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Aporphine Enantiomers

Interactions with D-1 and D-2 Dopamine Receptors

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SUMMARY

The R(−) and the S(+) enantiomers of apomorphine (APO) and *N*-*n*-propyl norapomorphine (NPA) interact with both the D-1 and the D-2 dopamine receptors. R(−)-APO, as well as R(−)- and S(+)-NPA, stimulates the D-1 dopamine receptor in carp retina; S(+)-APO blocks this dopamine receptor. Similarly, R(−)-APO, as well as R(−)- and S(+)-NPA, stimulates the D-2 dopamine receptor in the intermediate lobe of the rat pituitary gland; S(+)-APO blocks the intermediate lobe D-2 receptor. The interactions between these aporphine enantiomers and the D-1 and the D-2 dopamine receptors exemplify several manifestations of the previously described "*n*-propyl phenomenon." Because S(+)-APO is distinguished from the other tested aporphines by its ability to antagonize either the D-1 or the D-2 dopamine receptors, it is hypothesized that the presence of an *N*-methylated tertiary amine in a molecule of appropriate configuration can confer dopamine receptor antagonist activity to the molecule.

INTRODUCTION

Both the D-1 and D-2 dopamine receptors possess the property of stereoselectivity (1–4). The synthesis of the R(−) and S(+) enantiomers of the aporphines APO[2] and NPA has provided stereoselective probes for either category of dopamine receptor (5, 6) (Fig. 1). However, a systematic investigation of the pharmacological effects of these aporphines upon the D-1 and the D-2 dopamine receptors has not been reported prevously. Such a systematic investigation seemed worthwhile, since these aporphine enantiomers display a range of pharmacological activities. Both R(−)-APO and R(−)-NPA are agonists in behavioral and biochemical tests (5, 7, 8). S(+)-NPA is, at best, a weak dopamine receptor agonist in behavioral assays but is a partial agonist in a biochemical assay for D-1 agonist activity; S(+)-NPA is also active in dopamine receptor binding assays (6). In contrast, some (but not all) investigators report S(+)-APO to be a dopamine receptor antagonist in behavioral and biochemical assays (5, 7, 8).

The present study determines the ability of the S(+) and R(−) enantiomers of APO and NPA to stimulate or to block the D-1 and the D-2 dopamine receptors. The dopamine-sensitive adenylate cyclase activity of teleost retina is used as a biochemical model of the D-1 receptor (9, 10). If an aporphine enantiomer mimics the ability of dopamine to stimulate enzyme activity, then it is designated a D-1 agonist. Conversely, if a compound antagonizes the stimulatory effect of dopamine, then it is designated a D-1 antagonist. The dopaminergic inhibition of the release of IR-αMSH from the melanotroph of the intermediate lobe of the rat pituitary gland is used as a physiological model of the D-2 dopamine receptor (11, 12). The release of IR-αMSH is enhanced with (−)-isoproterenol, a beta-adrenergic agonist. If an aporphine mimics the ability of LY 171555, the active isomer of the selective D-2 agonist LY 141865 (3), to attenuate the isoproterenol-enhanced release of IR-αMSH, then the compound is a D-2 agonist (11). Conversely, if the compound abolishes the inhibitory effect of LY 171555, then it is a D-2 antagonist.

EXPERIMENTAL PROCEDURES

Materials. Drugs were obtained from the following sources: R(−)-APO, S(+)-APO, R(−)-NPA, S(+)-NPA, and S(+)-bulbocapnine, Research Biochemicals, Inc. (Wayland, Mass.); LY-171555, Eli Lily Laboratories (Indianapolis, Ind.); dopamine and (−)-isoproterenol, Sigma Chemical Company (St. Louis, Mo.); and YM-09151-2, Yamanouchi Pharamceutical Company (Tokyo, Japan). Carp (*Carrasius auratus*, 3 inches in length) were purchased from Three Springs Fishery (Lilypons,

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[2] The abbreviations used are: APO, apomorphine; NPA, *N*-*n*-propyl norapomorphine; αMSH, α-melanocyte-stimulating hormone; IR-αMSH, immunoreactive αMSH-like peptides; LY 171555, *trans*-(−)-4a*R*-4-4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H* (or 2*H*)-pyrazolo[3,4-*g*]-quinolone; YM-09151-2, cis-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide.

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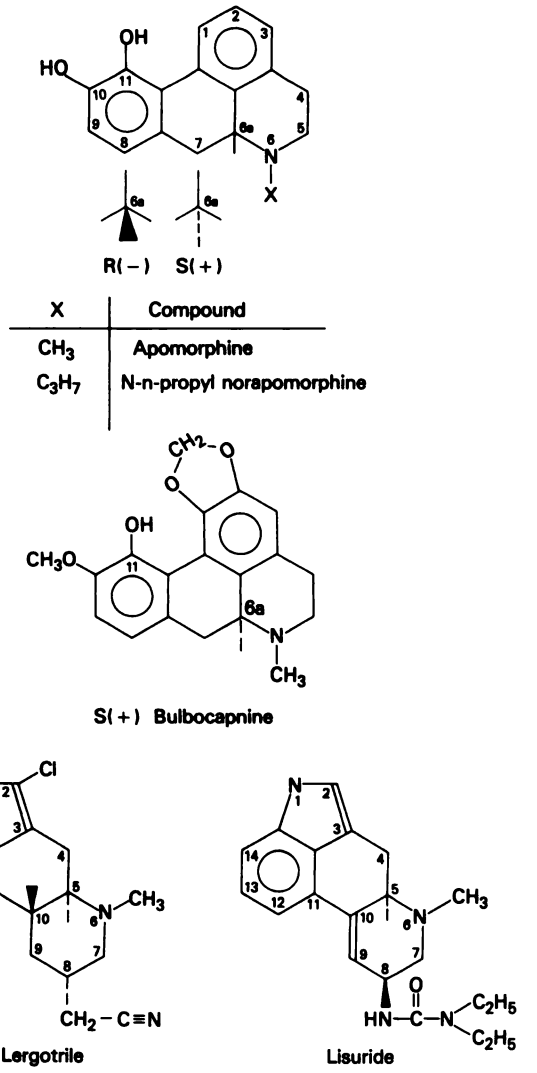

FIG. 1. *Structure of S(+)-bulbocapnine, lergotrile, lisuride, and the enantiomers of APO and NPA*

Md.). Male Sprague-Dawley rats (weighing between 300 and 350 g) were obtained from Taconic Farms (Germantown, N. Y.).

Data provided by Research Biochemicals Inc. state that the enantiomeric purity of R(−)- and S(+)-NPA was greater than 99.9%; the material used in the present study was the same as that used to generate the data presented in figure 1 of Neumeyer *et al.* (6). Data provided by Research Biochemicals Inc. states that the enantiomeric purity of R(−)- and S(+)-APO was greater than 98%.

Carp retinal adenylate cyclase activity. Adenylate cyclase activity was determined in a cell-free homogenate of carp retina using procedures previously described (9, 10).

Release of IR-αMSH from dispersed rat intermediate lobe cells. Dispersed intermediate lobe cells were isolated from rat neurointermediate lobes using the method of Goldman *et al.* (12) with the following modifications. Neurointermediate lobes were digested for two successive 45-min periods in 10 ml of 0.35% collagenase, 0.1% hyaluronidase, and 3% bovine serum albumin in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer at 37°. The resulting intermediate lobe tissue was then dissociated into single cells in 10 ml of 0.125% pancreatin in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer for 15 min at 37°. Following the hyaluronidase incubation, the cells were washed twice with Eagle's minimal essential medium with Earle's salts supplemented with 0.25% bovine serum albumin and placed in an incubator (37°, 95% air/5% carbon dioxide) for 2 hr.

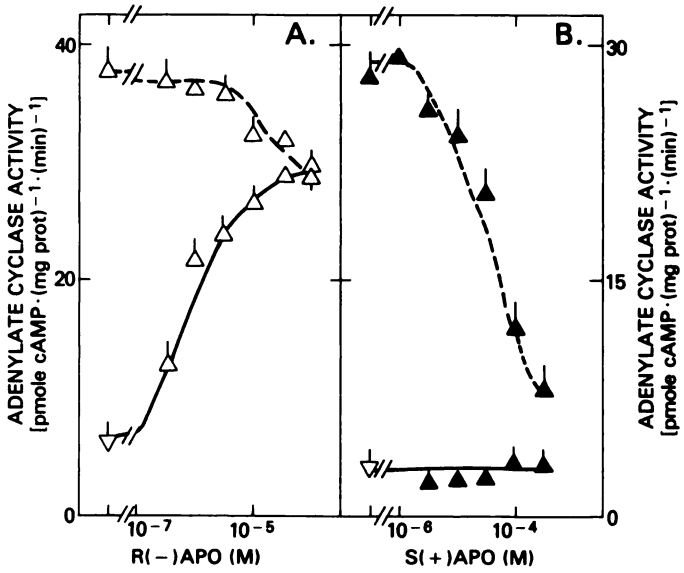

FIG. 2. *D-1 receptor: stimulation by R(−)-APO and blockade by S(+)-APO*

A. Using a cell-free homogenate of carp retina, adenylate cyclase activity was determined in the absence of added drugs (▽) or in the presence of either the indicated concentrations of R(−)-APO (△——△) or a combination of 10 μM dopamine and the indicated concentrations of R(−)-APO (△- - -△).

B. In a separate experiment, adenylate cyclase activity in a cell-free homogenate of carp retina was determined in the absence of added drugs (▽) or in the presence of either the indicated concentrations of S(+)-APO alone (▲——▲) or a combination of 10 μM dopamine and the indicated concentrations of S(+)-APO (▲- - -▲).

Data represent means ± standard error of four replicate samples.

All experimental incubations were carried out in a final volume of 1.0 ml Eagle's minimal essential medium with Earle's salts supplemented with 0.25% bovine serum albumin. Drugs were added in a volume of 10 μl immediately before the cells were added to the incubation tubes. The experimental incubations were terminated by centrifugation in a Microfuge (Beckman Instruments, Palo Alto, Calif.). IR-αMSH was quantified using a COOH-terminally directed anti-αMSH antibody (α-1, final dilution 1:75,000) which cross-reacted on an equimolar basis with desacetyl αMSH, αMSH, and *N,O*-diacetyl αMSH.[3]

RESULTS

Interactions between aporphines and the D-1 receptor in carp retina. R(−)-APO stimulated the D-1 receptor. R(−)-APO mimicked the ability of dopamine to enhance adenylate cyclase activity in a cell-free homogenate of carp retina (Fig. 2A). On a molar basis, R(−)-APO was equipotent with dopamine; half-maximal activation of adenylate cyclase activity was achieved with 2.3 μM R(−)-APO and 2 μM dopamine (Fig. 3; Table 1). However, R(−)-APO was a partial agonist, eliciting only 55% of the maximal response to dopamine (Fig. 3; Table 1). R(−)-APO displayed minimal activity as a D-1 antagonist; at a concentration of 100 μM, R(−)-APO attenuated

[3] M. Beaulieu, M. E. Goldman, K. Myazaki, E. A. Frey, R. L. Eskay, J. W. Kebabian, and T. E. Cote, unpublished observations.

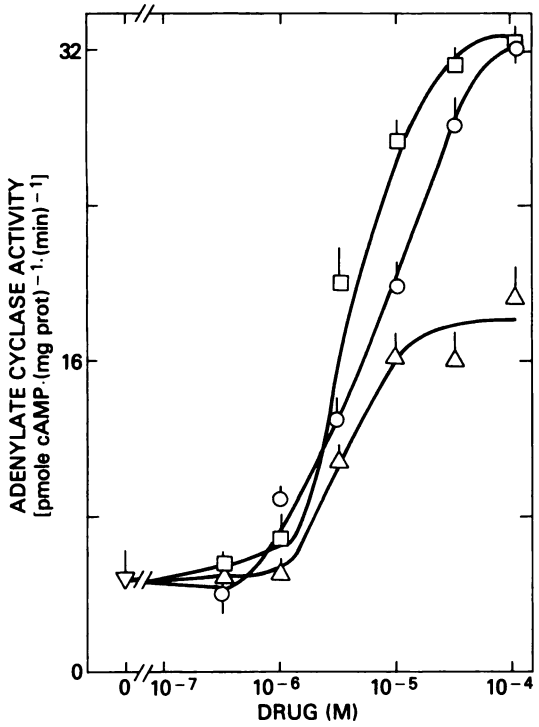

FIG. 3. *D-1 receptor: stimulation by R(−) enantiomers of APO or NPA*

Utilizing a cell-free homogenate of carp retina, adenylate cyclase activity was determined in the absence of added drugs (▽) or in the presence of the indicated concentrations of dopamine (□——□) R(−)-NPA (○——○) or R(−)-APO (△——△). Data represent means ± standard error of four replicate samples obtained in a single experiment.

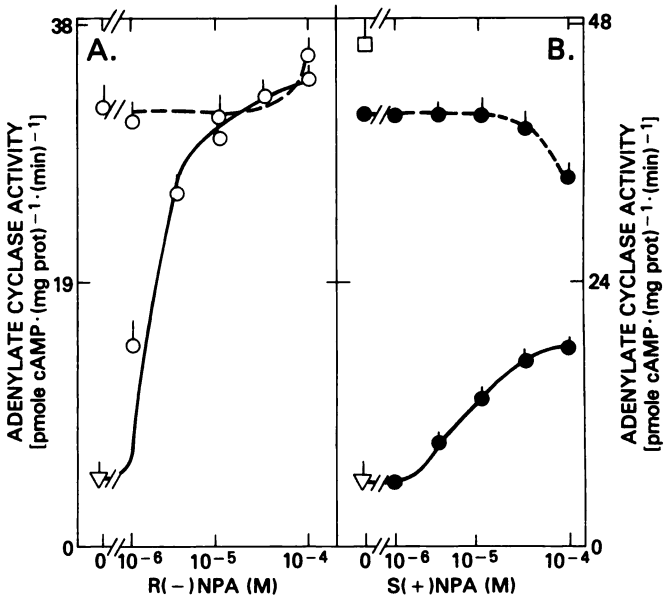

FIG. 4. *D-1 receptor: stimulation by either R(−)- or S(+)-NPA*

A. In an experiment similar to those shown in Fig. 2, adenylate cyclase activity in a cell-free homogenate of carp retina was determined in the absence of added drugs (▽) or in the presence of either the indicated concentrations of R(−)-NPA (○——○) or a combination of 10 μM dopamine and the indicated concentrations of R(−)-NPA (○- - -○).

B. In an experiment separate from that shown in A, adenylate cyclase activity was determined in the absence of added drugs (▽) or in the presence of either the indicated concentrations of S(+)-NPA (●——●) or a combination of 10 μM dopamine and the indicated concentrations of S(+)-NPA (●- - -●). Enzyme activity in the presence of 100 μM dopamine.

Data represent means ± standard error of four replicate samples obtained in a single experiment.

the response to 10 μM dopamine by less than 30% (Fig. 2A).

S(+)-APO blocked the D-1 receptor. S(+)-APO attenuated, in a dose-dependent manner, the stimulatory effect of dopamine upon the carp retinal adenylate cyclase activity (Fig. 2B). Assuming a kinetic competition between S(+)-APO and dopamine for the D-1 receptor, the affinity of S(+)-APO for this receptor was calculated to be 11.0 μM (Table 1). S(+)-APO did not stimulate the D-1 receptor (Fig. 2B, Table 1).

TABLE 1

Efficacy of aporphine enantiomers upon the D-1 and the D-2 receptors

Data for the D-1 receptor were obtained in experiments determining adenylate cyclase activity in cell-free homogenates of carp retina. For D-1 agonists, the value in parentheses is the maximal response to the indicated agent expressed as a percentage of the maximal response to dopamine (100 μM); affinity is the concentration of the indicated agent causing a half-maximal enhancement of enzyme activity. For S(+)-APO, affinity is the inhibitory constant (K_i) calculated from data obtained in experiments similar to that shown in Fig. 2B; the procedures used for this calculation have been described previously (13, 14).

Data for the D-2 receptor were obtained in experiments determining the release of IR-αMSH from ezymatically dispersed intermediate lobe cells. For the D-2 agonists, the value in parentheses is the maximal agonist-induced inhibition of (−)-isoproterenol-stimulated release of IR-αMSH expressed as a percentage of the inhibition due to 1 μM LY 171555; affinity is the concentration of the indicated agent causing half-maximal inhibition of (−)-isoproterenol-stimulated release of IR-αMSH. For S(+)-APO, affinity is the inhibition constant (K_i) calculated from experiments similar to that shown in Fig. 5B. The procedure used for this calculation has been described previously (13); in this calculation, the affinity of LY 171555 for the D-2 dopamine receptor was 27 nM (Y. Itoh, M. E. Goldman, and J. W. Kebabian, unpublished data).

All values are means ± standard error of data obtained in at least three separate experiments.

Compound	D-1 receptor		D-2 receptor	
	Action	Affinity	Action	Affinity
		μM		*nM*
R(−)-APO	Partial agonist (55%)	2.3 ± 0.6	Agonist (100%)	14.0 ± 6.0
S(+)-APO	Antagonist	12.8 ± 2.5	Antagonist	495 ± 99
R(−)-NPA	Agonist (100%)	5.0 ± 1.7	Agonist (100%)	0.3 ± 0.1
S(+)-NPA	Partial agonist (34%)	6.7 ± 2.0	Agonist (100%)	16.7 ± 6.7

TABLE 2

D-2 agonist activity of aporphine enantiomers: antagonism by YM-09151-2, a selective D-2 antagonist

Dispersed intermediate lobe cells (4.5×10^4) were incubated in the presence of (−)-isoproterenol (1 μM), R(−)-APO (100 nM), R(−)-NPA (100 nM), S(+)-NPA (100 nM), and YM-09151-2 (100 nM) as indicated. At the end of the experimental incubation, the amount of IR-αMSH released during the experimental incubation was determined by radioimmunoassay. Data represent means ± standard error ($n = 3$) for samples obtained in a single experiment.

Addition	IR-αMSH released in the presence of	
	No D-2 antagonist	YM-09151-2 (100 nM)
	ng/4.5 × 10⁴ cells/20 min	
None	0.74 ± 0.11	0.83 ± 0.11[a]
(−)-Isoproterenol	2.83 ± 0.88[b]	ND[c]
(−)-Isoproterenol + R(−)-APO	0.59 ± 0.04[a]	2.76 ± 0.22[d]
(−)-Isoproterenol + R(−)-NPA	0.68 ± 0.06[a]	2.75 ± 0.20[d]
(−)-Isoproterenol + S(+)-NPA	0.89 ± 0.07[a]	2.79 ± 0.10[d]

[a] No significant difference versus no D-2 antagonist.

[b] $p < 0.001$ versus no addition.

[c] ND, Not determined in the present experiment; however, in two separate experiments, YM-09151-2 (100 nM) did not affect the (−)-isoproterenol-stimulated (1 μM) release of IR-αMSH.

[d] $p < 0.001$ versus YM-09151-2 (100 nM).

Both enantiomers of NPA were D-1 agonists. R(−)-NPA was equipotent with dopamine on the basis of either maximal response or molar potency (Fig. 3; Table 1). In contrast, S(+)-NPA produced only 34% of the maximal response to dopamine (Fig. 4B; Table 1). Neither R(−)- nor S(+)-NPA blocked the D-1 receptor (Fig. 4A and B).

Interactions between aporphines and the D-2 receptor in rat intermediate lobe. In the present experiments, (−)-isoproterenol (1 μM) increased the release of IR-αMSH between 2.5- and 4.0-fold (Table 2; Figs. 5 and 6). This effect of (−)-isoproterenol was attenuated by 0.3 μM LY 171555 (Fig. 5, *inset*; Figs. 5 and 6); YM-09151-2, a selective antagonist of the D-2 receptor, prevented this effect of LY 171555 (Fig. 5, *inset*) (3, 10, 15).

R(−)-APO stimulated the D-2 receptor. This compound mimicked the ability of LY 171555 to attenuate the (−)-isoproterenol-stimulated release of IR-αMSH from dispersed rat intermediate lobe cells (Fig. 5A). Half-maximal inhibition of the (−)-isoproterenol-stimulated release of IR-αMSH was achieved with 14 nM R(−)-APO (Table 1). This effect of R(−)-APO could be abolished by YM-09151-2 (Table 2). R(−)-APO displayed no activity as an antagonist of the D-2 receptor; it did not reverse the inhibitory activity of LY 171555 upon (−)-isoproterenol-stimulated release of IR-αMSH (Fig. 5A).

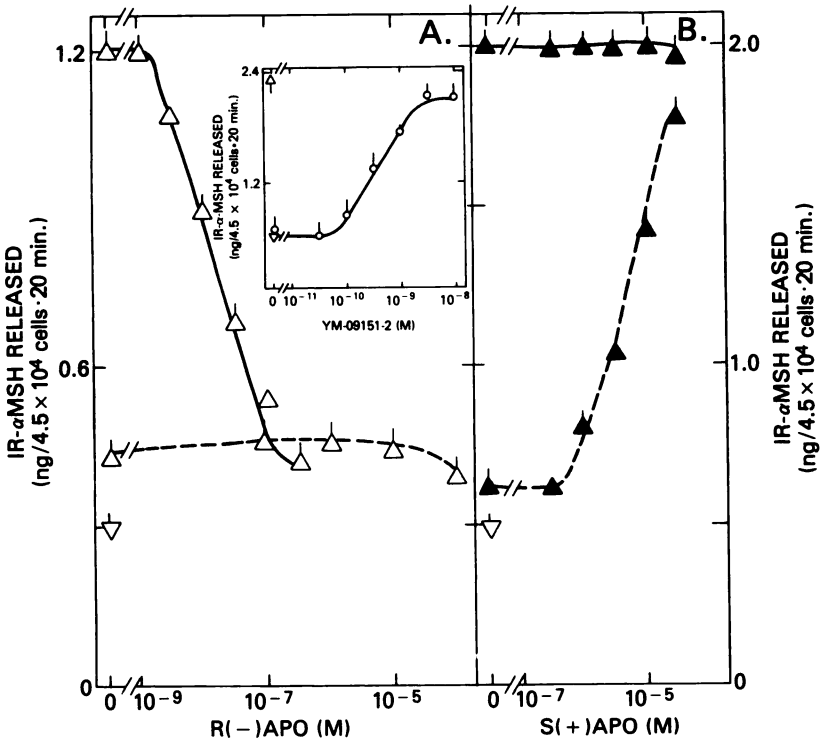

FIG. 5. *D-2 receptor: stimulation by R(−)-APO and blockade by S(+)-APO*

A. Dispersed intermediate lobe cells (4.5×10^4 cells/incubation tube) were incubated for 20 min in a volume of 1.0 ml in the absence of added drugs (▽) or in the presence of a combination of either (−)-isoproterenol (1 μM) and the indicated concentrations of R(−)-APO (△——△) or (−)-isoproterenol (1 μM), LY 171555 (300 nM), and the indicated concentrations of R(−)-APO (△- - -△). The experimental incubation with drugs was terminated by centrifugation, and the amount of IR-αMSH released by the cells into the incubation medium was determined by radioimmunoassay. *Inset.* Dispersed intermediate lobe cells were incubated for 20 min in the absence of added drugs (▽) or in the presence of either (−)-isoproterenol (1 μM, △) or (−)-isoproterenol and LY 171555 (1 μM and 0.3 μM, respectively) and the indicated concentrations of YM-09151-2 (○). Data represent means ± standard error ($n = 3$) of observations in a single experiment; similar observations were made in three additional experiments. Alone, YM-09151-2 does not diminish the (−)-isoproterenol-stimulated release of IR-αMSH (data not shown).

B. In an experiment similar to that shown in A, dispersed intemediate lobe cells were incubated in the absence of added drugs (▽) or in the presence of a combination of either (−)-isoproterenol (1 μM) and the indicated concentrations of S(+)-APO (▲——▲) or (−)-isoproterenol (1 μM), LY 171555 (300 nM), and the indicated concentrations of S(+)-APO (▲— — —▲).

Data in each panel represent means ± standard error of data obtained from three replicate samples. Each panel represents a separate experiment.

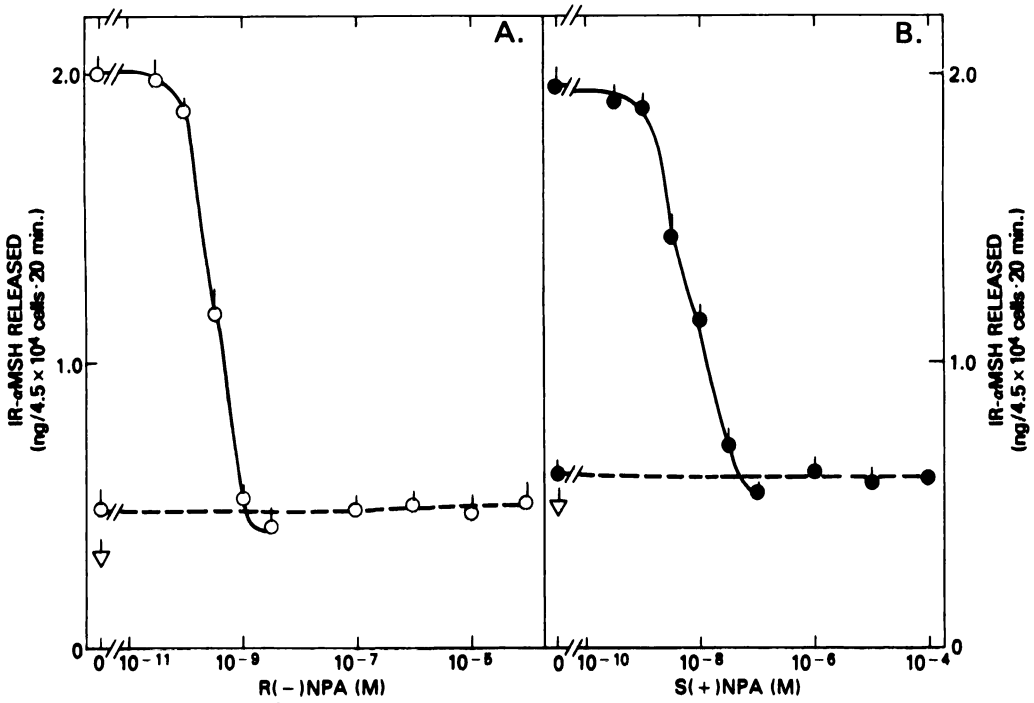

FIG. 6. *D-2 receptor: stimulation by R(−)- or S(+)-NPA*

A. In an experiment similar to those shown in Fig. 5, dispersed intermediate lobe cells were incubated in the absence of added drugs (▽) or in the presence of a combination of either (−)-isoproterenol (1 μM) and the indicated concentrations of R(−)-NPA (○——○) or (−)-isoproterenol (1 μM), LY 171555 (300 nM), and the indicated concentrations of R(−)-NPA (○- - -○). Data represent means ± standard error (n = 3) of the amount of IR-αMSH released during the experimental incubations.

B. In a separate experiment, dispersed intermediate lobe cells were incubated in the absence of added drugs (▽) or in the presence of a combination of either (−)-isoproterenol (1 μM) and the indicated concentrations of S(+)-NPA (●——●) or (−)-isoproterenol (1 μM), LY 171555 (300 nM), and the indicated concentrations of S(+)-NPA (●- — -●). Data represent means ± standard error (n = 3) of the amount of IR-αMSH released during the experimental incubations.

S(+)-APO blocked the D-2 receptor. S(+)-APO antagonized the inhibitory effect of LY 171555 on the (−)-isoproterenol-stimulated release of IR-αMSH (Fig. 5B). Assuming a kinetic competition between S(+)-APO and LY 171555 for the D-2 dopamine receptor, the affinity of S(+)-APO for the D-2 dopamine receptor was calculated to be 495 nM (Table 1). S(+)-APO did not stimulate the D-2 receptor; this aporphine did not mimic the inhibitory effect of LY 171555 upon (−)-isoproterenol-stimulated release of IR-αMSH (Fig. 5B).[4]

Both enantiomers of NPA were agonists upon the D-2 receptor. Either R(−)- of S(+)-NPA mimicked the ability of LY 171555 to attenuate (−)-isoproterenol-stimulated release of IR-αMSH (Fig. 6A and B). R(−)-NPA was approximately 45-fold more potent than S(+)-NPA as a D-2 agonist (Fig. 6; Table 1). Neither R(−)- nor S(+)-NPA displayed any activity as an antagonist of the D-2 receptor (Fig. 6A and B).

[4] Additional experiments determining adenylate cyclase activity in cell-free homogenates of cholera toxin-treated intermediate lobe tissue (10) demonstrated that R(−)-APO is a dopaminergic agonist with an apparent affinity toward the D-2 receptor of 360 ± 50 nM (mean ± standard error, n = 3); in this cell-free assay system, S(+)-APO is a D-2 antagonist with an apparent K_i toward the D-2 receptor of 8.5 ± 3.1 μM (mean ± standard error, n = 4) (Y. Itoh, and J. W. Kebabian, unpublished observations). Difference between the potencies of D-2 agonists and antagonists upon intact intermediate lobe cells and cell-free homogenates of intermediate lobe tissue have been observed previously (16, 17).

DISCUSSION

The present data provide insight into the structural and stereochemical requirements for dopaminergic antagonists. S(+)-APO is distinguished from the other aporphines tested by its ability to block either the D-1 or the D-2 dopamine receptor. Some (but not all) previously published behavioral studies suggest that S(+)-APO is a dopamine antagonist (5, 7); however, these behavioral studies do not identify the type of dopamine receptor blocked by S(+)-APO. The ability of S(+)-APO to block the D-1 dopamine receptor of teleost retina (or other tissues) has not been previously reported. The ability of S(+)-APO to block the intermediate lobe D-2 dopamine receptor is in accord with the previous demonstration that S(+)-APO blocks striatal dopamine receptors modulating the depolarization-evoked release of [^{3}H]acetylcholine or [^{3}H]dopamine (8). Both of these striatal dopamine receptors are D-2 receptors (18).

It is worthwhile to consider the structural features of S(+)-APO contributing to its ability to block dopamine receptors (Fig. 1). Since R(−)-APO is an agonist upon either the D-1 or the D-2 receptor and S(+)-APO blocks these receptors, one or more of the structural changes accompanying the stereochemical inversion of carbon atom 6a must cause the loss of dopamine agonist activity. The supposition that the S(+) configuration of APO causes it to possess dopamine receptor antagonist activity is in accord with the observation that S(+)-bulbocapnine, which possesses both an N-methyl group at position

6 and S(+) stereochemistry at position 6a (Fig. 1), blocks the D-1 receptor in mammalian neostriatum and carp retina as well as the D-2 receptor in the rat intermediate lobe (19).[5] When tested upon the DA-1 and the DA-2 dopamine receptors in the vascular system, S(+)-bulbocapnine also blocks these dopamine receptors (20).

The "*N*-methyl antagonist phenomenon" described above may account for the ability of lergotrile and lisuride to block the D-1 receptor (21–23). Since S(+)-NPA does not block either the D-1 or the D-2 dopamine receptors, it appears that the methyl substitution upon the tertiary amine at position 6 confers dopamine antagonist activity of S(+)-APO. Both lisuride and lergotrile possess an *N*-methylated tertiary amine (Fig. 1) and can be oriented so as to resemble S(+)-APO or S(+)-bulbocapnine. Since each of these ergots stimulates the D-2 receptor (11), it is obvious that the structural requirements for antagonists of the D-1 and the D-2 dopamine receptors are dissimilar.

The ability of *N*-*n*-propyl substitution to increase the dopamine agonist activity of apomorphine is well known (see ref. 24 for a summary). Likewise di-*N*-*n*-propyl dopamine is a dopamine agonist (25). Recently, this effect of *N*-propyl substitution was designated as the "*N*-propyl phenomenon" (26). The effects of R(−)-NPA and R(−)-APO demonstrated in the present study show that the D-1 and the D-2 dopamine receptors can be distinguished by their manifestation of the "*n*-propyl phenomenon." At the D-1 receptor, R(−)-NPA displays greater intrinsic activity than does R(−)-APO; however, the affinities toward the D-1 receptor of these two aporphines are similar (Table 1). At the D-2 receptor, the intrinsic activities of R(−)-NPA and R(−)-APO are similar; both compounds are full agonists. However, the affinity toward the D-2 receptor of R(−)-NPA is 45-fold greater than the affinity of R(−)-APO. These differences in the intrinsic activity and apparent affinity of the *N*-*n*-propylated and the *N*-methylated aporphines is in accord with the hypothesis that the D-1 and the D-2 dopamine receptors are distinct pharmacological entities (1).

[5] Y. Itoh, M. E. Goldman, and J. W. Kebabian, unpublished observations.

REFERENCES

1. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature* (*Lond.*) **277**:93–96 (1979).
2. Seiler, M. P., and R. Markstein. Further characterization of structural requirements for agonists at the striatal dopamine D-1 receptor: studies with a series of monohydroxyaminotetralins on dopamine-sensitive adenylate cyclase and a comparison with dopamine receptor binding. *Mol Pharmacol.* **22**:281–289 (1982).
3. Titus, R. D., E. C. Kornfeld, N. D. Jones, J. A. Clemens, E. B. Smalstig, R. W. Fuller, R. A. Hahn, M. D. Hynes, N. R. Mason, D. T. Wong, and M. M. Foreman. The resolution and absolute configuration of an ergoline-related dopamine agonist, *trans*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H(or 2H)-pyrazolo[3,4-g]quinoline. *J. Med. Chem.* **26**:1112–1116 (1983).
4. Kaiser, C. Stereoisomeric probes of the dopamine receptor, in *Dopamine Receptors*. American Chemical Society Symposium Series 224. American Chemical Society Press, Washington, D. C., 223–246 (1983).
5. Saari, W. S., S. W. King, and V. J. Lotti. Synthesis and biological activity of (6aS)-10,11-dihydroxyaporphine, the optical antipode of apomorphine. *J. Med. Chem.* **16**:171–172 (1973).
6. Neumeyer, J. L., D. Reischig, G. W. Arana, A. Campbell, R. J. Baldessarini, N. S. Kula, and K. J. Watling. Aporphines. 48. Enantioselectivity of (R)-(−)- and (S)-(+)-*N*-*n*-propylnorapomorphine on dopamine receptors. *J. Med. Chem.* **26**:516–521 (1983).
7. Riffee, W. H., R. E. Wilcox, R. V. Smith, P. J. Davis, and A. Brubaker. Inhibition of R(−)-apomorphine-induced stereotypic cage-climbing behavior in mice by S-(+)-apomorphine. *Adv. Biosci.* **37**:357–362 (1982).
8. Lehmann, J., R. V., Smith, and S. Z. Langer. Stereoisomers of apomorphine differ in affinity and intrinsic activity at presynaptic dopamine receptors modulating [^{3}H]dopamine and [^{3}H]acetylcholine release in slices of cat caudate. *Eur. J. Pharmacol.* **88**:81–88 (1983).
9. Watling, K. J., J. E. Dowling, and L. L. Iversen. Dopamine receptors in the retina may all be linked to adenylate cyclase. *Nature* (*Lond.*) **281**:578–580, (1979).
10. Tsuruta, K., E. A. Frey, C. W. Grewe, T. E. Cote, R. L. Eskay, and J. W. Kebabian. Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor. *Nature* (*Lond.*) **292**:463–465 (1981).
11. Cote, T. E., R. L. Eskay, E. A. Frey, C. W. Grewe, M. Munemura, J. C. Stoof, K. Tsuruta, and J. W. Kebabian. Biochemical and physiological studies of the beta-adrenoceptor and the D-2 dopamine receptor in the intermediate lobe of the rat pituitary gland: a review. *Neuroendocrinology* **35**:217–224 (1982).
12. Goldman, M. E., M. Beaulieu, J. W. Kebabian, and R. L. Eskay. α-Melanocyte-stimulating hormone-like peptides in the intermediate lobe of the rat pituitary gland: characterization of content and release in vitro. *Endocrinology* **112**:435–441 (1983).
13. Cheng, Y. C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
14. Clement-Cormier, Y. C., J. W. Kebabian, G. L. Petzold, and P. Greengard. Dopamine-sensitive adenylate cyclase in mammalian brain: a possible site of action of antipsychotic drugs. *Proc. Natl. Acad. Sci. U. S. A.* **71**:1113–1117 (1974).
15. Grewe, C. W., E. A. Frey, T. E. Cote, and J. W. Kebabian. YM-09151-2: a potent antagonist for a peripheral D_2-dopamine receptor. *Eur J. Pharmacol.* **81**:149–152 (1982).
16. Frey, E. A., T. E. Cote, C. W. Grewe, and J. W. Kebabian. [^{3}H]Sprioperidol identifies a D-2 dopamine receptor inhibiting adenylate cyclase activity in the intermediate lobe of the rat pituitary gland. *Endocrinology* **110**:1897–1904 (1982).
17. Meunier, H., and F. Labrie. The dopamine receptor in the intermediate lobe of the rat pituitary gland is negatively coupled to adenylate cyclase. *Life Sci.* **30**:963–968 (1982).
18. Stoof, J. C. Dopamine receptors in the neostriatum: biochemical and physiological studies, in *Dopamine Receptors*. American Chemical Society Symposium Series 224. American Chemical Society Press, Washington, D. C., 117–145 (1983).
19. Miller, R. J., P. H. Kelly, and J. L. Neumeyer. Aporphines. 15. Action of aporphine alkaloids on dopaminergic mechanisms in rat brain. *Eur. J. Pharmacol.* **35**:77–83 (1976).
20. Shepperson, N. B., N. Duval, R. Massingham, and S. Z. Langer. Differential blocking effects of several dopamine receptor antagonists for peripheral pre- and postsynaptic dopamine receptors in anesthetized dogs. *J. Pharmacol. Exp. Ther.* **221**:753–761 (1982).
21. Kebabian, J. W., D. B. Calne, and P. R. Kebabian. Lergotrile mesylate: an in vivo dopamine antagonist which blocks dopamine receptors in vitro. *Commun. Psychopharmacol.* **1**:311–318 (1977).
22. Pieri, L., H. H. Keller, W. Burkhard, and M. DaPrada. Effects of lisuride and LSD on cerebral monoamine systems and hallucinosis. *Nature* (*Lond.*) **272**:278–280 (1978).
23. Brown, E. M., M. F. Attie, S. Reen, D. G. Gardner, J. Kebabian, and G. D. Aurbach. Characterization of dopaminergic receptors in dispersed bovine parathyroid cells. *Mol. Pharmacol.* **18**:335–340 (1980).
24. Menon, M. K., W. G. Clark, and J. L. Neumeyer. Comparison of the dopaminergic effects of apomorphine and (−)*N*-*n*-propyl norapomorphine. *Eur. J. Pharmacol* **52**:1–9 (1978).
25. Goldberg, L. I., P. F. Sonneville, and J. L. McNay. An investigation of the structural requirements for dopamine-like renal vasodilation: phenylethylamines and apomorphine. *J. Pharmacol. Exp. Ther.* **163**:188–197 (1968).
26. Nichols, D. E. The development of novel dopamine agonists, in *Dopamine Receptors*. American Chemical Society Symposium Series 224. American Chemical Society Press, Washington, D. C., 202–218 (1983).

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