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EFFECTS OF ISOMERS OF HYDROXYAPORPHINES ON DOPAMINE METABOLISM IN RAT BRAIN REGIONS

ROSS J. BALDESSARINI,* ELDA R. MARSH, NORA S. KULA, RUSHI ZONG, YIGONG GAO and JOHN L. NEUMEYER

Departments of Psychiatry and Neuroscience Program, Harvard Medical School, Boston, MA; Laboratories for Psychiatric Research, Mailman Research Center, McLean Division of Massachusetts General Hospital, Belmont, MA 02178; and Section of Medicinal Chemistry, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115, U.S.A.

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Abstract—The effects of isomers of di- and monohydroxyaporphines on cerebral dopamine (DA) metabolism were evaluated in representative extrapyramidal (corpus striatum) and limbic (nucleus accumbens septi) tissues of rat brain by three methods: (1) changes in the ratio of homovanillic acid (HVA) to DA, (2) accumulation of L-dihydroxyphenylalanine (DOPA) after inhibiting its decarboxylation to DA under "open-loop" conditions, as well as (3) after γ-butyrolactone (GBL) pretreatment to provide selective effects at presynaptic DA autoreceptors. The DA-agonist *R*(−) isomers of the aporphines apomorphine (APO), *N*-*n*-propylnorapomorphine (NPA), and 11-hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa) showed consistent dose-dependent inhibition of DA synthesis in both brain regions with all models; the neuroleptic haloperidol had the opposite effect in the first two models only, as expected. The *S*(+) isomers of NPA and 11-OH-NPa have shown behavioral evidence of antidopaminergic activity, especially in the limbic system. Unlike the neuroleptic, *S*(+)NPA did not show DA-synthesis enhancing actions in accumbens or striatal tissue but, instead, inhibited DA synthesis like its *R*(−) antipode in all three test paradigms. *S*(+)11-OH-NPa given alone produced minor changes in the HVA/DA ratio and did not antagonize *R*(−)11-OH-NPa, weakly increased accumulation of DOPA in the second model, and had no effect in the third—all without regional selectivity. In the test of autoreceptor functioning, the dihydroxyaporphine *S*(+)NPA, but not *S*(+)11-OH-NPa, inhibited DA synthesis and this effect, in turn, was largely reversed by haloperidol, as were the inhibitory effects of the three *R*(−)aporphines tested. In this model, however, neither *S*(+)NPA nor *S*(+)11-OH-NPa antagonized the DA-synthesis inhibiting effect of *R*(−)APO as haloperidol did. Overall, these results are consistent with evidence that *R*(−)NPA and 11-OH-NPa have high affinity at D-2 receptor sites in rat brain and show behavioral effects of typical DA agonists. The non-stereoselective inhibitory effects of NPA on DA synthesis may reflect its activity as a weak DA agonist with very low intrinsic activity, but may also include a direct "catechol-effect" on tyrosine hydroxylase. In contrast, *R*(−)11-OH-NPa appears to be a stereoselective D-2 agonist, active at autoreceptors as well as postsynaptic receptors, that lacks the nonstereospecific effects on DA metabolism of its catechol-aporphine congener. It may be a useful probe for the further characterization of dopamine receptors and autoreceptors.

The *S*(+) enantiomer of the potent dopamine (DA) agonist *R*(−)*N*-*n*-propylnorapomorphine (NPA; 10,11-dihydroxy-*N*-*n*-propylnoraporphine), rather than being relatively inert neuropharmacologically, has shown activity as a DA *antagonist* in behavioral experiments [1], and as a very weak agonist in neurophysiological studies [2, 3]. Behavioral antagonist activity of *S*(+)NPA, including responses to direct intracerebral injection of DA, appears to be selective for the limbic system and not the extrapyramidal system of mammalian forebrain [4], but an explanation of this regional selectivity remains elusive [5, 6]. Reversal of agonist and antagonist activity, or otherwise dissimilar activity, between optical isomers of agents at DA receptors has been observed with several other enantiomeric pairs as well, including the antipodes of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP) (whose pharmacology is complex) [7], *cis*-1-methyl-5-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetrahydronaphthalene (5-OH-MDAT) [8], and others [9, 10], and a stereochemical theory to account for this isomeric difference has been proposed [6, 9, 10]. Such a relationship has been reported, more recently, to occur with isomers of the novel 11-monohydroxy analog of NPA, 11-hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa; see Fig. 1) [6, 11], in which the 11-OH group is analogous to the *meta*-hydroxy group of DA.

In preliminary experiments seeking evidence of regional alterations of DA metabolism by *S*(+)NPA, we found, paradoxically, that like the *R*(−)antipode, *S*(+)NPA *decreased* DA synthesis in nucleus accumbens septi (accumbens) and corpus striatum (striatum).† The lack of an increase in DA synthesis or metabolic turnover by *S*(+)NPA seems inconsistent with its apparent antidopaminergic activity, but might reflect a nonstereoselective direct neurochemical action on tyrosine hydroxylase and its

* Address correspondence to: Dr Ross J. Baldessarini, Mailman Research Center, McLean Hospital, 115 Mill St., Belmont, MA 02178.

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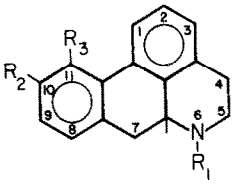

Fig. 1. Structure of aporphines.

Agent	R_1	R_2	R_3
Apomorphine (10,11-dihydroxyaporphine) (APO)	CH_3	OH	OH
10,11-Dihydroxy-*N*-*n*-propylnoraporphine (NPA)	$CH_2CH_2CH_3$	OH	OH
11-Hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa)	$CH_2CH_2CH_3$	H	OH

Optical isomerism is determined by the orientation of the proton at carbon 6a, between positions 6 and 7.

pterin cofactor, not receptor-mediated but possibly analogous to "end-product" inhibition of catecholamine synthesis by DA and other catechols [12, 13], or may reflect very weak agonistic activity [2, 3].

To test this hypothesis further, we carried out more systematic studies of DA synthesis and metabolic turnover with the enantiomers not only of the "catechol-aporphine" NPA, but also of its recently developed monohydroxy congener, 11-OH-NPa. We used three biochemical models, including the ratio of metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) to DA, the accumulation of L-dihydroxyphenylalanine (DOPA) after inhibiting its decarboxylation in an "open-loop" model in which postsynaptic, multisynaptic, and autoreceptor mechanisms can be expressed, as well as in a model believed to reflect presynaptic autoreceptor control selectively after inhibiting neurophysiological activity in ascending DA systems with γ-butyrolactone (GBL) before monitoring the accumulation of DOPA [14]. Moreover, to extend assessment of the actions of *S*(+)aporphines in limbic and extrapyramidal brain regions, we investigated DA metabolism in both the accumbens and the striatum of rat brain.

MATERIALS AND METHODS

Young adult, male, Sprague–Dawley albino rats (Charles River Laboratories, Wilmington, MA), initially weighing 200 g, were housed five per cage with free access to standard food (Purina Laboratory Rat Chow) and water in a controlled environment (lights on, 7:00 a.m. to 7:00 p.m.; temperature, 21–23°; humidity, 40–50%). Rats were adapted to the laboratory environment for at least 72 hr before treatment.

Test agents were dissolved in purified water, except that haloperidol was dissolved in a drop of 1 M lactic acid before diluting in water; physiological saline was used as a placebo-control. All treatments were given by intraperitoneal (i.p.) injection (preliminary experiments showed little difference in pharmacologic activity of 11-OH-NPa isomers by various routes of administration [15]). In initial experiments, metabolic turnover of DA was evaluated as the ratio of HVA/DA (or of [DOPAC + HVA]/DA) in brain regions of animals given a test aporphine 30 min earlier, but not otherwise pretreated. In a more direct test of the rate of synthesis of DA, the regional concentration of DOPA was assayed at 30 min following pretreatment with a large, centrally effective dose of the inhibitor of aromatic L-amino acid decarboxylase, NSD-1015 (*m*-hydroxybenzylhydrazine dihydrochloride; Sigma Chemical Co., St. Louis, MO; 100 mg/kg, i.p.), and test agents were administered at 35 min before the animals were killed. In a test of the interactions of test agents with putative presynaptic autoreceptors of DA in neurophysiologically isolated nigrostriatal and mesolimbic DA projection systems [14], additional pretreatment with GBL (Sigma Chemical Co.; 750 mg/kg, i.p.) was given at 40 min before the rats were killed, and brain regions again were assayed for DOPA 30 min after NSD-1015. When more than one test agent was given (e.g. haloperidol followed by an aporphine), GBL was administered at 40 min, and the two test agents at 45 min (antagonists), and 35 min (agonists) before sacrifice. *R*(−)Apomorphine hydrochloride hemihydrate (APO) was obtained from McFarlan-Smith (Edinburgh, Scotland); *R*(−) and *S*(+)*N*-*n*-propylnorapomorphine (NPA) were obtained from Research Biochemicals, Inc. (RBI, Natick, MA); and *R*(−) and *S*(+)11-hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa) were synthesized and characterized in the Section of Medicinal Chemistry at Northeastern University. Haloperidol was a gift of McNeil Laboratories (Ft. Washington, PA).

Brains of rats killed by decapitation were removed and rapidly dissected on ice. Portions of corpus striatum (*ca.* 40 mg) and nucleus accumbens septi (*ca.* 10 mg) were weighed electronically, frozen on dry ice, and stored at −70° until prepared for assay. Individual tissue samples were homogenized in 1.0 mL of 0.1 N perchloric acid (PCA) solution containing 0.1 mM disodium EDTA, 0.4 mM sodium metabisulfite and 3,4-dihydroxybenzylamine (Sigma Chemical Co.) as an internal standard. After centrifugation at 9000 *g* for 5 min, the supernatant fraction was divided into two portions (used for assay of DA and DOPA, or of DOPAC and HVA). The high performance liquid chromatographic (HPLC) separation and electrochemical (EC) detection

methods used were slightly modified from those reported previously [16–18]. Each assay system consisted of a Rainin (Woburn, MA) Rabbit HP Solvent Delivery System, separated on a Rainin C18 reverse phase column (4.6 mm × 25 cm; 5 μm particle size), linked to a Bioanalytical Systems (BAS, Lafayette, IN) model LC-4B amperometric detector.

DA and DOPA were assayed as follows: 0.4 mL of sample supernatant was added to 1.0 mL of 2 M Tris–HCl (pH 8.6) containing 0.05 mM disodium EDTA and 10 mg of acid-washed alumina (Bioanalytical Systems) and mixed for 10 min. Following centrifugation at 9000 *g* for 5 min, the supernatant fraction was discarded and the alumina pellet was washed with 6 mM Tris–HCl (pH 8.6), then 5 mM potassium dihydrogen phosphate (pH 7.0); the supernatant fraction was aspirated to waste after each wash. To elute catechols, 0.4 mL of 0.1 N PCA was added to the alumina pellets, mixed for 10 min, and centrifuged again at 9000 *g* for 10 min. An aliquot of this acid extract supernatant (100 μL) was injected into the HPLC system. The mobile phase consisted of 0.5 M sodium acetate, 4.2 mM 1-heptane sulfonic acid, 0.2 mM disodium EDTA and 1.5% (v/v) acetonitrile (all HPLC grade, Fisher Scientific, Medford, MA) adjusted to pH 3.5 with glacial acetic acid, and was run at 1.0 mL/min. The catechols were detected at +0.55 V using a glassy carbon working electrode versus an Ag/AgCl reference electrode.

DOPAC and HVA were assayed by injecting 100 μL of supernatant fraction of the original tissue sample extract directly into the HPLC system. This mobile phase consisted of 0.1 M sodium acetate, 0.2 mM disodium EDTA, and 12% (v/v) methanol (all HPLC grade, Fisher Scientific), adjusted to pH 4.6 with glacial acetic acid and was run at 1.2 mL/min. These acidic compounds were detected at +0.75 V using the same type of electrode system.

Amperometric EC signals from unknown samples were quantified by a microcomputer program that compared EC signals from experimental samples to those from pure standards (Sigma Chemical Co.). These methods routinely provide adequate separation and sensitivity to detect sub-ng levels of analytes in ≤10 mg of brain tissue. Overall recovery averaged 91% for DA and 79% for DOPA. Within-sample ratios of acidic metabolite to parent amine were computed as a convenient, widely employed index of metabolite turnover ([HVA]/[DA]) [16–18]. Similar effects also were found with [DOPAC + HVA]/[DA]. In the present experiments, the raw EC signal strength with standard solutions showed a within-experiment and between-days variance (SD/mean) of ≤5%; in addition, results between pairs of independent replicate experiments on saline-treated control subjects correlated closely ($r > 0.95$).

Data are presented as means ± SE. To facilitate presentation of large numbers of conditions and comparisons, as well as replications of virtually all experimental conditions, data routinely are expressed as the mean ± SE percent of a relevant placebo-treated control condition, with an average of seven to nine subjects per condition, and only representative data are shown. Saline control results were found not to vary significantly between experiments and so were pooled. SE values for percentage-of-control data were computed by a method that accounts for variance in numerator and denominator [19]. Treatment effects were evaluated by nonparametric methods for ratio data (Mann–Whitney U-test); parametric data were evaluated by *t*-test when appropriate, and results were considered statistically significant when $P \leq 0.05$ (two-tailed). Correlations were evaluated by least-squares linear regression to determine Pearson's *r*.

RESULTS

Results obtained from HPLC–EC assays of DA and its metabolites in regions of brain tissue from control (saline-treated) rats were in good agreement with expectations based on previous publications [14, 16, 17]. Control values (after saline injections) for DA averaged 8.39 ± 0.60 μg/g for striatal tissue and 6.88 ± 0.55 μg/g wet tissue wt for accumbens (N = 30). Other relevant control data are shown in the figure legends.

In the first model of evaluating DA metabolism by changes in the ratio of its acidic metabolites to DA itself, similar results were obtained with the ratio HVA/DA (generally with slightly greater sensitivity and precision) as with [DOPAC + HVA]/DA, and only the former ratio is presented. For example, in striatal tissue, treatment with *R*(−)APO (1.0 mg/kg) reduced the ratio [HVA + DOPAC]/DA to 49%, and HVA/DA to 46% of saline control values, whereas acute treatment with haloperidol (1.0 mg/kg) increased these ratios to 221% and 275% of control, respectively. Also, when values for these two ratios involving 38 conditions of drug treatments and doses for both brain regions were assessed overall, the results correlated very closely ($r = 0.973$). As expected, the ratio of HVA/DA increased significantly in both brain regions after treatment with the DA antagonist haloperidol, and decreased after treatment with the DA agonist *R*(−)aporphines, APO, NPA, and 11-OH-NPa; this effect of *R*(−)-NPA was dose dependent and this agent appeared to be particularly potent. *S*(+)NPA also significantly decreased the metabolite/DA ratio, but *S*(+)11-OH-NPa had little or no effect, in doses up to 10 mg/kg alone, and did not alter the effect of *R*(−)11-OH-NPa. These effects were very similar in both brain regions tested (Fig. 2).

In the second model (Fig. 3), the accumulation of DOPA for 30 min after inhibiting its decarboxylation followed a similar pattern as found with the first model. That is, this index of DA synthesis showed a marked increase after haloperidol, and dose-dependent decreases after treatment with the *R*(−)aporphines APO, NPA and 11-OH-NPa; *R*(−)11-OH-NPa appeared to require somewhat higher doses than *R*(−)NPA. Similar to its actions in the first model, *S*(+)NPA also *decreased* the accumulation of DOPA, but appeared to be somewhat less potent than its *R*(−)isomer. In contrast to the DOPA-decreasing effect of *S*(+)NPA and the large increase induced by a small dose of haloperidol, *S*(+)11-OH-NPa increased the accumulation of DOPA only at a dose of 10 mg/kg.

In the third model, believed to reflect DA auto-

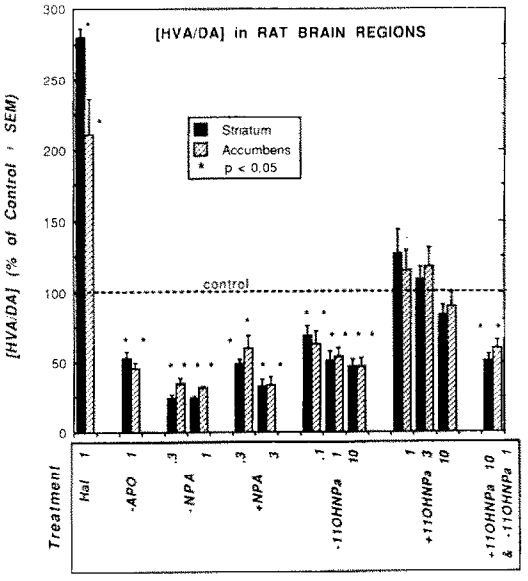

Fig. 2. [HVA/DA] in rat brain regions. Rats (mean N = 8) were pretreated 30 min before sacrifice with saline (control condition) or a test agent (shown in the bottom panel), as follows: haloperidol (Hal, 1 mg/kg), *R*(−)-apomorphine (−APO, 1 mg/kg), *R*(−)*N*-*n*-propylnorapomorphine (−NPA, 0.3 or 1 mg/kg), *S*(+)*N*-*n*-propylnorapomorphine (+NPA, 0.3 or 3 mg/kg), *R*(−)11-hydroxy-*N*-*n*-propylnoraporphine (−11-OH-NPa, 0.1, 1, or 10 mg/kg), *S*(+)11-hydroxy-*N*-*n*-propylnoraporphine (+11-OH-NPa, 1, 3 or 10 mg/kg), or +11-OH-NPa (10 mg/kg) with −11-OH-NPa (1 mg/kg). Data are the mean ± SE percent of control (saline condition) for corpus striatum (solid bars) and nucleus accumbens septi (shaded bars). Statistically significant differences from the saline-control condition, by U-test ($P \leq 0.05$) are indicated by an asterisk (*). Mean ± SE control values (N = 30) for HVA/DA = 0.080 ± 0.007 for striatum and 0.092 ± 0.009 for accumbens.

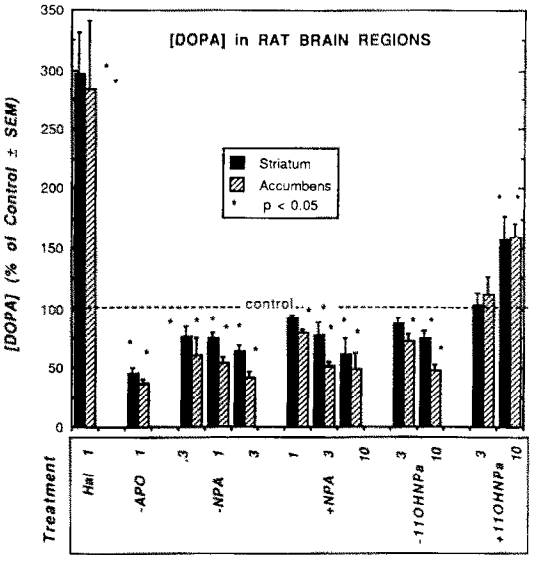

Fig. 3. [DOPA] in rat brain regions. Rats (mean N = 7) were treated with NSD-1015 to prevent conversion of DOPA to DA, and extrapyramidal (striatum, solid bars) and limbic (accumbens, shaded bars) brain regions were assayed for accumulation of DOPA for 30 min. Pretreatments given at 35 min before sacrifice (see bottom panel) were: haloperidol (Hal, 1 mg/kg), *R*(−)-apomorphine (−APO, 1 mg/kg), *R*(−)*N*-*n*-propylnorapomorphine (−NPA, 0.3, 1, or 3 mg/kg), *S*(+)*N*-*n*-propylnorapomorphine (+NPA, 1, 3, or 10 mg/kg, *R*(−)11-hydroxy-*N*-*n*-propylnoraporphine (−11-OH-NPa, 3 or 10 mg/kg), or *S*(+)11-hydroxy-*N*-*n*-propylnoraporphine (+11-OH-NPa, 3 or 10 mg/kg). Data are mean percent of saline control values ± SE; statistical significance (U-test, $P \leq 0.05$) is indicated by an asterisk (*). Control values (N = 18) for accumulation of DOPA = 1.60 ± 0.07 μg/g/30 min for striatum and 1.77 ± 0.11 μg/g/30 min for accumbens, and values for DA were 8.39 ± 0.60 μg/g and 6.88 ± 0.55 μg/g, respectively.

receptor mechanisms, the accumulation of DOPA was measured after pretreatment with GBL. GBL induced an additional increase of DOPA accumulation over that induced by NSD-1015 alone, by 99 ± 5.8% in striatum and 46 ± 1.8% in accumbens, respectively ($P < 0.05$ by *t*-test). The findings obtained (Fig. 4) were consistent with those of the first two "open-loop" models which can include reflections of postsynaptic and multisynaptic mechanisms as well as those mediated by presynaptic autoreceptors. Again, the DA agonist *R*(−)isomers of APO, NPA, and 11-OH-NPa all induced dose-dependent reductions of the accumulation of DOPA, suggesting that they all have agonist activity at DA autoreceptors in both brain regions tested. In this model, haloperidol alone failed to exert a significant DA-synthesis stimulating effect, as expected, but pretreatment with this typical, partly D-2 selective, neuroleptic blocked the DA-synthesis diminishing action of the *R*(−)isomers of APO, NPA and 11-OH-NPa, and also largely antagonized the DA-synthesis inhibiting action of *S*(+)NPA (Fig. 4). When *S*(+)NPA was given with *R*(−)APO, there was no reversal of the reduced accumulation of DOPA, which was similar to, or slightly greater than (not significant), that observed with APO alone. *S*(+)11-OH-NPa—up to a dose of 10 mg/kg, or about ten times higher than behavioral ED_{50} values [15]—had no effect on DOPA accumulation after GBL nor significant antagonistic interaction with *R*(−)APO (Fig. 4).

DISCUSSION

The results of the present experiments indicate that: (1) the methods employed were sensitive to the DA-synthesis and metabolism increasing and decreasing effects of standard DA agonists (*R*[−]APO and NPA) and an antagonist (haloperidol); (2) the *R*(−) isomer of 11-OH-NPa mimicked the effects of *R*(−)APO and NPA; (3) the autoreceptor effects of these DA agonist aporphines were reversed by haloperidol pretreatment; (4) *S*(+)NPA not only failed to block the DA-synthesis decreasing actions of the *R*(−) isomers of APO or NPA, but (5) this dihydroxy ("catechol") aporphine exerted relatively nonstereoselective DA-synthesis inhibiting actions not found with the monohydroxy

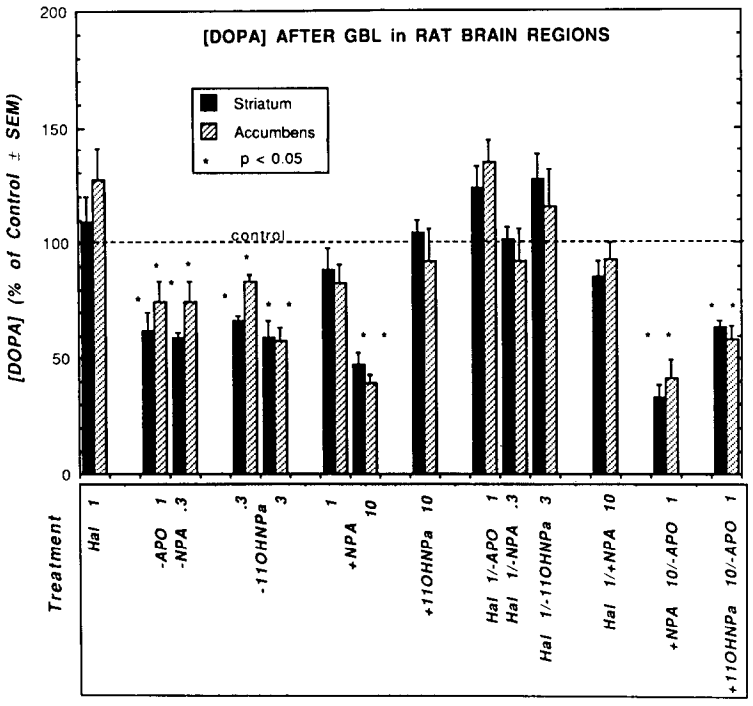

Fig. 4. [DOPA] in rat brain regions after pretreatment with GBL. Rats (mean N = 9) were pretreated with GBL (at −40 min) to block activity in ascending cerebral DA pathways, and treated with NSD-1015 (as for Fig. 3) 10 min later; brain regions (striatum, solid bars; and accumbens, shaded bars) were assayed for DOPA accumulation 30 min later. Test treatments (see bottom panel) given at 35 min before sacrifice were: haloperidol (Hal, 1 mg/kg), *R*(−)apomorphine (−APO, 1 mg/kg), *R*(−)*N*-*n*-propylnorapomorphine (−NPA, 0.3 mg/kg), *R*(−)11-hydroxy-*N*-*n*-propylnoraporphine (−11-OH-NPa, 0.3 or 3 mg/kg), *S*(+)*N*-*n*-propylnorapomorphine (+NPA, 1 or 10 mg/kg), or *S*(+)11-hydroxy-*N*-*n*-propylnoraporphine (+11-OH-NPa, 10 mg/kg); or the combination (at 45 and 35 min before sacrifice) of Hal (1 mg/kg) with −APO (1 mg/kg), −NPA (0.3 mg/kg), or −11-OH-NPa (3 mg/kg; or of +NPA (10 mg/kg) or +11-OH-NPa (10 mg/kg) with −APO (1 mg/kg; all i.p.). Data are mean ± SE percent of control values from saline and GBL-treated rats. Mean control DOPA accumulation rate (N = 36) = 3.18 ± 0.06 μg/g/30 min for stratium and 2.59 ± 0.15 μg/g/30 min for accumbens. Statistical significance (U-test; $P \leq 0.05$) is indicated by an asterisk (*); there was also a significant difference between the −APO-, −NPA-, 11-OH-NPa, or +NPA-alone conditions vs Hal added.

congener *S*(+)11-OH-NPa. Several of these findings require clarification and comment.

The very limited ability of *S*(+)11-OH-NPa to increase DA turnover (Figs. 2 and 3) or to block the DA-synthesis inhibiting effects of *R*(−)11-OH-NPa (Fig. 2) or APO (Fig. 4) seems inconsistent with its apparent antidopaminergic activity found recently in behavioral experiments [6, 11, 15]. Since the ED_{50} values for behavioral effects of the isomers of *R*(−)11-OH-NPa were 1–2 mg/kg, i.p. [11, 15], the doses given in the present experiments (up to 10 mg/kg) should have been adequate to yield DA antagonistic effects. The lack of antagonism by *S*(+)11-OH-NPa or *R*(−)APO (Fig. 4) is unlikely to reflect direct inhibition of tyrosine hydroxylase by the catechol-aporphine since the effect of APO in the autoreceptor model was reversed with haloperidol (Fig. 4). Moreover, *S*(+)11-OH-NPa failed to alter the HVA/DA decreasing action of the monohydroxy-aporphine *R*(−)11-OH-NPa (Fig. 2). Thus, the significance of the lack of consistent DA metabolism-increasing actions, or of an antagonistic effect of *S*(+)11-OH-NPa versus DA-agonist *R*(−)aporphines observed here is not clear. Perhaps the apparently antidopaminergic behavioral effects of the *S*(+)*N*-*n*-propylnoraporphines, in contrast to typical neuroleptic agents, reflect activity as very weak partial DA agonists [2, 3, 18] or, possibly, they may be mediated by undefined actions other than effects at DA receptors. Neither *S*(+)aporphine tested was regionally selective neurochemically, nor found to antagonize the DA-synthesis diminishing action of *R*(−)APO (Figs. 2–4), and so the present findings leave the behavioral evidence of apparently *limbic-selective* antidopaminergic actions of the *S*(+)*N*-*n*-propylnoraporphines [1, 4–6, 15] unexplained.

Evidence of antidopaminergic activity of *S*(+)NPA, although strongly supported in behavioral experiments [1, 4], may not have been observable in the present biochemical experiments if this dihydroxyaporphine has other actions tending to decrease synthesis of DA. The striking difference in stereoselectivity between the dihydroxyaporphine NPA and its monohydroxy congener 11-OH-NPa in

the present experiments, as well as *in vitro* experiments with rat striatal tissue [13], may reflect the tendency for many catechols to inhibit tyrosine hydroxylase by a direct biochemical effect, probably at the site of interaction of its pterin cofactor [20–23]. This view is consistent with previous observations of inhibition of tyrosine hydroxylase by catecholaporphines [21–23], but not a monohydroxy DA-agonist aminotetralin analog of DA [24]. On the other hand, in both the present experiments (Fig. 4) and our *in vitro* studies [13], neuroleptic DA antagonists with strong anti-D-2 activity largely blocked the DA-synthesis inhibiting actions of *S*(+)- as well as *R*(−)NPA, suggesting again that *S*(+)NPA may act as a *dopaminergic agonist with very weak intrinsic activity* (i.e. potentially active as a *functional antagonist* with respect to agonists of higher intrinsic activity, such as *R*[−] aporphines or DA itself) as has been suggested recently in neurophysiological experiments which evaluated DA autoreceptor mechanisms *in vivo* [2, 3].

The present findings extend the impression [11, 15] that the monohydroxyaporphine *R*(−)11-OH-NPa is an effective DA agonist, at autoreceptors as well as postsynaptic receptors, while the *S*(+) isomer lacks such activity and shows no evidence of important DA antagonistic activity in the present experiments. Very recently, it has been found that, while *R*(−)11-OH-NPa is even a more potent, full DA agonist than is *R*(−)APO, the *S*(+)isomer, unlike *S*(+)NPA, was inactive in an *in vivo* neurophysiological test of nigral DA autoreceptor-mediated mechanisms; *S*(+)11-OH-NPa, however, did produce a rightward shift (competitive antagonism) of an equimolar dose of *R*(−)APO [25]. Both enantiomers of 11-OH-NPa show selective affinity for D-2 receptor sites in radioreceptor assays [11]. This activity accords well with the current theory that DA autoreceptors regulating DA synthesis at the tyrosine hydroxylase step are of the D-2 type [14]. DA autoreceptors remain incompletely characterized, and the molecular basis of autoreceptor-mediated actions controlling DA synthesis and release from nerve terminals remains unknown [14, 24]. We propose that compounds such as *R*(−)11-OH-NPa (or other analogs), as effective stereoselective non-catechol D-2 agonists, represent an advantageous probe for further exploration of DA receptors and, particularly, autoreceptor mechanisms.

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REFERENCES

1. Campbell A, Baldessarini RJ, Teicher MH and Neumeyer JL, Behavioral effects of apomorphine isomers in the rat: Selective locomotor-inhibitory effects of *S*(+)*N*-*n*-propylnorapomorphine. *Psychopharmacology* **88**: 158–164, 1986.
2. Cox RF, Neumeyer JL and Waszczak BL, Effects of *N*-*n*-propylnorapomorphine enantiomers on single unit activity of substantia nigra pars compacts and ventral tegmental area dopamine neurons. *J Pharmacol Exp Ther* **247**: 355–362, 1988.
3. Cox RF and Waszczak BL, 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.
4. Campbell A, Baldessarini RJ, Teicher MH and Neumeyer JL, *S*(+)Aporphines: selective inhibition of excitatory effects of dopamine injected into the limbic system of the rat. *Neuropharmacology* **24**: 391–399, 1985.
5. Kula NS, Baldessarini RJ, Bromley S and Neumeyer JL, Effects of isomers of aporphines on dopamine receptors in striatal and limbic tissue of rat brain. *Life Sci* **37**: 1051–1057, 1985.
6. Neumeyer JL, Froimowitz M, Baldessarini RJ, Campbell A and Gao Y, Neuropharmacology and stereochemistry of dopamine receptor agonist and antagonist enantiomeric pairs. *J Recept Res* **31**: 83–96, 1988.
7. Wikstrom H, Sanchez D, Lindberg P, Hacksell U, Arvidsson L-E, Johansson AM, Thorberg S-O, Nilsson JLG, Svensson K, Hjorth S, Clark D and Carlsson A, Resolved 3-(3-hydroxyphenyl)*N*-*n*-propylpiperidine and its analogues: central dopamine receptor activity. *J Med Chem* **27**: 1030–1036, 1984.
8. Johansson AM, Arvidsson L-E, Hacksell U, Nilsson JLG, Svensson K, Hjorth S, Clark D, Carlsson A, Sanchez D, Andersson B and Wikstrom H, Novel dopamine receptor agonists and antagonists with preferential action of autoreceptors. *J Med Chem* **28**: 1049–1053, 1985.
9. Froimowitz M, Neumeyer JL and Baldessarini RJ, A stereochemical explanation of the dopamine agonist and antagonist activity of stereoisomeric pairs. *J Med Chem* **29**: 1570–1573, 1986.
10. Froimowitz M and Baldessarini RJ, A stereochemical and conformational model of dopaminergic agonist and antagonist activity: further evaluation. *J Pharm Sci* **76**: 557–564, 1987.
11. Gao Y, Zong R, Campbell A, Kula NS, Baldessarini RJ and Neumeyer JL, Synthesis and dopamine agonist and antagonist effects of (*R*)(−) and (*S*)(+)-11-hydroxy-*N*-*n*-propylnoraporphine. *J Med Chem* **31**: 1392–1396, 1988.
12. Musacchio JM, Enzymes involved in the biosynthesis and degradation of catecholamines. In: *Handbook of Psychopharmacology* (Eds. Iversen LL, Iversen SD and Snyder SH), Vol. 3, Chap. 1, pp. 1–35. Plenum Press, New York, 1975.
13. Booth R, Kula NS and Baldessarini RJ, Effects of mono- and dihydroxyaporphines on tyrosine hydroxylase activity in striatal tissue of rat brain *in vitro*, *Soc Neurosci Abstr* **15**: 427, 1989.
14. Wolf ME and Roth RH, Dopamine autoreceptors. In: *Dopamine Receptors* (Eds. Creese I and Fraser CM), Chap. 3, pp. 45–96. Alan R. Liss, New York, 1987.
15. Campbell A, Baldessarini RJ and Neumeyer JL, *R*(−) and *S*(+) Stereoisomers of 11-hydroxy- and 11-methoxy-*N*-*n*-propylnoraporphine: Central dopaminergic behavioral activity in the rat. *Neuropharmacology*, in press.
16. Finklestein SP, Campbell A, Stoll AL, Baldessarini RJ, Stinus L, Paskevich PA and Domesick VB, Changes in cortical and subcortical levels of monoamines and their metabolites following unilateral ventrolateral cortical lesions in the rat. *Brain Res* **271**: 279–288, 1983.
17. Baldessarini RJ, Kula NS, Francoeur D, Finklestein SP, Murphy F and Neumeyer JL, Synthetic analgesics and other phenylpiperidines: effects on uptake and storage of dopamine and other monoamines in mouse forebrain tissue. *Life Sci* **39**: 1765–1777, 1986.

18. Campbell A, Baldessarini RJ, Cremens MC, Teicher MH, Marsh E and Kula NS, Bromocriptine antagonizes behavioral effects of cocaine in the rat. *Neuropsychopharmacology* **2**: 209–224, 1989.
19. McLean RA and Welch BL, A common error in assessing the significance of percentage change in neuropharmacology. *J Pharm Pharmacol* **23**: 643–645, 1971.
20. Weiner N, Tyrosine-3-mono-oxygenase (tyrosine hydroxylase). In: *Aromatic Amino Acid Hydroxylases: Biochemical and Physiological Aspects* (Ed. Youdim MBH), pp. 141–190. John Wiley, New York, 1979.
21. Iversen LL, Rogawski MA and Miller RJ, Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic mechanisms in the rat striatum. *Mol Pharmacol* **12**: 251–262, 1976.
22. Brautigam M, Kittner B and Laschinski G, Effects of apomorphine enantiomers and of lisuride on 3,4-dihydroxyphenylalanine production in striatal synaptosomes. *Mol Pharmacol* **28**: 515–520, 1985.
23. Saller CF and Salama AI, Apomorphine enantiomers' effects on dopamine metabolism: receptor and non-receptor related actions. *Eur J Pharmacol* **121**: 181–188, 1986.
24. Waggoner WG, McDermed J and Leighton HJ, Presynaptic regulation of tyrosine hydroxylase activity in rat striatal synaptosomes by dopamine analogs. *Mol Pharmacol* **18**: 91–99, 1980.
25. Martin LP, Cox RF and Waszczak BL, Efficacy and potency comparisons among aporphine enantiomers: effects on dopamine neurons in substantia nigra of rat. *Neuropharmacology* **29**: 135–144, 1990.