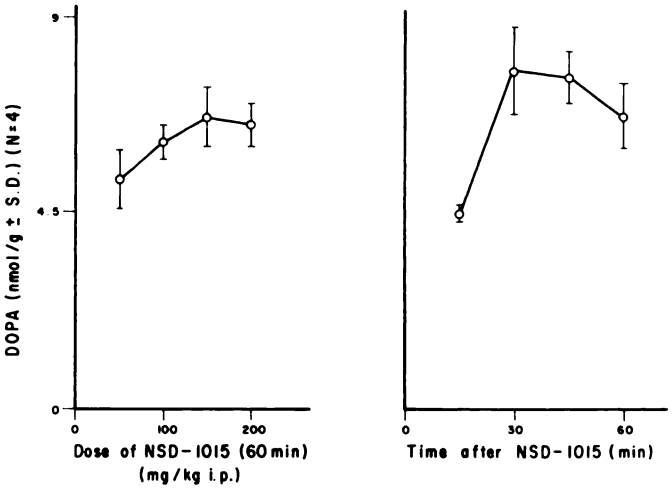

FIG. 1. *Concentration of dopa in rat corpus striatum plotted as a function of the dose of NSD-1015 (left) or the time after administration of NSD-1015, 100 mg/kg (right)*

Values are averages of results from four animals.

of eight dopamine agonists administered in doses that induced between 51% and 64% inhibition of the GBL-induced increase in the rate of dopa accumulation when given 5 min before GBL. As may be seen (Fig. 2), the duration of action of the aporphine-type compounds was relatively short, lasting for less than 2 hr, whereas the activity of the ergot derivatives persisted for at least 4 hr after treatment. Furthermore, the magnitude of the inhibitory action of pergolide and bromocriptine increased with time after treatment. There was a wide range of potencies in the compounds studied, with *N-n*-propylnorapomorphine displaying more than 1000 times the potency of 3-PPP in reversing the effect of GBL.

Two compounds known to have dopamine agonist activity in other systems, the ester of ADTN (15) and DPI (16) were not active in the *in vivo* assay for dopamine autoreceptor activity at nonlethal doses (Table 1).

In order to determine whether the effect of the dopamine agonists was mediated via an action at a dopamine receptor, rats were pretreated with haloperidol prior to administration of the dopamine receptor agonists. As shown in Fig. 3, haloperidol pretreatment reversed the inhibitory action of all eight active compounds. Haloperidol administered alone enhanced the rate of accumulation of dopa induced by GBL administration by 18% [GBL alone = 42.2 ± 4.2 nmoles/g·hr ($N = 9$); GBL plus haloperidol = 49.8 ± 9.2 nmoles/g·hr ($N = 9$); $p < 0.01$].

In vitro assay of dopamine autoreceptors. The synaptosomal tyrosine hydroxylase assay was evaluated and found to be linear with respect to tissue concentration with up to 1.25 mg/ml and with time for up to 60 min (Fig. 4).

As shown in Fig. 5, addition of *N-n*-propylnorapomorphine or apomorphine to the incubation mixture resulted in marked inhibition of the reaction, and this was reversed by preincubation for 5 min[3] of the samples with

[3] Studies of the binding of haloperidol to receptors have shown that equilibrium is reached within this time (17).

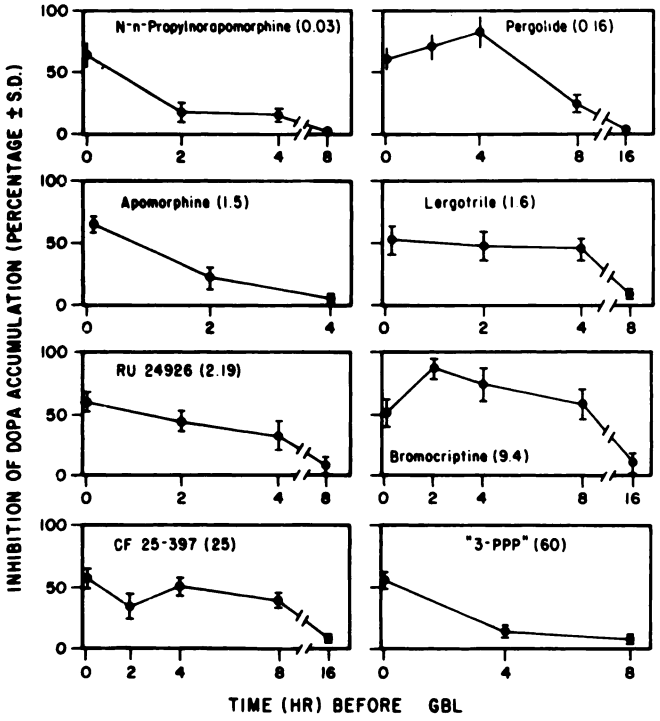

FIG. 2. *Duration of action of dopamine agonists as inhibitors of the GBL-induced increase in the rate of dopa accumulation*

Agonists were administered at various times before GBL. NSD-1015 was given 5 min after GBL, and rats were killed 30 min later. Each point is the average of results from 6–12 rats. Doses shown in parentheses are micromoles per kilogram (i.p.).

haloperidol, as indicated by the shift to the right in the dose-response curve. Other dopamine receptor-blocking drugs also reversed the inhibitory action of apomorphine on the rate of synaptosomal tyrosine hydroxylation. As shown in Table 2, chlorprothixene was the most potent, and metoclopramide the weakest.

ADTN and RU-24926 also inhibited the rate of synaptosomal tyrosine hydroxylation, as shown in Fig. 6. However, in contrast to the inhibitory action of the aporphines (Fig. 5), the inhibition induced by ADTN or RU-24926 was not reversed by haloperidol (Fig. 6).

Unlike the aporphines, the ergot-type dopamine agonists did not inhibit the rate of synaptosomal tyrosine hydroxylation more than 50% at concentrations less than 100 μM. These results are illustrated in Fig. 7, which also

TABLE 1

Effect of DPI or ADTN ester on the GBL-induced increase in dopamine synthesis rate in vivo

Treatment	Dose	Pretreatment time[a]	Lethality	Inhibition of dopa accumulation	
				% ± SD	N
	μmoles/kg i.p.	*min*	*dead/total*		
DPI	3	5	0/6	0	
	10	5	2/6	22 ± 22	4
	30	5	2/6	11 ± 18	4
	100	5	3/6	27 ± 16	3
ADTN ester	200	55	0/6	5 ± 6	6
	400	55	0/5	12 ± 9	5
	400	115	3/6	33 ± 19	3
	400	235	4/6	0	2

[a] Time interval before administration of GBL.

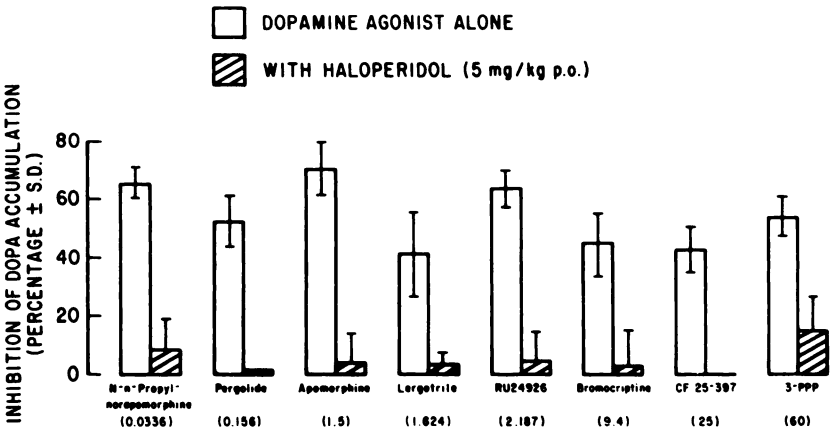

FIG. 3. *Reversal by haloperidol of the inhibitory effect of dopamine agonists on the GBL-induced increase in the rate of dopa accumulation*
Values are averages of results from six rats. Haloperidol (5 mg/kg p.o.) was administered 1 hr before the dopamine agonists, which were administered either 10 min (apomorphine, *N-n*-propylnorapomorphine, and 3-PPP) or 1 hr (other compounds) before GBL.

shows the relatively weak activity of 3-PPP in this system. In order to determine whether the ergolines bind as antagonists to the receptor that controls the rate of dopamine synthesis *in vitro*, lergotrile and bromocriptine were tested for ability to reverse the effect of apomorphine but, as shown in Table 2, neither compound was active when tested. Other compounds tested and found to be inactive (<50% inhibition at 10 μM) as inhibitors of synaptosomal tyrosine hydroxylation include adrenergic agonists (isoproterenol, amphetamine, methylphenidate, and clonidine), amino acids (γ-aminobutyric acid, muscimol, taurine, glycine, aspartate), purines (2-chloroadenosine, dibutyryl cyclic AMP), and cholinomimetics (carbachol, arecoline).

In an effort to assess other possible actions of dopamine receptor agonists that could account for their ability to inhibit synaptosomal tyrosine hydroxylation, some of the compounds were tested for inhibition of other metabolic processes for dopamine. As shown in Table 3, none of the compounds was as active an inhibitor of soluble tyrosine hydroxylase as α-methyltyrosine, although apomorphine and ADTN did exert a weak inhibitory action on the soluble enzyme at high concentrations. ADTN and RU-24926 inhibited the uptake of [^{3}H]dopamine, with potencies nearly equal to that of nomifensin (Table 3). 3-PPP also inhibited uptake of dopamine but was less potent than ADTN or RU-24926 (Table 3). None of the compounds tested inhibited accumulation of [^{3}H]tyrosine by synaptosomes (Table 3).

DISCUSSION

The results of this study show that dopamine receptor agonists from four different chemical classes [i.e., aporphine, ergots, a piperidine (3-PPP), and a tertiary amine (RU-24926)] reverse the increase in dopamine synthesis rate that occurs in the corpus striatum of rats treated with GBL. This finding extends the findings of other investigators who have obtained similar results with apomorphine (13), lergotrile (18), bromocriptine (19), and 3-PPP (20). In general, the duration of action of the ergot compounds was nearly twice that of either the aporphines or 3-PPP. The aporphines were the most potent class of

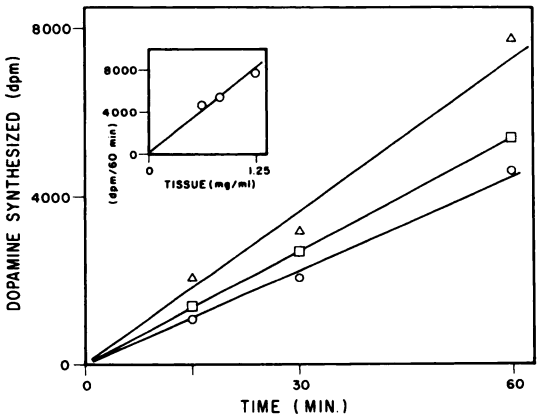

FIG. 4. *Synthesis of dopamine in striatal synaptosomes plotted as a function of time*
The concentrations of tissue (original wet weight in milligrams per milliliter during incubation) were 0.625 (○), 0.82 (□) and 1.25 (△). The *inset* shows the 60-min data plotted as a function of the tissue concentration. Each point is the average of four determinations.

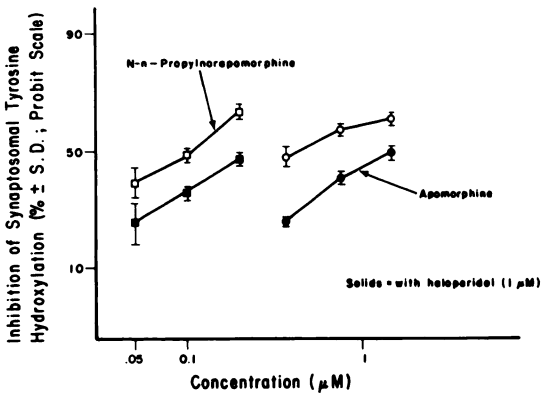

FIG. 5. *Inhibition of synaptosomal tyrosine hydroxylation by N-n-propylnorapomorphine* (□, ■) *and apomorphine* (○, ●)
The IC_{50} values (micromolar) with 95% confidence limits were 0.10 (0.07–0.14) for *N-n*-propylnorapomorphine and 0.42 (0.05–0.66) for apomorphine. The shift in the concentration-response curves induced by haloperidol (■, ●) was statistically significant ($p < 0.05$; *t*-test).

TABLE 2

Reversal of the apomorphine-induced inhibition of synaptosomal tyrosine hydroxylation

Apomorphine was added at a concentration of 1 μM. Values are averages of two or three samples, and were corrected for changes induced by the neuroleptic alone, which induced 21–38% inhibition. Variability was less than ±5%.

Compound	Concentration	% Reversal
	μM	
Chlorprothixene	1	89
Chlorpromazine	1	61
Tiapride	1	56
Sulpiride	1	52
Clozapine	1	24
Metoclopramide	1	22
Lergotrile	100	0
Bromocriptine	10	0

compounds, whereas 3-PPP was the least potent of the active compounds.[4] The inhibitory effect *in vivo* of these dopaminergics was prevented by pretreatment of rats with haloperidol, which indicates that the inhibitory effect was mediated by stimulation of a dopamine receptor.

The ester of ADTN was not active in reversing the increase in dopamine synthesis rate induced by administration of GBL. This finding was unexpected in view of the fact that ADTN is a potent dopaminergic agonist in both the *in vitro* adenylate cyclase system (21) and in the *in vivo* turning rat model when given intraventricularly (22). The ester of ADTN, when administered parenterally to rats, is hydrolyzed *in vivo* to ADTN, which accumulates in brain (23). Administration of ADTN has also been shown to reduce the concentration of HVA in the corpus striatum of rats and to attenuate the increase in concentration of HVA induced by administration of GBL (15). However, changes in the level of HVA may not accurately reflect changes in the rate of synthesis of dopamine and, like other catechols, ADTN may inhibit catechol *O*-methyltransferase to lower the level of HVA. The present results, in which the synthesis rate of dopamine was measured by a direct method (i.e., accumulation of dopa after decarboxylase inhibition) suggest that ADTN does not stimulate the receptor that controls the rate of dopamine synthesis *in vivo*. This suggestion is supported by the present *in vitro* results (see Fig. 6 and discussion below) and by results of other studies (8, 9) which show that the inhibitory effect of ADTN on dopamine synthesis rate *in vitro* is blocked by an inhibitor of dopamine uptake and therefore is not mediated by a dopamine receptor located upon the neuronal membrane.

DPI was also inactive as an inhibitor of the increase in dopamine synthesis rate induced by GBL, even though the compound is a potent agonist at the inhibitory dopamine receptors in the brains of the invertebrate *Helix aspersa* (16). Although the reason for the lack of an effect of DPI is not clear from the present results, it is possible that the compound does not adequately cross the blood-brain barrier or, if it does, is unable to stimulate dopamine receptors in mammalian brain.

[4] Differences in rates of absorption, distribution, and metabolism as well as affinity for the receptors may contribute to differences in potency.

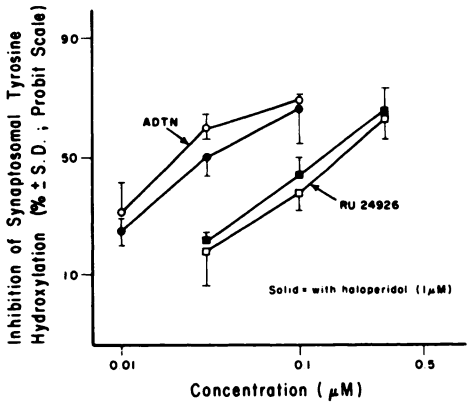

FIG. 6. *Inhibition of synaptosomal tyrosine hydroxylation by ADTN* (○, ●) *and RU-24926* (□, ■).

The IC_{50} values (micromolar) with 95% confidence limits were 0.04 (0.02–0.06) for ADTN and 0.13 (0.10–0.16) for RU-24926. Haloperidol (●, ■) did not significantly affect the IC_{50} values.

In the *in vitro* assay of the autoreceptor control of dopamine synthesis rate, only the aporphine-type dopamine agonists inhibited the rate of synaptosomal tyrosine hydroxylation by interaction with dopamine receptors on the presynaptic membrane, since these were the only compounds whose inhibitory effects were reversed by addition of haloperidol to the incubation medium (see Fig. 5). ADTN and RU-24926 did inhibit the reaction *in vitro*, but by a mechanism that was not reversible by haloperidol (see Fig. 6) and therefore did not involve a dopamine receptor located on the neuronal membrane. The mechanism by which ADTN and RU-24926 inhibit synaptosomal tyrosine hydroxylation was not determined in the present study. Neither compound appreciably inhibited the uptake of [^{3}H]tyrosine into synaptosomes. ADTN is a weak inhibitor of soluble tyrosine hydroxylase (8, 9) (Table 3) and has affinity for the dopamine transport system, as judged by its ability to block transport of [^{3}H]dopamine (24) (Table 3), and may, therefore, be taken up into synaptosomes of the corpus striatum and

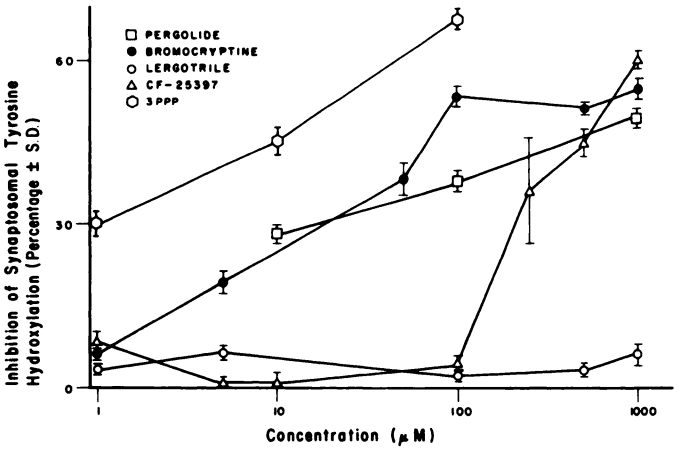

FIG. 7. *Effect of ergots and 3-PPP on synaptosomal tyrosine hydroxylation*

Values are averages of three or four determinations with standard deviations.

TABLE 3

Effects of dopamine receptor agonists on metabolic processes for dopamine

Compound	Concentration	Inhibition (% ± SD or IC_{50} with 95% CL)[a]		
		Soluble tyrosine hydroxylase	[^{3}H]Dopamine uptake	[^{3}H]Tyrosine uptake
	μM			
Reference agent				
D,L-α-methyltyrosine[b]	5	0		
	50	70 ± 22		
	100	89 ± 30	—	—
Nomifensin	—	—	IC_{50} = 0.1[c] (0.02–0.03)	—
Dopamine agonist				
N-*n*-propylnorapomorphine	10	—	32 ± 9	—
	50	—	77 ± 2	—
Apomorphine	10	—	9 ± 11	—
	50	26 ± 12	60 ± 29	20 ± 9
	100	34 ± 18	—	—
ADTN	0.5	—	IC_{50} = 0.13 (0.08–0.18)	18 ± 6
	10	22 ± 12		—
	50	36 ± 14		—
	100	26 ± 16		—
RU-24926	10	—	IC_{50} = 0.18 (0.007–0.95)	18 ± 11
	50	0		—
	100	0		—
3-PPP	—	—	IC_{50} = 1.6 (1.2–2.0)	—

[a] IC_{50} values were determined by regression analysis of 12–15 determinations at three or four concentrations. Other values are means of three or four determinations.

[b] IC_{50} in synaptosomal tyrosine hydroxylase assay was 1 μM.

[c] IC_{50} values are micromolar.

be sufficiently concentrated within dopaminergic neurons to inhibit directly the synthetic reaction. RU-24926 is also a relatively potent inhibitor of [^{3}H]dopamine uptake (Table 3) and may also be accumulated within dopamine nerve terminals, possibly to inhibit the rate of synthesis of dopamine by a mechanism not evaluated in the present studies (e.g., by inhibition of dopa decarboxylase or particulate tyrosine hydroxylase). Further studies are needed to determine precisely the mechanism of action of these compounds to inhibit the rate of dopamine synthesis.

The finding that all four ergot derivatives used in the present study were active in the *in vivo* model of the dopamine autoreceptor, but not in the *in vitro* model, agrees with results of Kebabian and Kebabian (25) showing that 10 μM lergotrile and lisuride do not inhibit synaptosomal tyrosine hydroxylation. One possible explanation for this paradoxical result is that these compounds are metabolically activated *in vivo*. Formation of an active metabolite of lergotrile has previously been suggested as a possible explanation for the finding that the parenteral administration of lergotrile stimulates postsynaptic dopamine receptors, whereas no such activity was observed when the compound was given directly into brain (26). Our results show a relatively slow onset of maximal activity of pergolide and bromocriptine *in vivo*, which is consistent with such an interpretation; however, this was not observed to occur with the other ergots, lergotrile and CF 25-397 (see Fig. 2). Furthermore, ergots are active either as dopamine agonists in other *in vitro* systems such as the rabbit retina (27) and hypothalamic cultures (28), or as antagonists in the apomorphine-stimulated adenylate cyclase system (29). Our results indicate that, in contrast to the adenylate cyclase system, ergots do not block apomorphine in the synaptosomal tyrosine hydroxylase system (see Table 2). Thus it appears that the *in vitro* regulation of dopamine synthesis rate is controlled by dopamine receptors to which the ergot-type dopaminergic compounds do not bind either as agonists or antagonists.

The poor correlation between the *in vivo* and *in vitro* activity of dopamine agonists at the receptor that controls the rate of dopamine synthesis may also be explained by the operation of neuronal circuitry in the intact brain that is not present in the *in vitro* system because of the drastic isolation procedures involved. The possibility of any influence of neuronal feedback on the regulation of dopaminergic neuronal activity is presumably excluded by treatment of rats with GBL. Nevertheless, as Nowycky and Roth (10) have pointed out, it may not be possible to exclude an effect of dopamine agonists on a trans-synaptic feedback mechanism, initiated by an effect of the dopamine agonists on postsynaptic receptors.

The results of the present study may also be interpreted to suggest that the rate of synthesis of dopamine is regulated by more than one class of receptor. The existence of several classes of dopamine receptors has already been inferred from the results of studies that used radioactive ligands to identify sites on the membrane with high affinity for dopamine agonists and antagonists (see ref. 17). In addition, it is known that the

susceptibility of tyrosine hydroxylase to inhibition by dopamine can be altered by changes in impulse flow through dopaminergic neurons (30), and it is tempting to speculate on the possibility that a normally quiescent class of dopamine autoreceptors is "unmasked" by treatments (e.g., GBL) that influence impulse flow through this pathway. The fact that the aporphines are active in both the synaptosomal tyrosine hydroxylase assay and in the clinic as dopamine autoreceptor agonists (5), whereas the ergots are not active either in the *in vitro* preparation or in humans (31) suggests that the synaptosomal tyrosine hydroxylase system may be a better predictor of potential clinical efficacy.

ACKNOWLEDGMENT

The authors are grateful to Dr. Neeti Bohidar, Biometrics Research, Merck Sharp & Dohme, for performing the statistical analysis and for advice in the random design of experiments.

REFERENCES

1. Usden, E., and W. E. Bunney. in *Pre- and Postsynaptic Receptors* (E. Usden and W. E. Bunney, Jr., eds.). Marcal Dekker, New York (1975).
2. Kebabian, J. W. and D. B. Calne. Multiple receptors for dopamine. *Nature (Lond.)* **277**:93–96 (1979).
3. Aghajanian, G. K., and B. S. Bunney. Dopamine autoreceptors: pharmacological characterization by microiontophoretic single cell recording studies. *Nauyn Schmeidebergs Arch. Pharmacol.* **297**:1–7 (1977).
4. Carlsson, A. Receptor-mediated control of dopamine metabolism, in *Pre- and Postsynaptic Receptors* (E. Usden and W. E. Bunney, Jr., eds.). Marcel Dekker, New York (1975).
5. Tamminga, C. A., M. H. Schaffer, R. C. Smith, and J. M. Davis. Schizophrenic symptoms improve with apomorphine. *Science (Wash. D. C.)* **200**:567–568 (1978).
6. Starke, K., W. Reimann, A. Zumstein, and G. Hertting. Effect of dopamine re-eptor agonists and antagonists on release of dopamine in the rat caudate nucleus in vitro. *Nauyn Schmeidebergs Arch. Pharmacol.* **305**:27–36 (1978).
7. Kehr, W., A. Carlsson, and M. Lindqvist. A method for the determination of 3,4-dihydroxyphenylalanine (DOPA) in brain. *Nauyn Schmeidebergs Arch. Pharmacol.* **274**:273–280 (1972).
8. Iversen, L. L., M. A. Rogawski, and R. J. Miller. Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic mechanisms in the rat striatum. *Mol. Pharmacol.* **12**:251–262 (1976).
9. Waggoner, W. G., J. McDermed, and H. J. Leighton. Presynaptic regulation of tyrosine hydroxylase activity in rat striatal synaptosomes by dopamine analogs. *Mol. Pharmacol.* **18**:91–99 (1980).
10. Nowycky, M. C., and R. H. Roth. Dopaminergic neurons: role of presynaptic receptors in the regulation of transmitter biosynthesis. *Prog. Neuropsychopharmacol.* **2**:139–158 (1978).
11. Walters, J. R., and R. H. Roth. Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow. *J. Pharmacol. Exp. Ther.* **191**:82–91 (1974).
12. Roth, R. H., J. R. Walters, L. C. Murrin, and V. H. Morganroth. Dopamine neurons: role of impulse flow and presynaptic receptors in the regulation of tyrosine hydroxylase, in *Pre- and Postsynaptic Receptors* (E. Usden and W. E. Bunney, Jr., eds.). Marcel Dekker, New York, 5–48 (1975).
13. Walters, J. R. and R. H. Roth. Dopaminergic neurons: and *in vivo* system for measuring drug interactions with presynaptic receptors. *Nauyn Schmeidebergs Arch. Pharmacol.* **296**:5 (1976).
14. Nagatsu, T., M. Levitt, and S. Udenfriend. Tyrosine hydroxylase, the initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **239**:2910–2917 (1964).
15. Horn, A. S., D. DeKaste, D. Dijkstra, H. Rollema, M. Feenstra, B. H. C. Westerink, C. Grol, and A. Westerink. A new dopaminergic prodrug. *Nature (Lond.)* **276**:405–407 (1978).
16. Stryker-Boudier, H. I. J., L. Teppema, P. Cools, and J. VanRossum. (3,4-Dihydroxyphenylamino)-2-imidazoline (DPI), a new potent agonist at dopamine receptors mediating neuronal inhibition. *J. Pharm. Pharmacol.* **27**: 882–883 (1975).
17. Titeler, M., and P. Seeman. In vitro measurement of brain receptors for dopamine and neuroleptics, in *The Neurobiology of Dopamine* (P. S. Horn, J. Korf, and B. H. C. Westerink, eds.). Academic Press, New York, 179–195 (1980).
18. Walters, J. R., J. M. Lakoski, M. D. Baring, and N. Eng. Dopamine neurons: effect of lergotrile on unit activity and transmitter synthesis. *Eur. J. Pharmacol.* **60**:199–210 (1979).
19. Marek, K. L., and R. H. Roth. Ergot alkaloids: interaction with presynaptic dopamine receptors in the neostriatum and olfactory tubercles. *Eur. J. Pharmacol.* **62**:137–146 (1980).
20. Hjorth, S., A. Carlsson, P. Lindberg, D. Sanchez, H. Wikström, L.-E. Arvidsson, U. Hacksell, J. L. G. Nilsson, and U. Svensson. New centrally acting DA-receptor agonist with selectivity for autoreceptors. Presented at the Eighteenth Annual Meeting, American College of Neuropsychopharmacology, San Juan, Puerto, Rico, December 12–14 (1979).
21. Miller, R., A. Horn, L. Iversen, and R. Pinder. Effects of dopamine-like drugs on rat striatal adenyl cyclase have implications for CNS dopamine receptor topography. *Nature (Lond.)* **250**:238–241 (1974).
22. Woodruff, G. N., A. O. Elkhawad, A. R. Grossman, and R. J. Walker. Further evidence for the stimulation of rat brain dopamine receptors by a cyclic analog of dopamine. *J. Pharm. Pharmacol.* **26**:740–741 (1974).
23. Horn, A. S., P. Kelly B. H. C. Westerink, and D. Dijkstra. A prodrug of ADTN: selectivity of dopaminergic action and brain levels of ADTN. *Eur. J. Pharmacol.* **60**:95–99 (1979).
24. Horn, A. S. The conformation of dopamine at its uptake site: further studies with rigid analogs. *J. Pharm. Pharmacol.* **26**:735–737 (1974).
25. Kebabian, J. W. and P. R. Kebabian. Lergotrile and lisuride: *in vivo* dopaminergic agonists which do not stimulate the presynaptic dopamine autoreceptor. *Life Sci.* **23**:2199–2204 (1978).
26. McDivitt, J. T., and P. E. Setler. Dopaminergic effects of lergotrile: possible involvement of a metabolite. *Neuropsychopharmacology* **19**:537–542 (1980).
27. Schorderet, M. Direct evidence for the stimulation of rabbit retina dopamine receptors by ergot alkaloids. *Neurosci. Lett.* **2**:87–91 (1976).
28. Pasteels, J. L., A. Danguy, M. Frérotte, and F. Ectors. Inhibition de la sécrétion de prolactine par l'ergo-cornire et la 2-bromo-α-ergoloryptine: action directe sur l'hypophyse en culture. *Ann Endocrinol. (Paris)* **32**:188–192 (1971).
29. Markstein, R., P. L. Herrling, H. R. Burki, H. Asper, and W. Ruch. The effect of bromocriptine on rat striatal adenylate cyclase and rat brain monoamine metabolism. *J. Neurochem.* **32**:1163–1172 (1978).
30. Morganroth, V. H., J. R. Walters, and R. H. Roth. Dopaminergic neurons—alteration in the kinetic properties of tyrosine hydroxylase after cessation of impulse flow. *Biochem. Pharmacol.* **25**:655–661 (1976).
31. Tamminga, C. A., and M. H. Schaffer. Treatment of schizophrenia with ergot derivatives. *Psychopharmacology* **66**:239–242 (1979).

Send reprint requests to: Mrs. A. B. Pflueger, Neuropsychopharmacology Section, Department of Pharmacology, Merck Institute for Therapeutic Research, West Point, Pa. 19486.