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Presynaptic Inhibition of Dopamine Synthesis in Rat Striatal Tissue by Enantiomeric Mono- and Dihydroxyaporphines

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SUMMARY

Presynaptic autoreceptor-mediated modulation of dopamine (DA) synthesis was evaluated as the inhibition of tyrosine hydroxylase activity by enantiomeric mono- and dihydroxyaporphines with minced striatal tissue from rat brain. The isomers of *N-n*-propylnorapomorphine (NPA) both inhibited tyrosine hydroxylase activity [IC_{50} = 0.3 and 1.0 μM for (*R*)-(−)- and (*S*)-(+)-NPA, respectively; *R/S* potency = 3.6]. Their effects were fully blocked by the nonselective DA receptor antagonist fluphenazine, as well as by the D_2-selective antagonist spiperone, but not by the D_1 antagonist SCH-23390. These results suggest a D_2-type autoreceptor-mediated inhibition of DA synthesis, with limited enantiomeric selectivity of this catechol-aporphine. The corresponding monohydroxy analogs, (*R*)-(−)- and (*S*)-(+)-11-hydroxy-*N-n*-propylnoraporphine (11-OH-NPa) were about 100 times less potent (IC_{50} = 42 and 87 μM, respectively) than the NPA isomers in fully inhibiting the enzyme activity in normal tissue but, after depletion of endogenous DA by acute *in vivo* pretreatment with reserpine (which did not alter the number of D_1 or D_2 specific binding sites), (*R*)-(−)-11-OH-NPa was a highly potent but partial agonist (IC_{25} = 7 nM). Fluphenazine and spiperone fully antagonized the inhibition of tyrosine hydroxylase by (*R*)-(−)-11-OH-NPa in reserpinized tissue, but SCH-23390 was ineffective. Actions mediated by endogenous DA probably contribute to the effect of high concentrations of (*R*)-(−)-11-OH-NPa to evoke a full inhibition of DA synthesis, but its high potency partial agonist effects appear to be mediated by D_2-autoreceptors. (*S*)-(+)-11-OH-NPa was a very weak partial agonist in reserpinized tissue, with an IC_{25} = 30 μM (essentially the same as normal tissue); thus, (*R*)-(−)-11-OH-NPa was >4,000 times more potent than its *S*-(+)-enantiomer in the absence of endogenous DA. These results demonstrate that NPA, which contains a catechol moiety, acts as a full agonist to inhibit striatal DA synthesis via a presynaptic autoreceptor of the D_2 type, with only slight stereoselectivity, and that its monohydroxy analog is a very potent but partial D_2 autoreceptor agonist, with very high stereoselectivity.

The phenomenon of presynaptic regulation of DA synthesis by dopaminergic "autoreceptors" was proposed in 1972 by Kehr *et al.* (1). There is compelling evidence based on both *in vitro* (2, 3) and *in vivo* (4–6) experiments that DA agonists can modulate DA synthesis via presynaptic receptor-mediated inhibition of tyrosine hydroxylase (EC 1.14.16.2), the rate-limiting step in the biosynthesis of DA (7), perhaps by increasing the K_m of the enzyme for its cofactor (8, 9). The classic DA agonist apomorphine (*R*)-(−)-APO (Fig. 1) and other aporphine analogs of DA have been used to characterize the stereospecific functional and biochemical pharmacology of dopaminergic receptors (10–18). The *N-n*-propyl analog of APO, NPA (Fig. 1), has been reported to be somewhat more potent than APO in behavioral models of dopaminergic activity (19), to show greater affinity and selectivity for D_2-type receptors (20), and to have much greater selectivity for DA autoreceptors than postsynaptic receptors (21). Aporphines are rigid molecules that structurally incorporate the *trans*-α-rotamer of DA (Fig. 1), the conformation believed to be active at central DA receptors (10, 22, 23). Furthermore, it should be noted that the aporphines possess a chiral center at the 6a-carbon (Fig. 1) and that absolute configuration is critically important for affinity and activity at DA receptors (10, 22, 23). The *R*-(−)-enantiomer of APO and of NPA is the preferred configuration in the behavioral (12, 17), neurophysiological (16, 17), and *in vivo* neurochemical (6) expression of central dopaminergic activities and shows enantiomeric selectivity at D_1 and especially at D_2 recep-

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ABBREVIATIONS: DA, dopamine; APO, apomorphine; NPA, *N-n*-propylnorapomorphine; DOPA, L-dihydroxyphenylalanine; GBL, γ-butyrolactone; 3-PPP, 3-(3-hydroxyphenyl)-*N-n*-propylpiperidine; 11-OH-NPa, 11-hydroxy-*N-n*-propylnoraporphine; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

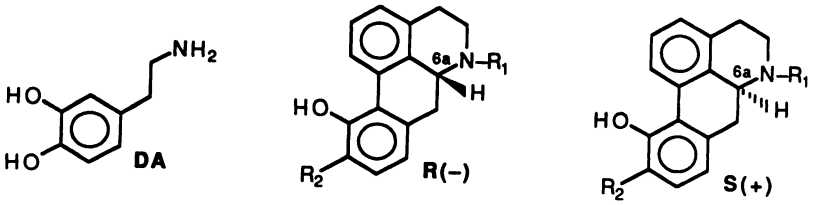

Fig. 1. Structures of the *trans*-α-rotamer of DA and aporphine enantiomers described in text. APO, $R_1 = CH_3$, $R_2 = OH$; NPA, $R_1 = CH_2CH_2CH_3$, $R_2 = OH$; 11-OH-NPa, $R_1 = CH_2CH_2CH_3$, $R_2 = H$.

tor sites (10, 11, 24). However, rather than being inert pharmacologically, the *S*-(+)-isomers of APO, and especially of NPA, have shown activity as DA antagonists in behavioral studies (12, 19) or as very weak partial agonists in neurophysiological studies (16, 17). Recently, the *R*-(−)- and *S*-(+)-enantiomers of 11-OH-NPa (Fig. 1) were synthesized, separated, and found to have binding affinities to D_1 and D_2 receptors and behavioral activity similar to (*R*)-(−)- and (*S*)-(+)-NPA, respectively (12, 19, 25, 26). Thus, the catechol moiety in APO and NPA evidently is not an absolute requirement for dopaminergic activity. However, the 11-OH group, which corresponds to the *meta*-OH of DA, appears be a crucial contributor to D_2 receptor affinity, because 10-OH-substituted aporphines are much less potent in binding to D_2 receptors (27, 28). The effects of *R*-(−)-antipodes of NPA and APO on the presumably presynaptic modulation of DA synthesis via inhibition of tyrosine hydroxylase have been examined previously *in vivo* (4, 6, 21) and *in vitro* (4, 29). In these studies, (*R*)-(−)-NPA was more potent than (*R*)-(−)-APO, and the autoreceptor-mediated inhibition was blocked by the relatively selective D_2 antagonist haloperidol and the nonselective antagonist (+)-butaclamol. Only recently have the *S*-(+)-isomer of NPA and the *R*-(−)- and *S*-(+)-enantiomers of 11-OH-NPa been evaluated for activity at DA autoreceptors. In an *in vivo* model of DA autoreceptor function (30) based on accumulation of DOPA after pretreatment with *m*-hydroxyphenylhydrazine (NSD-1015) and γ-butyrolactone (GBL), (*R*)-(−)- and (*S*)-(+)-NPA and (*R*)-(−)-11-OH-NPa all inhibited DA synthesis in rat corpus striatum and nucleus accumbens septi, whereas (*S*)-(+)-11-OH-NPa had no effect up to doses of 10 mg/kg, intraperitoneally (6). Haloperidol blocked the inhibitory effects of the active aporphines after pretreatment with GBL (6), consistent with a D_2 autoreceptor mechanism. In an effort to overcome potential confounding variables inherent in *in vivo* studies, the present work examined the *in vitro* effects of the *R*-(−)- and *S*-(+)-enantiomers of NPA and 11-OH-NPa on presynaptic regulation of DA synthesis in striatal minces from normal and DA-depleted (reserpinized) rats.

Materials and Methods

Animals. Young adult (250–300 g) male albino Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were adapted, for at least 1 week before use, to housing in wire cages, in groups of four or five, under controlled conditions of 20–22°, 40–50% humidity, and lights on from 7:00 a.m. to 7:00 p.m. Animals had free access to commercial food pellets and fresh tap water. Some animals received injections of reserpine free base (dissolved in a minimal volume of glacial acetic acid and diluted in normal saline) at 5 mg/kg, intraperitoneally, 20 and 2 hr before sacrifice. This regimen produced >90% depletion of striatal DA, as measured by high pressure liquid chromatography with electrochemical detection assays (6) of randomly selected striatal samples.

Drugs and chemicals. The enantiomers of 11-OH-N-n-propylnoraporphine (11-OH-NPa) were synthesized at Northeastern University as previously reported (25) and the isomers of NPA and APO were provided by Research Biochemicals, Inc. (Natick, MA). Other pharmaceuticals were purchased from, or generously donated by their manufacturers as follows: (+)-amphetamine sulfate, Sigma Chemical Co. (St. Louis, MO); (+)-butaclamol, Ayerst Laboratories (New York); fluphenazine-HCl, E.R. Squibb Corp. (New Brunswick, NJ); (*cis*)-(*Z*)-fluphenthixol, Lundbeck Laboratories (Denmark); GBR-12909-$(HCl)_2$, Gist-Brocades Laboratories (Delft, Netherlands); pargyline-HCl, Abbott Laboratories (North Chicago, IL); reserpine, Aldrich Chemical Co. (Milwaukee, WI); (+)-SCH-23390-HCl, Schering Corp. (Bloomfield, NJ); and spiperone, Janssen Pharmaceutica (Beerse, Belgium). The radiolabeled compounds [7-^{3}H]3,4-dihydroxyphenylethylamine (DA) (40.0 Ci/mmol), [^{3}H]SCH-23390 [(*R*)-(+)-[*N-methyl*-^{3}H]8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-7-ol-benzazepine] (60.4 Ci/mmol), [*benzene ring*-^{3}H]spiperone (27.5 Ci/mmol), and L-[1-^{14}C]tyrosine (48.6 mCi/mmol) were purchased from DuPont-NEN, Inc. (Boston, MA). All other drugs and chemicals were obtained from Fisher Scientific (Pittsburgh, PA) in the highest available purity.

Tyrosine hydroxylase activity in striatal minces. Activity of tyrosine hydroxylase as a measure of DA synthesis was assessed by modification of published procedures, which measure the formation of $^{14}CO_2$ evoked during the synthesis of DA from L-[1-^{14}C]tyrosine, based on the marked substrate preference of aromatic L-amino acid decarboxylase for L-DOPA over L-tyrosine (31–33). Corpus striatum of rats killed by decapitation was rapidly dissected on ice, weighed, finely minced (approximately 0.5 mm^3) in 0.32 M sucrose with a razor blade on an ice-cold Parafilm surface to produce a fine slurry, and subsequently diluted to 25 mg/ml with ice-cold assay buffer containing the following (mM): NaCl (124.0), KCl (5.0), K_2HPO_4 (1.4), $MgCl_2$-$6H_2O$ (1.3), $CaCl_2$-$2H_2O$ (0.8), sodium ascorbate (0.15), sucrose (80.0), (+)-glucose (11.0), and HEPES buffer (20.0). The pH was adjusted to 7.0 by bubbling with air/CO_2 gas (95:5 by volume). Aliquots of this suspension (300 μl, approximately 7.5 mg of tissue) were added to test tubes containing various concentrations of a test aporphine (0–200 μM) diluted in assay buffer so that the volume of each tube before addition of [^{14}C]tyrosine was 400 μl. Assay tubes were preincubated 5 min at 37° with mechanical shaking. In some experiments, a DA antagonist or uptake blocker was preincubated with tissue 5 min at 37° before addition of an aporphine. The reaction was initiated with 0.250 μCi (in 100 μl of assay buffer) of [^{14}C]tyrosine to provide a final concentration of 20 μM substrate in 0.5-ml total volume. Assay tubes were immediately capped (via a rubber tubing connector, 60 mm × 16 mm o.d.) with an inverted 5-ml polyethylene scintillation vial containing a strip (1 × 9 cm) of Whatman no. 3 filter paper impregnated with a 20% (by volume) methanolic solution of phenylethylamine to "trap" evolved $^{14}CO_2$. Incubation at 37° with shaking was continued for 45 min. The reaction was terminated and $^{14}CO_2$ was liberated from the suspension by acidification by injection (via the rubber tubing) of 1 ml of 1 N perchloric acid. After 8 hr, each scintillation vial containing the filter paper strip was filled with 4 ml of scintillation fluid (Poly-Fluor; Packard Instrument Co., Downers Grove, IL) and assayed by scintillation counting for ^{14}C at an efficiency of approximately 93%. For each set of experimental conditions, a value of ^{14}C cpm/assay tube was determined, minus a blank value (matched conditions but tissue omitted; usually ≤10% of basal cpm). Typically, four to six replicates were run for each condition or drug concentration, and each condition was repeated independently at least once, with final data (as percentage of corresponding basal control values) being pooled and expressed as mean ±

standard error; values of drug effects, as IC_{50} ± standard error, were obtained using the ALLFIT program adapted to a microcomputer (34).

Release of previously accumulated [^{3}H]DA from striatal minces. Rat striatal minces were suspended at 50 mg/ml in the same assay buffer used for tyrosine hydroxylase activity, with 100 μM pargyline added as an inhibitor of monoamine oxidase to prevent oxidative metabolism of DA. The suspension was preincubated at 37° for 5 min and, after the addition of 100 nM [^{3}H]DA, incubated aerobically for 10 min. High affinity uptake of [^{3}H]DA was stopped by addition of 20-fold excess ice-cold wash buffer (same as previous buffer with glucose omitted), and the suspension was centrifuged at 1500 × *g* for 10 min. The minces were collected and washed twice with a large excess (approximately 100 volumes) of ice-cold wash buffer and subsequently resuspended in assay buffer at 10 mg/ml. The suspension was kept on ice and divided into 1.5-ml aliquots in 10-ml polyethylene vials containing 50 μl of various concentrations of aporphines (0–50 μM) or (+)-amphetamine (10 μM) diluted in buffer. The assay mixtures were incubated at 37° for 10 min with shaking and then placed on ice. Subsequently, to each assay vial were added 5 ml of cold wash buffer, and the vials were centrifuged at 1500 × *g* for 15 min. A 100-μl aliquot of the supernatant was removed from each and counted for ^{3}H at approximately 65% efficiency. Mean spontaneous "basal" release of ^{3}H (cpm) was determined for each assay, and drug-induced release is expressed as mean percent of basal release ± standard error (three experiments). ^{3}H counts were shown chromatographically (silica gel/*n*-butanol/acetic acid/water, 25:4:10, by volume) to be >90% [^{3}H]DA.

Radioreceptor assays with striatal homogenates. The procedures to determine specific high affinity binding of the selective D_1 ligand [^{3}H]SCH-23390 and D_2 ligand [^{3}H]spiperone to rat striatal tissue homogenates were similar to those described elsewhere (20, 35). Briefly, striata of reserpine- or saline-pretreated rats were homogenized (Teflon on glass) in 50 mM Tris buffer (at pH 7.4 with 120mM NaCl for D_1, and pH7.7 for D_2 assays for homogenizations, centrifugations, and washings) and centrifuged at 27,000 × *g* in 100 volumes of buffer. After washing, the pellet was suspended in 10 volumes of buffer, vortexed for 15 sec, incubated at 37° for 10 min, and then kept on ice. For binding to D_1 receptors, glass tubes (triplicate) received 0.05–10 nM [^{3}H]SCH-23390, 200 μl of striatal tissue, and enough Tris buffer to bring the final volume of each tube to 1 ml. To determine nonspecific binding, (*cis*)-(*Z*)-fluphenthixol was included at 300 nM in each test condition. Tubes, kept on ice until assay, were incubated in a shaking water bath at 30° for 30 min. For the assay of binding to D_2 sites, tubes received 0.01–0.5 nM [^{3}H]spiperone, 600 μl of striatal tissue, and enough Tris buffer to bring the final volume to 1.8 ml. Nonspecific binding was determined by including (+)-butaclamol (200 nM) in each test condition. Tubes were incubated for 15 min at 37°. After incubation, D_1 or D_2 assay samples were filtered in a cell harvester (Brandel Corp., Gaithersburg, MD) through glass fiber sheets (S and S no. 32), which were washed twice with 5 ml of ice-cold Tris buffer, punched into 3-cm circles, and counted for ^{3}H at approximately 50% efficiency. Specific binding in the D_1 assay was calculated as the difference between total ^{3}H counts bound after incubation with [^{3}H]SCH-23390 alone and those remaining in the presence of (*cis*)-(*Z*)-fluphenthixol. The same approach was used in the D_2 assay using [^{3}H]spiperone and (+)-butaclamol as the blank. The results using six concentrations of radioligand were plotted in linearized form as specific binding (*B*) versus the ratio of bound to free ligand (*B*/*F*), to provide computed values of apparent K_d (negative slope) and B_{max} (*y*-intercept), expressed as the mean ± standard error of at least three independent determinations.

Results

Inhibition of tyrosine hydroxylase activity in striatal minces. In agreement with previous reports (31–33), preliminary experiments confirmed that the amount of tissue and the length of time of incubation (up to 1 hr) versus the rate of production of $^{14}CO_2$ gave linear relationships ($r \geq 0.9$) in these assays (data not shown). The average basal production of $^{14}CO_2$ (approximately 4.0 pmol/45 min/mg of wet weight of striatum) was similar to that in other reports with striatal minces, slices, and crude synpatosomal preparations (5, 33).

IC_{50} values for inhibition of tyrosine hydroxylase activity by DA and its aporphine analogs in untreated rat striatal tissue are shown in Table 1. IC_{50} values for the isomers of APO are included for comparison with those of NPA and 11-OH-NPa; that for (*R*)-(−)-APO (0.75 μM) is similar to previously published values obtained with preparations of striatal synaptosomes (IC_{50} of approximately 0.6 μM) (36, 37) and slices (IC_{50} of approximately 0.7 μM) (38, 39). Inhibition versus log concentration curves for the enantiomers of NPA and 11-OH-NPa are shown in Fig. 2A. The concentration-response curve for (*R*)-(−)-APO (not shown) was 2.6 times to the right of that of (*R*)-(−)-NPA (IC_{50} of 0.75 versus 0.29 μM), whereas the less potent *S*-(+)-isomers of APO and NPA had very similar IC_{50} values (1.5 and 1.0 μM, respectively). The inhibition versus log concentration curve for DA (IC_{50} of 0.17 μM; curve not shown) was nearly superimposable on the curve for (*R*)-(−)-NPA (IC_{50} of 0.29 μM). In normal tissue, the isomers of 11-OH-NPa, overall, were about 100 (83 to 144) times less potent than the isomers of the dihydroxyaporphines APO and NPA, although the inhibition curve obtained with (*R*)-(−)-11-OH-NPa appeared to be biphasic (Fig. 2A). The isomeric potency ratios (*R*/*S*) within enantiomeric pairs were very similar for APO, 11-OH-NPa, and NPA (2.1, 2.1, and 3.6 fold, respectively; Table 1).

Concentration-response curves for the NPA and 11-OH-NPa enantiomers, obtained with striatal minces from reserpine-pretreated rats, are shown in Fig. 2B. Because IC_{50} for the 11-OH-NPa isomers was not reached with reserpinized tissue even at 200 μM, a comparison of IC_{25} values between tissues from normal and reserpine-treated rats for these analogs is shown in Table 2. In reserpinized compared with normal tissue, there was a 2000-fold increase in potency for (*R*)-(−)-11-OH-NPa and no change in apparent potency for its *S*-(+)-isomer, associated with a corresponding large (>2000 times) increase in stereoselectivity (*R*/*S* potency ratio = 4300 and 2.1 in reserpinized and normal tissue, respectively). These results indicate that reserpine pretreatment, which depleted endogenous DA by ≥90%, reduced total efficacy of the 11-OH-NPa enantiomers and suggest that an indirect DA-mediated inhibition of tyrosine hydroxylase activity may be involved in the inhibition of DA synthesis by high concentrations of 11-OH-NPa in normal brain tissue.

The NPA isomers behaved quite differently from their corresponding monohydroxy analogs in reserpinized striatal tissue.

TABLE 1

Inhibition of tyrosine hydroxylase activity in normal rat striatum by DA and aporphine isomers

IC_{50} values are means ± standard errors.

Compound	IC_{50}	*R*/*S* potency
	μM	
DA	0.17 ± 0.03	
(*R*)-(−)-APO	0.75 ± 0.09	
(*S*)-(+)-APO	1.54 ± 0.58	2.05
(*R*)-(−)-NPA	0.29 ± 0.04	
(*S*)-(+)-NPA	1.04 ± 0.03	3.59
(*R*)-(−)-11-OH-NPa	41.9 ± 4.90[a]	
(*S*)-(+)-11-OH-NPa	86.8 ± 2.79	2.07

[a] Also includes a high affinity component (IC_{20} approximately 10 nM; Fig. 2A).

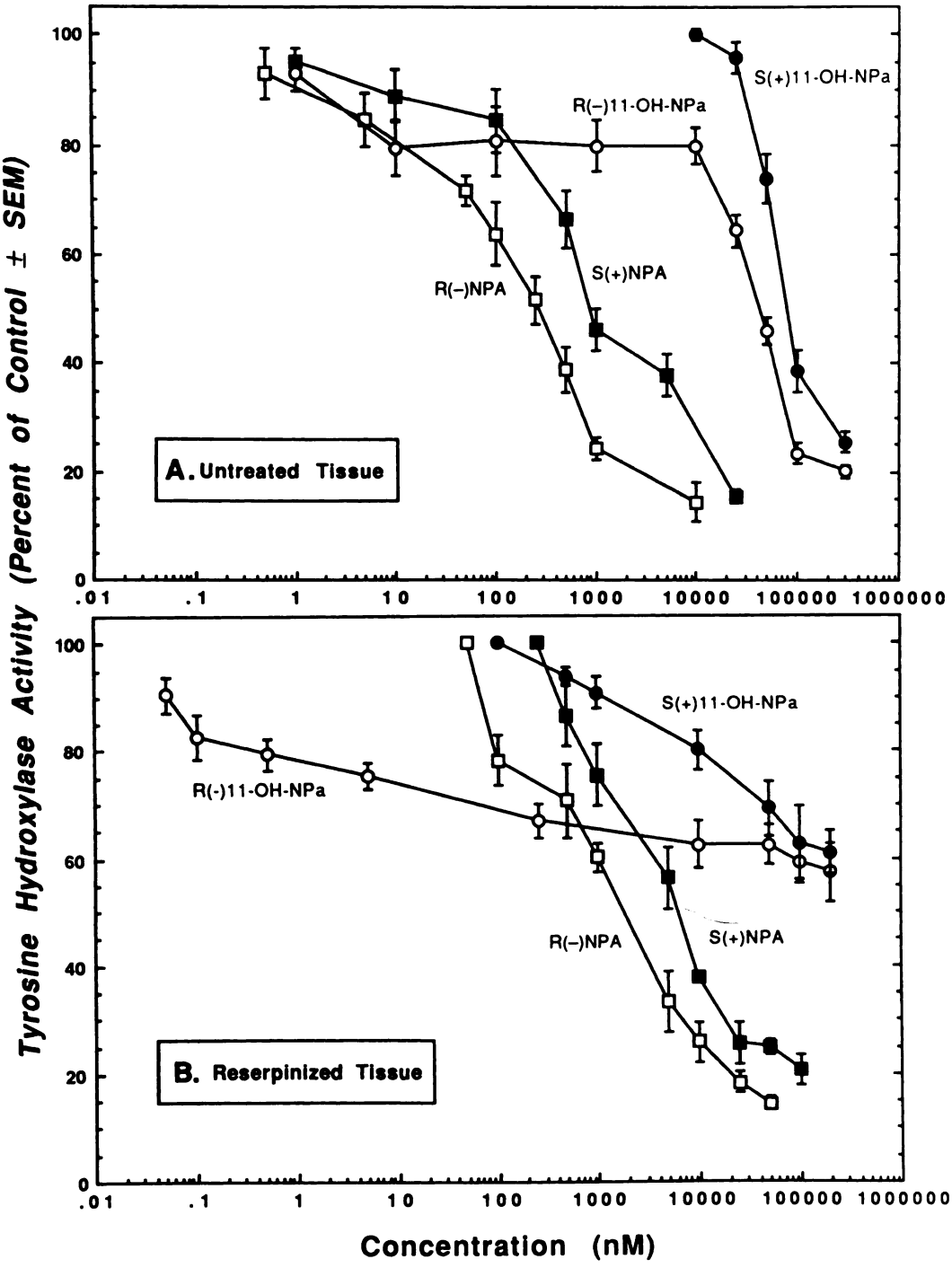

Fig. 2. Concentration-response curves for inhibition of striatal tyrosine hydroxylase activity from normal (A) and reserpine-pretreated (B) rats by aporphines (see structures in Fig. 1). Data are mean ± standard error of 8–12 determinations at the indicated concentrations. Control tyrosine hydroxylase activity for normal and reserpine-pretreated tissue was approximately 4 and 3 pmol of $^{14}CO_2$/45 min/mg of tissue, respectively.

The IC_{50} values for (*R*)-(−)- and (*S*)-(+)-NPA were 1.90 and 6.80 μM, respectively, following reserpine pretreatment (Table 2 and Fig. 2B). These values are both 6.5 times greater than the IC_{50} values obtained with untreated tissue, but the stereoselectivity remained constant (*R*/*S* ratio = 3.6 in both reserpinized and normal tissue; Table 2).

Antagonism of inhibition of tyrosine hydroxylase activity by aporphine and DA. When preincubated with normal striatal minces, moderate concentrations of the nonselective D_1 and D_2 antagonist fluphenazine fully blocked the inhibitory effects on tyrosine hydroxylase of both NPA isomers at concentrations near their IC_{50} value (Table 3). In normal tissue, fluphenazine at 2 and 3 μM fully blocked the effects of (*R*)-(−)-NPA (0.25 μM) and (*S*)-(+)-NPA (1.5 μM), respectively. This antagonism is in agreement with results from synaptosomal preparations (4, 5, 37) and is consistent with a presynaptic receptor-mediated effect on DA synthesis by both NPA isomers.

The inhibition of tyrosine hydroxylase activity by 0.25 μM DA was fully antagonized by 1 μM fluphenazine only in the presence of 1 μM GBR-12909, a potent and selective antagonist of high affinity uptake of DA (40) (Table 3). Because fluphenazine alone did not fully block the inhibitory effect of DA, the catecholamine may have accumulated in striatal nerve terminals to produce a direct catechol-mediated "end product" inhibitory effect on tyrosine hydroxylase activity. On the other hand,

TABLE 2

Inhibition of tyrosine hydroxylase activity by aporphine isomers in reserpinized versus normal striatal tissue

Aporphine	IC_{25}[a]	
	Reserpinized	Normal
	μM	
(*R*)-(−)-11-OH-NPa	0.007	14.0
(*S*)-(+)-11-OH-NPa	30.0	30.0
R/*S* potency	4300	2.14
Aporphine	IC_{50}	
	Reserpinized	Normal
	μM	
(*R*)-(−)-NPA	1.90	0.29
(*S*)-(+)-NPA	6.80	1.04
R/*S* potency	3.58	3.59

[a] Estimated from concentration-response curves in Fig. 2.

TABLE 3

Antagonism by fluphenazine (FLU) or GBR-12909 of inhibition of striatal tyrosine hydroxylase activity by aporphines and DA

Values are mean ± standard error.

Compound (concentration)	Tyrosine hydroxylase activity
μM	% of control
FLU (2)	98.9 ± 0.6
(*R*)-(−)-NPA (0.25)	55.6 ± 3.1[a]
FLU (2) + (*R*)-(−)-NPA (0.25)	96.8 ± 1.8
FLU (3)	93.8 ± 2.9
(*S*)-(+)-NPA (1.5)	50.4 ± 2.9[a]
FLU (3) + (*S*)-(+)-NPA (1.5)	93.1 ± 2.4
FLU (2)[b]	99.4 ± 0.5
(*R*)-(−)-11-OH-NPa (1)[b]	64.7 ± 3.4[a]
FLU (2) + (*R*)-(−)-11-OH-NPa (1)[b]	97.1 ± 2.9
FLU (1)	98.8 ± 1.2
GBR-12909 (1)	95.2 ± 3.1
DA (0.25)	49.0 ± 4.2[a]
FLU (1) + DA (0.25)	64.0 ± 6.9[a]
GBR-12909 (1) + DA (0.25)	62.3 ± 7.3[a]
FLU (1) + GBR-12909 (1) + DA (0.25)	95.3 ± 3.6

[a] $p < 0.001$ by *t* test; 10 replications.
[b] Reserpinized tissue used.

the DA-uptake blocker GBR-12909 alone also did not fully antagonize the inhibitory effect of DA on tyrosine hydroxylase activity (Table 3), consistent with a mechanism that, at least in part, evidently is autoreceptor mediated.

At less than equimolar concentrations, fluphenazine only partially antagonized the inhibition of tyrosine hydroxylase activity by NPA and 11-OH-NPa and, at concentrations of >3 μM, fluphenazine itself (as well as >0.5 μM spiperone, (−)-sulpiride, and (+)-SCH-23390) inhibited basal enzyme activity by ≥10% (data not shown). Thus, experiments involving interactions of DA receptor antagonists with 11-OH-NPa were limited to the more potent *R*-(−)-isomer with reserpinized tissue, in view of its lower potency in normal tissue and the very low potency of (*S*)-(+)-11-OH-NPa in both DA-depleted and normal tissue. With reserpinized tissue, fluphenazine (2 μM) fully blocked the inhibitory effects of 1 μM (*R*)-(−)-11-OH-NPa (Table 3). Similarly, the same concentration of fluphenazine also fully antagonized the effect of 2 μM (*R*)-(−)-NPA (approximately IC_{50}) with reserpinized tissue (data not shown). Thus, in DA-depleted striatal tissue, the inhibition of tyrosine hydroxylase activity by the more potent *R*-(−)-isomers of NPA and 11-OH-NPa was abolished by the DA receptor antagonist fluphenazine, again suggesting a DA receptor-mediated mechanism. The high IC_{50} values for the *S*-(+)-enantiomers of NPA and 11-OH-NPa in reserpine-treated striatal tissue (6.8 and 30 μM, respectively; Table 2) precluded the possibility of unambiguous receptor-blocking experiments with high concentrations of DA receptor antagonists, which themselves interfered with enzyme activity.

In an effort to establish the type of DA receptor mediating the presynaptic effects of (*R*)-(−)- and (*S*)-(+)-NPA in normal striatal tissue, selective D_1 and D_2 antagonists were used. As shown in Fig. 3, spiperone (0.5 μM), a relatively D_2-selective antagonist (13), fully antagonized the inhibitory effect of both NPA enantiomers (near their IC_{50} concentrations), whereas the D_1-selective antagonist SCH-23390 (15), also at 0.5 μM, had no effect. Thus, the autoreceptor-mediated regulation of DA synthesis by the enantiomers of NPA appears to be of the D_2 type. Again, in order to avoid the inhibitory effects of high concentrations of DA receptor antagonists, we were limited to evaluating only the *R*-(−)-enantiomer of 11-OH-NPa in reserpinized tissue, so that all drug concentrations could be kept at ≤0.5 μM. In DA-depleted tissue, 0.5 μM spiperone but not SCH-23390 also fully blocked the inhibitory effect of (*R*)-(−)-11-OH-NPa (0.5 μM) on tyrosine hydroxylase activity (Fig. 3). Apparently then, in the absence of endogenous DA, the monohydroxyaporphine (*R*)-(−)-11-OH-NPa also inhibits DA synthesis via a presynaptic D_2 autoreceptor.

To assess whether the (*S*)-(+)-aporphines themselves might possess antagonist properties at striatal DA autoreceptors *in vitro*, we evaluated their ability to interfere with the inhibitory effects of a moderate concentration (0.25 μM) of (*R*)-(−)-NPA on tyrosine hydroxylase activity. At concentrations that caused little (≤10%) inhibition themselves, neither the *S*-(+)-enantiomer of NPA (0.25 μM) nor that of 11-OH-NPa (25 μM) antagonized the inhibitory effect of (*R*)-(−)-NPA on tyrosine hydroxylase activity in normal striatal minces (data not shown). In other experiments, (*S*)-(+)-NPA at 2 μM inhibited tyrosine hydroxylase activity by 67% and did not antagonize the inhibition by 0.5 μM (*R*)-(−)-NPA (61%; data not shown).

Release of previously accumulated [^{3}H]DA from striatal minces. It has been reported that increase of Ca^{2+}-independent spontaneous efflux of previously accumulated [^{3}H]DA from striatal slices by (+)-amphetamine is not under autoreceptor control (41, 42). At low concentrations (<1 μM), (+)-amphetamine appears to promote release of DA via an exchange diffusion mechanism and, at higher concentrations (>10 μM), the amine passively diffuses into neurons and diplaces DA from intraneuronal binding sites (42). In view of the high IC_{50} values for inhibition of tyrosine hydroxylase activity by 11-OH-NPa, we sought to establish whether high concentrations of aporphines might release DA from striatal terminals, similar to (+)-amphetamine, and so promote a DA-mediated negative feedback inhibition of tyrosine hydroxylase, perhaps by increasing an intracellular free pool of DA with direct access to the enzyme and/or via autoreceptor-mediated effects of DA released to extracellular sites. Thus, we assessed the abilities of the aporphines to increase the efflux of previously accumulated [^{3}H]DA from striatal minces over spontaneous outflow and compared them with (+)-amphetamine as a positive control agent (Table 4). As reported previously with striatal slices (42), 10 μM (+)-amphetamine induced a substantial release of [^{3}H]DA, equal to

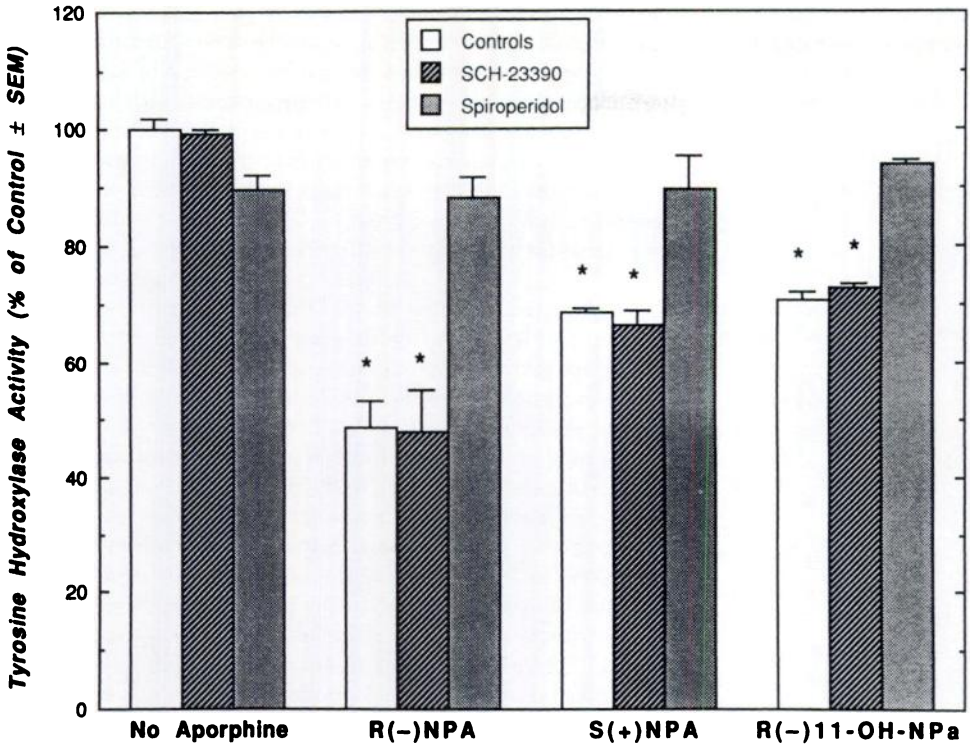

Fig. 3. D_2-selective antagonism of (*R*)-(−)- and (*S*)-(+)-NPA and (*R*)-(−)-11-OH-NPa (see structures in Fig. 1). Spiroperidol ▨ or SCH-23390 (■) was preincubated with striatal tissue from normal (NPA isomers) or reserpine-pretreated rats [(*R*)-(−)-11-OH-NPa] for 5 min at 37° before addition of (*R*)-(−)- or (*S*)-(+)-NPA or (*R*)-(−)-11-OH-NPa. Control conditions were no drug, DA antagonist alone, or aporphine alone. All drug concentrations were 0.5 μM. Data are mean ± standard error of 6–10 determinations. * $p < 0.001$ by *t* test.

TABLE 4

Release of previously accumulated [^{3}H]DA from striatal minces by (+)-amphetamine and aporphines

Values are mean ± standard error.

Compound	Concentration	Efflux of [^{3}H]DA
	μM	% of basal
(+)-Amphetamine	10	250 ± 14*
(*R*)-(−)-NPA	1.0	115 ± 4
	50	170 ± 4*
(*S*)-(+)-NPA	10	109 ± 5
	50	175 ± 6*
(*R*)-(−)-11-OH-NPa	10	100 ± 3
	50	155 ± 6*
(*S*)-(+)-11-OH-NPa	10	100 ± 4
	50	136 ± 5*

* $p < 0.01$ by *t* test; three replications.

about 250% of the basal efflux (i.e., a 2.5-fold increase). At 1 μM (more than 3 times its IC_{50} for inhibition of tyrosine hydroxylase activity), (*R*)-(−)-NPA had an insignificant effect and induced a [^{3}H]DA efflux equal to 115% of spontaneous basal release. (*S*)-(+)-NPA, even at 10 μM (10 times its IC_{50} versus tyrosine hydroxylase activity), triggered a release equal to only 109% of the basal release of [^{3}H]DA. At 10 μM, neither (*R*)-(−)- nor (*S*)-(+)-11-OH-NPa elicited any measurable increase over basal efflux of [^{3}H]DA. Only when concentrations of ≥50 μM were reached did any of the aporphines evoke a significant increase (to 136–175% of basal release; $p < 0.01$ by *t* test) in the efflux of [^{3}H]DA (Table 4). GBR-12909 had no effect on the ability of high concentrations of the aporphines to release previously accumulated [^{3}H]DA (data not shown). These results suggest that, at high concentrations (>10 μM), the aporphines tested increased the efflux of [^{3}H]DA from striatal tissues by a nonspecific action, perhaps by altering diffusion across cell membranes, as also has been suggested for high concentrations of (+)-amphetamine (42). In agreement with the hypothesis that a Ca^{2+}-independent process mediates effects of high concentrations of (+)-amphetamine (42), exclusion of $CaCl_2$ and addition of EDTA (5 mM) to the assay buffer did not influence basal efflux of [^{3}H]DA or alter the effects of 50 μM levels of either isomer of NPA or 11-OH-NPa (data not shown). Also, in agreement with results reported by Liang and Rutledge (42), the present increase in efflux of [^{3}H]DA promoted by (+)-amphetamine (10 μM) was not significantly different ($p > 0.1$ by *t* test) in reserpine-pretreated and untreated animals and reserpinization had no effect on the efflux of [^{3}H]DA induced by the aporphine analogs tested (data not shown).

Effect of reserpine on D_1- and D_2-specific binding sites. Chronic reserpine administration to rats is reported to produce a 20–25% increase in striatal D_2 receptor binding assayed with [^{3}H]haloperidol (43) or [^{3}H]spiperone (44). The possibility that the acute reserpine regimen used in the present experiments (5 mg/kg, intraperitoneally, at 20 and 2 hr before sacrifice) might have altered striatal populations of D_2 autoreceptors, and so altered sensitivity to aporphines, was investigated by measurement of the specific binding of the D_2 antagonist [^{3}H]spiperone; for comparison, D_1 sites were assessed with [^{3}H]SCH-23390. The values of mean apparent B_{max} and K_d values calculated from linearized plots of *B* versus *B/F* for [^{3}H]spiperone and [^{3}H]SCH-23390 binding to rat striatal membranes are shown in Table 5. The number of D_2 sites labeled and the affinity constant for [^{3}H]spiperone were very similar with tissue from control and acute reserpine-pretreated rats (B_{max} = 50.5 and 55.3 fmol/mg of tissue and K_d = 30.0 and 28.0 pM for control and reserpinized rats, respectively; Table 5), as reported also by Chugani et al. (45). Similarly, the binding characteristics of [^{3}H]SCH-23390 to D_1 sites also were virtually unchanged (B_{max} = 34.1 and 27.4 fmol/mg of tissue and K_d = 0.39 and 0.35 nM for control and reserpinized rats, respectively). These results (Table 5) indicate that the reserpine treatment

TABLE 5

Specific binding of [^{3}H]SCH-23390 (D_1) and [^{3}H]spiperone (D_2) to receptors in striatum of normal and acutely reserpinized rats

Values are means ± standard error of three determinations.

Group	[^{3}H]SCH-23390 (D_1)		[^{3}H]Spiperone (D_2)	
	B_{max}	K_d	B_{max}	K_d
	fmol/mg of tissue	nM	fmol/mg of tissue	pM
Saline	34.1 ± 2.7	0.39 ± 0.02	50.5 ± 3.5	30.0 ± 2.0
Reserpine[a,b]	27.4 ± 1.9	0.35 ± 0.02	55.3 ± 2.8	28.0 ± 2.0

[a] Rats received reserpine 5 mg/kg, intraperitoneally, 2 and 20 hr before sacrifice.
[b] Values are not statistically different from saline-treated group ($p > 0.05$ by t test).

used to obtain DA depletion in these experiments did not alter the number or D_2:D_1 ratio of DA receptor types, as measured by specific binding of selective antagonists.

Discussion

The *R*-(−)- and *S*-(+)-enantiomers of the dihydroxyaporphine NPA demonstrated apparently receptor-mediated inhibition of DA synthesis with only weak (3.6-fold) stereoselectivity, as measured by inhibition of tyrosine hydroxylase activity in striatal minces (Tables 1 and 2). This inhibitory effect of both enantiomers was fully antagonized by the nonselective DA antagonist fluphenazine and by the D_2-selective antagonist spiperone. The D_1-selective antagonist SCH-23390, on the other hand, had no effect against the NPA isomers at equimolar concentrations. These observations agree with studies in which isolated nerve ending (synaptosomal) preparations were used (4, 5, 37) and are consistent with presynaptic regulation of striatal DA synthesis mediated by a D_2-type receptor for which both (*R*)-(−)- and (*S*)-(+)-NPA (as well as the isomers of APO) evidently are agonists, differing in enantiomeric potency only by about 3.6-fold in normal and reserpinized tissue, and with (*R*)-(−)-NPA being nearly equipotent to DA itself (IC_{50} of approximately 0.3 and 0.2 μM, respectively). Presynaptic D_2 regulation of DA synthesis also was strongly suggested by previous experiments using rat striatal synaptosomes, in which the inhibition of tyrosine hydroxylase by the D_2-selective agonist lisuride was reversed stereospecifically by the D_2 antagonist (−)-sulpiride (46). Furthermore, the D_2 nature of the putative DA autoreceptor is supported by *in vivo* experiments involving measurements of the accumulation of DOPA after pretreatment with an amino acid decarboxylase inhibitor and the nerve impulse inhibitor GBL to eliminate postsynaptic neurophysiological feedback mechanisms affecting the dopaminergic pathways ascending from midbrain (30). Watanabe *et al.* (47) found that the D_1 agonist SKF-38393 had no effect on *in vivo* striatal accumulation of DOPA, whereas (*R*)-(−)-APO, which stimulates both D_1 and D_2 receptors, inhibited accumulation of DOPA. Furthermore, the effect of (*R*)-(−)-APO was blocked by the potent and highly selective D_2 antagonist (*cis*)-YM-09151-2 but not by the D_1 antagonist SCH-23390 (47). Also consistent with a D_2 receptor mechanism are our results obtained with aporphines in a similar *in vivo* model of autoreceptor function in the rat, in which haloperidol, a relatively selective D_2 antagonist, blocked the DOPA-lowering effects of (*R*)-(−)- and (*S*)-(+)-NPA and (*R*)-(−)-11-OH-NPa (6).

It should be noted that, at the concentrations of NPA employed in the present studies, we did not observe evidence of a direct, non-receptor-mediated inhibition of tyrosine hydroxylase (7, 36), because the actions of (*R*)-(−)- and (*S*)-(+)-NPA were fully antagonized by fluphenazine and spiperone. Other investigators have found that (*R*)-(−)-APO inhibits soluble tyrosine hydroxylase in "cell-free" preparations with exogenous pterin cofactor added, but only at much higher concentrations (20- to 50-fold) than are required to inhibit the enzyme activity in synaptosomes (2, 3). Apparently then, a weak direct "catechol-type" inhibition of tyrosine hydroxylase by dihydroxyaporphines may occur in the absence of an intact cell-contained enzyme but, when cell membrane integrity is maintained and only endogenous pterin cofactor is present, receptor-mediated inhibition of tyrosine hydroxylase predominates. Although some investigators reported only partial reversal by DA antagonists of the inhibition of synaptosomal tyrosine hydroxylase by (*R*)-(−)-APO (3, 7, 36), it has been suggested that synaptosomal preparations are thermolabile and may degrade or their autoreceptors may become functionally uncoupled under adverse conditions (7, 46). Thus, in other synaptosomal studies, presumably in which elevated temperatures were avoided (4, 46), haloperidol fully reversed the inhibitory effects of (*R*)-(−)-APO and (*R*)-(−)-NPA on tyrosine hydroxylase, supporting an autoreceptor-mediated mechanism. In contrast, as expected, DA displayed evidence of both an apparently direct (presumably catechol-mediated) and an indirect (receptor-mediated) inhibition of tyrosine hydroxylase in our tissue mince assay system, with full antagonism of the DA effect occuring only in the presence of the DA receptor antagonist fluphenazine plus the DA-uptake blocker GBR-12909 (Table 3).

Interestingly, the *in vitro* autoreceptor model examined in the present study displayed only weak (approximately 3-fold) stereoselective preference for (*R*)-(−)-NPA over its *S*-(+)-enantiomer. This result contrasts to neurophysiological studies (inhibition of DA cell firing, which may involve somatodendritic autoreceptors and/or postsynaptic DA receptor stimulation of a striatonigral negative feedback loop) with NPA, in which the *S*-(+)-enantiomer was approximately 1000 times less potent than (*R*)-(−)-NPA as an agonist (16, 17). Likewise, studies of striatal DA receptor binding (25) and rat pituitary gland D_2 receptor stimulation (13) showed a strong stereochemical preference (approximately 50 times) for (*R*)-(−)- over (*S*)-(+)-NPA and, in behavioral studies, (*S*)-(+)-NPA even showed activity as a DA antagonist (12, 19). However, in our previous work assessing *in vivo* DA autoreceptor function as a decrease in formation of DOPA after pretreatment with GBL, the *R*/*S* enantiomeric potency ratio for NPA also was about 3 (6). This observation suggests that, in contrast to its *R*-(−)-isomer, (*S*)-(+)-NPA may have much greater efficacy at striatal nerve terminal autoreceptors modulating DA synthesis than at postsynaptic or other D_2 sites and might be a relatively selective striatal autoreceptor agonist.

Mechanisms to account for apparent discrepancies in the activity of (*S*)-(+)-NPA in different models of dopaminergic function may include presynaptic receptor reserve (16, 48). This hypothesis suggests that, when the receptor pool is relatively large, as is proposed for striatal autoreceptors, which are reported to have a receptor reserve of approximately 70% (9, 16), higher intrinsic activity of weak or partial dopaminergic agonists may be revealed. However, in models of the function of postsynaptic DA receptors, where there is much less receptor reserve (48), a compound with appreciable agonistic activity at DA autoreceptors may even appear to be an antagonist in

competition with a potent full agonist such as (*R*)-(−)-NPA or DA itself. Such an interpretation may, for example, also account for the properties of the (+)- and (−)-isomers of 3-PPP. In the *in vivo* autoreceptor model of DA synthesis (striatal accumulation of DOPA after NSD-1015 and GBL), both isomers of 3-PPP have been found to be agonists, and the potency of (+)-3-PPP was only twice that of the (−)-isomer (49). However, in behavioral and biochemical models of postsynaptic function, (−)-3-PPP has been reported to act as an antagonist, whereas (+)-3-PPP retained agonist activity (49), somewhat analogous to the actions of (*R*)-(−)- and (*S*)-(+)-NPA. The results from the present *in vitro* study and our previous *in vivo* experiments (6) indicate that the presumably autoreceptor-mediated inhibition of DA synthesis by (*R*)-(−)-NPA was not antagonized by its *S*-(+)-enatiomer and so seem to be consistent with the suggestion that (*S*)-(+)-NPA is a relatively selective agonist at the D_2 autoreceptor mediating inhibition of DA synthesis, perhaps dependent on the significant reserve shown to exist for striatal autoreceptors. Furthermore, (*S*)-(+)-NPA appears to be more potent (IC_{50} = 1.04 μM; Table 1) than the putative autoreceptor-selective agent (−)-3-PPP, which has an IC_{50} of >300 μM for inhibition of tyrosine hydroxylase activity in striatal synaptosomes (50) and striatal minces (51).

The shift to the right (approximately 6.5-fold) of the dose-response curves for the NPA isomers after pretreatment with reserpine was somewhat unexpected, because behavioral studies (evaluating stereotypy), even with acutely reserpinized rats, have shown evidence of increased potency over untreated animals for the closely related DA agonist (*R*)-(−)-APO (52). On the other hand, such behavioral models are unable to distinguish between the D_1 and D_2 or pre- and postsynaptic effects of (*R*)-(−)-APO and do not control for other nondopaminergic actions that may occur. Recently, it was reported that acute reserpine treatment decreased binding of [^{3}H]spiperone in *ex vivo* autoradiographic studies of rat striatum without altering its *in vitro* binding to striatal tissue homogenates, perhaps by agonist-mediated D_2 receptor internalization (45). Thus, it may be that, after acute reserpine treatment, D_2 receptors are less prevalent at the surface of neuronal membranes (due to receptor internalization) and that this change was not detected by measurement of binding of [^{3}H]spiperone to striatal tissue homogenates (Table 5), which presumably estimates total (surface and some internal) D_2 (both pre- and some postsynaptic) binding. A decreased number of D_2 receptors located on the surface of striatal membranes might account for the decreased potency of NPA isomers in reserpinized striatal minces.

In contrast to our previous *in vivo* results, where (*R*)-(−)-11-OH-NPa was about 10-fold less potent than (*R*)-(−)-NPA at inhibiting DOPA accumulation in striatum after GBL pretreatment (6), both isomers of 11-OH-NPa were about 100-fold less potent than their corresponding NPA analogs in their ability to fully inhibit striatal tyrosine hydroxylase activity in normal tissue *in vitro*. Furthermore, whereas the dose-response curves for (*R*)-(−)- and (*S*)-(+)-NPA and for (*S*)-(+)-11-OH-NPa were more or less of a standard sigmoidal type spanning 2–3 log units, the apparently biphasic curve found with (*R*)-(−)-11-OH-NPa (Fig. 2A) indicates that two different, perhaps overlapping, mechanisms are operative. Whereas the loss of efficacy of (*R*)-(−)-11-OH-NPa in DA-depleted tissue from reserpinized animals (Fig. 2B) suggests that endogenous DA contributes to the effect of high concentrations of this monohydroxyaporphine compound, the 2000-fold increase in its potency at lower concentrations (IC_{25} = 7 nM; Table 2) indicates that (*R*)-(−)-11-OH-NPa has high affinity and some intrinsic activity at striatal DA autoreceptors modulating DA synthesis. Moreover, this effect apparently is mediated by receptors of the D_2 type, because fluphenazine and spiperone, but not SCH-23390, fully blocked the activity of (*R*)-(−)-11-OH-NPa in reserpinized tissue. The 2000-fold increase in stereoselectivity (*R* > *S*) between the 11-OH-NPa isomers after reserpine pretreatment also seems consistent with a presynaptic receptor-mediated mechanism. A weak, presumably nonreceptor-mediated, DA-releasing or -displacing effect of (*R*)-(−)-11-OH-NPa may contribute to its DA-sensitive regulation of tyrosine hydroxylase activity at high concentrations (≥50 μM). The lower potency of (*R*)-(−)-11-OH-NPa in this *in vitro* model (in unreserpinized tissue) compared with the *in vivo* GBL model warrants further investigation into the mechanism of autoreceptor-mediated inhibition of DA synthesis in both models. Other discrepancies between *in vitro* and *in vivo* autoreceptor models have been noted by others; for example, Meller *et al.* (48) have recently reported that presynaptic receptor reserve was found to be quite large using the *in vivo* GBL model but absent in a synaptosomal *in vitro* model (9).

Like its *R*-(−)-enantiomer, (*S*)-(+)-11-OH-NPa also showed a loss of efficacy for inhibiting tyrosine hydroxylase activity after reserpinization (Fig. 2B). Thus, both isomers of 11-OH-NPa were similar in their requirement for intact stores of endogenous DA to effect full inhibition of tyrosine hydroxylase. We were unable to test for a receptor-mediated mechanism with the (*S*)-(+)-monohydroxyaporphine, given its lack of potency in both normal and reserpinized striatal tissue. In other experiments (data not shown), the DA-uptake blocker GBR-12909 (up to 10 μM) had little or no effect ($p > 0.1$ by *t* test; four determinations) on the inhibition of tyrosine hydroxylase activity by (*R*)-(−)-11-OH-NPa, (*R*)-(−)-NPA, or (*S*)-(+)-NPA (at their respective IC_{50} values) but the effect of (*S*)-(+)-11-OH-NPa (at 75 μM; approximately IC_{50}) was completely blocked by GBR-12909 (10 μM; $p < 0.002$ by *t* test; four determinations). Thus, at high concentrations, (*S*)-(+)-11-OH-NPa may enter the striatal nerve terminal to effect inhibition of tyrosine hydroxylase, perhaps exerting a direct or DA-mediated inhibition of the enzyme.

Inhibition of tyrosine hydroxylase by endogenous DA may arise as the result of shifting intraneuronal pools of DA. The aporphines tested here appear to behave similarly to (+)-amphetamine when present at concentrations of ≥50 μM, at which they increased the basal Ca^{2+}-independent efflux of previously accumulated [^{3}H]DA (Table 4). Because these high concentrations of 11-OH-NPa are within the range for inhibition of tyrosine hydroxylase activity (Table 1), it cannot be ruled out that the isomers of 11-OH-NPa may increase intracellular free cytoplasmic pools of DA and so induce a DA-mediated inhibition of the enzyme at high *in vitro* concentrations. The NPA isomers, on the other hand, had IC_{50} values for inhibiting tyrosine hydroxylase well below concentrations required to increase basal efflux of [^{3}H]DA appreciably.

In summary, we conclude that (*R*)-(−)- and (*S*)-(+)-NPA both can inhibit striatal DA synthesis via a D_2-type autoreceptor mechanism with only minor stereoselectivity (*R* > *S* approximately 3-fold). (*R*)-(−)-11-OH-NPa appears to be a potent partial agonist at the D_2 autoreceptor but requires the presence

of endogenous DA to fully inhibit tyrosine hydroxylase activity at high concentrations, probably by receptor-independent mechanisms. The *S*-(+)-enantiomer of 11-OH-NPa appeared to have very low affinity or intrinsic activity at striatal presynaptic D_2 receptors. Apparently, the autoreceptor modulating DA synthesis can accommodate and respond to agonists of the 10,11-dihydroxyaporphine type, such as APO or NPA, regardless of stereochemical conformation, whereas the partial agonist 11-OH-NPa is much more highly preferred in the *R*-(−)-configuration. These results differ from binding studies with corpus striatum membrane preparations, in which the affinity of the NPA enantiomers at D_2 sites (perhaps largely postsynaptic) was much more stereoselective for the *R*-(−)-isomer (*R*/*S* potency of approximately 50/1) and where (*R*)-(−)-NPA and (*R*)-(−)-11-OH-NPa had about equal affinities (25). The present results also contrast with neurophysiological studies, which show (*R*)-(−)-NPA to be about 1000 times more potent than (*S*)-(+)-NPA, but again only about 2.5 times more potent than (*R*)-(−)-11-OH-NPa, at inhibiting DA cell firing in the substantia nigra (16, 17). Also, (*R*)-(−)-NPA was reported to be about 50 times more potent than (*S*)-(+)-NPA at stimulating rat pituitary D_2 receptors (11), and the NPA enantiomers have shown opposite effects in behavioral measures of presumably postsynaptic dopaminergic function (12, 19). Taken together, these results suggest that the structural requirements for agonist activity at striatal D_2-type autoreceptors modulating DA synthesis may be different from other presynaptic (somatodendritic) or postsynaptic D_2 receptors. In this regard, it has recently been reported that the gene for the D_2 receptor produces two receptor isoforms by alternative splicing of messenger RNA, providing new molecular evidence of D_2 receptor diversity (53, 54). If a separate D_2 autoreceptor molecular type exists and has specific agonist structural requirements, then these characteristics may permit development of autoreceptor-selective agents.

References

1. Kehr, W., A. Carlsson, M. Lindqvist, T. Magnusson, and C. Atack. Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. *J. Pharm. Pharmacol.* **24**:744–746 (1972).
2. 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 (1975).
3. 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).
4. Haubrich, D. R., and A. B. Pflueger. The autoreceptor control of dopamine synthesis: an *in vitro* and *in vivo* comparison of dopamine agonsts. *Mol. Pharmacol.* **21**:114–120 (1982).
5. Baldessarini, R. J., J. L. Neumeyer, M. H. Teicher, N. S. Kula, A. Campbell, and E. Marsh. Do autoreceptors control dopamine synthesis in rat forebrain? Effects of aporphine analogs of dopamine. *Neurosci. Abstr.* **13**:192 (1987).
6. Baldessarini, R. J., E. R. Marsh, N. S. Kula, R. Zong, Y. Gao, and J. L. Neumeyer. Effects of isomers of hydroxyaporphines on dopamine metabolism in rat brain regions. *Biochem. Pharmacol.*, in press.
7. Wolf, M. E., and R. H. Roth. Dopamine autoreceptors, in *Dopamine Receptors* (I. Creese and C. M. Fraser, eds.). Alan R. Liss Inc., New York, 45–96 (1987).
8. Strait, K. A., and R. Kuczenski. Dopamine autoreceptor regulation of the kinetic state of striatal tyrosine hydroxylase. *Mol. Pharmacol.* **29**:561–569 (1986).
9. Bohmaker, K., T. Puza, M. Goldstein, and E. Meller. Absence of spare autoreceptors regulating dopamine agonist inhibition of tyrosine hydroxylation in slice of rat striatum. *J. Pharmacol. Exp. Ther.* **248**:97–123 (1989).
10. Neumeyer, J. L., S. J. Law, and J. S. Lamont. Apomorphine and related aporphines as probes of the dopamine receptor, in *Apomorphine and Other Dopamimetics, Vol. 1. Basic Pharmacology* (G. L. Gessa and G. U. Corsini, eds.). Raven Press, New York, 209–218 (1981).
11. Neumeyer, J. L., S. J. Lal, and R. J. Baldessarini. Historical highlights of the chemistry, pharmacology, and early clinical uses of apomorphine, in *Apomorphine and Other Dopamimetics, Vol. 1. Basic Pharmacology* (G. L. Gessa and G. U. Corsini, eds.). Raven Press, New York, 1–17 (1981).
12. Campbell, A., R. J. Baldessarini, M. H. Teicher, and J. L. Neumeyer. *S*(+)Apomorphines: selective inhibition of excitatory effects of dopamine injected into the limbic system of the rat. *Neuropharmacology* **24**:391–399 (1985).
13. Goldman, M. E., and J. W. Kebabian. Aporphine enantiomers: interactions with D_1 and D_2 receptors. *Mol. Pharmacol.* **25**:18–23 (1984).
14. Leff, S. E., M. W. Hamblin, and I. Creese. Interactions of dopamine agonists with brain D_1 receptors labeled by ^{3}H-antagonists: evidence for the presence of high and low affinity agonist-binding states. *Mol. Pharmacol.* **27**:171–183 (1985).
15. Hess, E. J., and I. Creese. Biochemical characterization of dopamine receptors, in *Dopamine Receptors* (I. Creese and C. M. Fraser, eds.). Alan R. Liss Inc., New York, 1–27 (1987).
16. Cox, R. F., and B. L. Waszczak. Differences in dopamine receptor reserve for *N*-*n*-propylnorapomorphine enantiomers: single unit recording studies after partial inactivation of receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Mol. Pharmacol.* **34**:125–131 (1989).
17. Martin, L. P., R. F. Cox, and B. L. Waszczak. Efficacy and potency comparisons among aporphine enantiomers: effects on dopamine neurons in substantia nigra of rat. *Neuropharmacology* **29**:135–143 (1990).
18. Bunney, B. S. The electrophysiological pharmacology of midbrain dopmainergic systems, in *The Neurobiology of Dopamine* (A. S. Horn, J. Korf, and B. H. C. Westerink, eds.). Academic Press, New York, 417–452 (1979).
19. Campbell, A., R. J. Baldessarini, M. J. Teicher, and J. L. Neumeyer. Behavioral effects of apomorphine isomers in the rat: selective locomotor-inhibitory effects of *S*(+)*N*-*n*-propylnorapomorphine. *Psychopharmacology* **88**:158–164 (1986).
20. Faedda, G., N. S. Kula, and R. J. Baldessarini. Pharmacology of binding of ^{3}H-SCH-23390 to D_1 dopaminergic receptor sites in rat striatal tissue. *Biochem. Pharmacol.* **38**:473–480 (1989).
21. Argiolas, A., G. Mereu, G. Serra, M. R. Melis, F. Fadda, and G. L. Gessa. *N*-*n*-Propylnorapomorphine: an extremely potent stimulant of dopamine autoreceptors. *Brain Res.* **231**:109–116 (1982).
22. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1980).
23. Kaiser, C., and T. Jain. Dopamine receptors: functions and subtypes. *Med. Res. Rev.* **5**:145–229 (1985).
24. Neumeyer, J. L., D. Reischig, G. W. Arana, A. Campbell, R. J. Baldessarini, N. S. Kula, and K. G. Watling. Enantioselectivity of *R*(−) and *S*(+)*N*-*n*-propylnorapomorphine on dopamine receptors. *J. Med. Chem.* **26**:516–521 (1983).
25. Gao, Y., R. Zong, A. Campbell, N. S. Kula, R. J. Baldessarini, and J. L. Neumeyer. Synthesis and dopamine agonist and antagonist effects of *R*(−) and *S*(+)-11-hydroxy-*N*-*n*-propylnoraporphine. *J. Med. Chem.* **31**:1389–1396 (1988).
26. Campbell, A., R. J. Baldessarini, Y. Gao, R. Zong, and J. L. Neumeyer. *R*(−) and *S*(+) Stereoisomers of 11-hydroxy- and 11-methoxy-*N*-*n*-propylnoraporphine: central dopaminergic behavioral activity in the rat. *Neuropharmacology*, in press.
27. Arana, G. W., R. J. Baldessarini, and J. L. Neumeyer. Structure-activity characteristics for high-affinity dopamine-agonist binding at central dopamine receptors. *Acta Pharm. Suec.* **2**(suppl.):25–36 (1983).
28. Arana, G. W., R. J. Baldessarini, J. S. Lamont, D. Amlicke, and J. L. Neumeyer. Pharmacology of high-affinity binding of [^{3}H](±)2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to bovine caudate nucleus tissue. *Biochem. Pharmacol.* **32**:2873–2880 (1983).
29. Bitran, M., and G. Bustos. On the mechanism of presynaptic autoreceptor-mediated inhibition of transmitter synthesis in dopaminergic nerve terminals. *Biochem. Pharmacol.* **31**:2851–2860 (1982).
30. Walters, J. R., and R. H. Roth. Dopaminergic neurons: an *in vivo* system for measuring drug interactions with presynaptic receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **296**:5–14 (1976).
31. Waymire, J. C., R. Bjur, and N. Weiner. Assay of tyrosine hydroxylase by coupled decarboxylation of DOPA formed from 1-^{14}C-L-tyrosine. *Anal. Biochem.* **43**:599–600 (1971).
32. Harris, J. E., and R. J. Baldessarini. Amphetamine-induced inhibition of tyrosine hydroxylation in homogenates of rat corpus striatum. *J. Pharm. Pharmacol.* **25**:755–757 (1973).
33. Harris, J. E., R. J. Baldessarini, and R. H. Roth. Amphetamine-induced inhibition of tyrosine hydroxylation in homogenates of rat corpus striatum. *Neuropharmacology* **14**:457–471 (1975).
34. Teicher, M. MED-65: ALLFIT (APPLESOFT): Adaptation of the ALLFIT Program of DeLean, Munson, and Rodbard for the Apple II Microcomputer. Biomedical Computing Technology Center, Vanderbilt University, Nashville, TN (1983).
35. Kula, N. S., R. J. Baldessarini, S. Bromley, and J. L. Neumeyer. Effects of isomers of apomorphines on dopamine receptors in striatal and limbic tissue of rat brain. *Life Sci.* **37**:1051–1057 (1985).
36. Brautigam, M., B. Kittner, and G. Laschinski. Effects of apomorphine enantiomers and of lisuride on 3,4-dihydroxyphenylalanine production in striatal synaptosomes. *Mol. Pharmacol.* **28**:515–520 (1985).
37. Bronaugh, R. L., J. Tabak, T. Ohashi, and M. Goldstein. The effect of butaclamol and of other neuroleptic agents on the apomorphine-elicited inhibition of synaptosomal tyrosine hydroxylase activity. *Psychopharmacol. Commun.* **1**:501–510 (1975).

38. Delanoy, R. L., and A. J. Dunn. Effects of haloperidol and apomorphine on catecholamine metabolism in brain slices. *Biochem. Pharmacol.* **31**:3297–3305 (1982).
39. Westfall, T., L. Naes, and C. Paul. Relative potency of dopamine agonists on autoreceptor function in various brain regions of the rat. *J. Pharmacol. Exp. Ther.* **224**:199–205 (1983).
40. Heikkila, R. E., and L. Manzino. Behavioral properties of GBR 12909 and GBR 13098: specific inhibitors of dopamine uptake. *Eur. J. Pharmacol.* **103**:241–248 (1984).
41. Kamal, L. A., S. Arbilla, A. M. Falzin, and S. Z. Langer. (+)Amphetamine inhibits electrically evoked release of [^{3}H]dopamine from slices of the rabbit caudate. *J. Pharmacol. Exp. Ther.* **227**:446–458 (1983).
42. Liang, N. Y., and C. O. Rutledge. Comparison of the release of [^{3}H]dopamine from isolated corpus striatum by (+)amphetamine, fenfluramine and unlabeled dopamine. *Biochem. Pharmacol.* **31**:983–992 (1982).
43. Burt, D. R., I. Creese, and S. H. Snyder. Antischizophrenic drugs: chronic treatment increases dopamine receptor binding in brain. *Science (Wash. D. C.)* **196**:326–327 (1977).
44. Norman, A. B., G. Battaglia, and I. Creese. Differential recovery rates of rat D_2 dopamine receptors as a function of aging and chronic reserpine treatment following irreverable modification: a key to receptor regulatory mechanisms. *J. Neurosci.* **7**:1484–1491 (1987).
45. Chugani, D. D., R. F. Ackermann, and M. E. Phelps. *In vivo* [^{3}H]spiperone binding: evidence for accumulation in corpus striatum by agonist-mediated receptor internalization. *J. Cereb. Blood Flow Metab.* **8**:291–303 (1988).
46. Tissari, A. J., L. Atzori, and M. T. Galdieri. Inhibition of dopamine synthesis in striatal synaptosomes by lisuride: stereospecific reversal by (−)-sulpiride. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **322**:89–91 (1983).
47. Watanabe, H., H. Suda, S. Sekihara, and Y. Nomura. D_1 type of dopamine autoreceptors are not involved in the regulation of dopamine synthesis in the striatum. *Jpn. J. Pharmacol.* **43**:327–330 (1987).
48. Meller, E., A. Enz, and M. Goldstein. Absence of receptor reserve at striatal dopamine receptors regulating cholinergic neuronal activity. *Eur. J. Pharmacol.* **155**:151–154 (1988).
49. Clark, D., S. Hjorth, and A. Carlsson. Dopamine receptor agonists: mechanisms underlying autoreceptor selectivity. *J. Neural Transm.* **62**:1–52 (1985).
50. Fowler, C. J., G. Thorell, M. Andersson, and O. Magnusson. Is inhibition of striatal synaptosomal tyrosine hydroxylation by dopamine agonists a measure of dopamine autoreceptor function? *Naunyn-Schmiedeberg's Arch Pharmacol.* **331**:12–19 (1985).
51. Booth, R.G., and R.J. Baldessarini. Adenosine A_2 autoreceptor stimulation of tyrosine hydroxylase activity in rat striatal minces is reversed by dopamine D_2 autoreceptor activation. *Eur. J. Pharmacol.*, in press, 1990.
52. Chipkin, R. E., R. D. McQuade, and L. C. Iorio. D_1 and D_2 dopamine site up-regulation and apomorphine-induced stereotypy. *Pharmacol. Biochem. Behav.* **28**:477–482 (1987).
53. Giros, B., P. Sokoloff, M. P. Martres, J. F. Riou, L. J. Emortine, and J. C. Schwartz. Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. *Nature (Lond.)* **342**:923–926 (1989).
54. Monsma, F. J., L. D. McVittie, C. R. Gerfen, L. C. Mahan, and D. R. Sibley. Multiple D_2 receptors produced by alternative RNA splicing. *Nature (Lond.)* **342**:926–929 (1989).