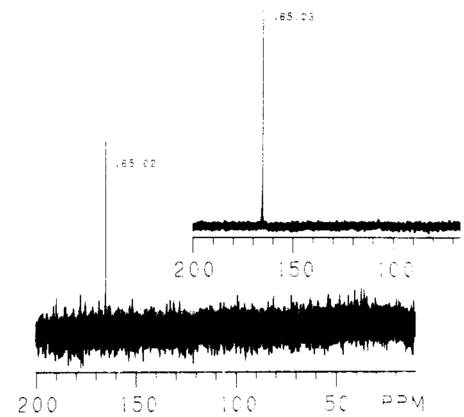

Figure 3. ^{13}C NMR spectrum of the [^{13}C]HCN (as [^{13}C]KCN) formed in the oxidation of [^{13}C]cyanamide by catalase/glucose-glucose oxidase. The inset shows authentic [^{13}C]KCN in 0.1 N KOD/D_2O. Incubations were carried out in sealed Erlenmeyer flasks with a suspended center well (Kontes, Vineland, NJ) containing 400 μL of 0.1 N KOD in D_2O in a shaking water bath at 37 °C for 1 h. The incubation mixture consisted of potassium phosphate buffer (100 mM, pD 7.4), bovine liver catalase (4 mg, 56 400 units), ^{13}C-free glucose oxidase (0.1 mg, 10.8 units), glucose (10 mM), [^{13}C]cyanamide (93 μmol), and bovine methemoglobin (16.8 mg) in a total volume of 2.0 mL. The reactions were initiated by the addition of glucose oxidase and were quenched by the addition of 0.5 mL of concentrated phosphoric acid through the rubber septum. This released the [^{13}C]HCN bound to methemoglobin for collection in the center well. After further equilibration at 37 °C for 30 min, the reaction mixture was allowed to stand overnight. The contents of the KOD trap from two identical reactions were then combined for determination of [^{13}C]cyanide by FT/NMR on a Nicolet NT-300WB NMR spectrometer. Control incubations lacked either catalase or glucose oxidase.

demethylation of *N*,*N*-dimethylaniline and aminopyrine by catalase/organic hydroperoxides, are well documented.[15] However, we are unaware of any reactions of catalase comparable to the postulated N-hydroxylation of cyanamide.

All attempts to prepare **2** chemically have so far been unsuccessful due to its instability. However, a stable *N*,*O*-dibenzoyl derivative of **2** has now been prepared, and this dibenzoyl derivative has been shown to inhibit yeast AlDH in vitro after bioactivation by esterase action intrinsic to this enzyme.[16] Together with data indicating that *C*-nitroso compounds (RN=O) (which can be considered *substituted* nitroxyls) are also good inhibitors of yeast AlDH without bioactivation[17] and that cyanide in concentrations up to 5 mM does not inhibit the enzyme, the present results lend credence to our hypothesis[7,8] that nitroxyl (**3**) produced in the oxidation of **1** is *the* inhibitor of AlDH.

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Synthesis and Dopamine Receptor Affinity of (*R*)-(−)-2-Fluoro-*N*-*n*-propylnorapomorphine: A Highly Potent and Selective Dopamine D_2 Agonist

(*R*)-(−)-Apomorphine (APO) and its *N*-*n*-propyl analogue (*R*)-(−)-*N*-*n*-propylnorapomorphine (NPA) are considered standard centrally active dopamine (DA) agonists.[1,2] Our past efforts have focused on delineating the portions of the aporphine molecular structure that are critical to interactions with DA receptors and responsible for dopaminergic properties with a goal of developing more potent and selective agonists or antagonists. Previously a series of novel 2-substituted *R*-(−) and *S*-(+) apomorphine derivatives were prepared and evaluated as ligands for DA receptors in mammalian brain. (*R*)-(−)-2-Fluoroapomorphine (2-F-APO), *R*-(−)-2-OCH_3-NPA (**4**), and 2-OH-NPA (**3**) were found to be relatively potent and selective for the D_2 receptor subtype.[3,4] To further elucidate the structural requirements of fluorine-substituted apomorphines for DA receptors, we now report the synthesis and preliminary biological evaluation of (*R*)-(−)-2-fluoro-*N*-*n*-propylnorapomorphine (2-F-NPA, **2**) and its comparison with other analogues for affinity and selectivity to D_1 and D_2 receptor sites in corpus striatum tissue from rat forebrain (Figure 1).

Chemistry

Synthesis of 2-F-NPA (**2**, Figure 1) was achieved by minor modifications of the procedure developed for the synthesis of 2-fluoroapomorphine.[4] The desired starting material for this sequence was the previously reported precursor 2-hydroxy-10,11-(methylenedioxy)-*N*-*n*-propylnoraporphine (**7**), which was prepared in five high-yielding steps from the opium alkaloid thebaine.[3] Conversion of the phenolic group at the 2-position in **7** to the key intermediate, the 2-aminoaporphine **11**, was achieved via a modified Smiles rearrangement reaction[5] (Scheme I).

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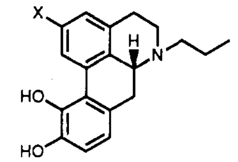
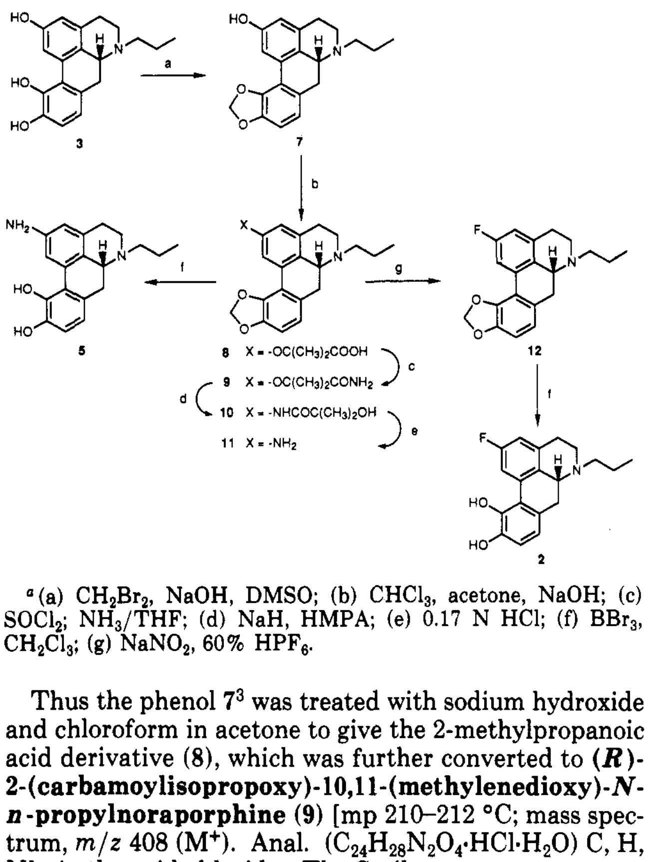


1 NPA, X = H
2 2-F-NPA, X = F
3 2-OH-NPA, X = OH
4 2-OCH3-NPA, X = OCH3
5 2-NH2-NPA, X = NH2
6 2-Br-NPA, X = Br

Figure 1.

Scheme I[a]



[a] (a) CH_2Br_2, NaOH, DMSO; (b) $CHCl_3$, acetone, NaOH; (c) $SOCl_2$; NH_3/THF; (d) NaH, HMPA; (e) 0.17 N HCl; (f) BBr_3, CH_2Cl_3; (g) $NaNO_2$, 60% HPF_6.

Thus the phenol **7**[3] was treated with sodium hydroxide and chloroform in acetone to give the 2-methylpropanoic acid derivative (**8**), which was further converted to **(*R*)-2-(carbamoylisopropoxy)-10,11-(methylenedioxy)-*N*-*n*-propylnoraporphine (9)** [mp 210–212 °C; mass spectrum, m/z 408 (M^+). Anal. ($C_{24}H_{28}N_2O_4{\cdot}HCl{\cdot}H_2O$) C, H, N] via the acid chloride. The Smiles rearrangement reaction of the propionamide **9** was again affected with sodium hydride in hexamethylphosphoric triamide (HMPA) with retention of the configuration at the chiral **6a** carbon. The product (**10**) was not isolated but was subjected to acid hydrolysis to give **(*R*)-2-amino-10,11-(methylenedioxy)aporphine dihydrochloride (11)** [mp 221–223 °C; mass spectrum, m/z 322 (M^+). Anal. ($C_{20}H_{22}N_2O_2{\cdot}2HCl{\cdot}0.5H_2O$) C, H, N]. The further conversion of this protected 2-aminoaporphine to its 2-fluoro congener **(*R*)-2-fluoro-10,11-(methylenedioxy)-*N*-*n*-propylnoraporphine hydrochloride (12)**, [mp 275–279 °C; mass spectrum, m/z 325 (M^+). Anal. ($C_{20}H_{20}NO_2F{\cdot}HCl{\cdot}0.25H_2O$) C, H, N] was achieved by using the Schiemann reaction through the thermal decomposition of the diazonium hexafluorophosphate salt.[6] The target catechol derivatives **(*R*)-(−)-2-fluoro-*N*-*n*-propylnorapomorphine hydrobromide (2)** [mp 200–202 °C; $[\alpha]^{25}_D$ −28.8° (*c* 0.13, MeOH); mass spectrum, m/z 313 (M^+). Anal. ($C_{19}H_{20}NO_2F{\cdot}HBr{\cdot}0.5H_2O$) C, H, N] and **2-amino-*N*-*n*-propylnorapomorphine dihydrobromide (5)** [215–217 °C; mass spectrum, m/z 310 (M^+). Anal. ($C_{19}H_{22}N_2O_2{\cdot}2HBr{\cdot}H_2O$) C, H, N] were synthesized by demethylenation of **11** and **12** with boron tribromide in methylene chloride. ^1H NMR spectra were recorded for each compound and were consistent with the expected structures. The 2-bromo derivative of (*R*)-(−)-*N*-*n*-propylnorapomorphine (2-Br-NPA, **6**) was prepared from thebaine by the procedure of Berenyi et al.[7] who did not report biological activity of this compound. This 2-bromo analogue was thus prepared from thebaine[7] and evaluated together with several other 2-substituted and standard (*R*)-(−)-10,11-dihydroxyaporphines (Table I).

Table I. Affinity and Selectivity of Dopamine Agonists for D_1 and D_2 Dopamine Receptors in Rat Brain Corpus Striatum Membranes[a]

no.	compound	IC_{50}, nM D_1	IC_{50}, nM D_2	D_2/D_1 potency ratio
	(*R*)-(−)-apomorphine	444	66.7	6
1	(*R*)-(−)-NPA	640	4.80	133
2	(*R*)-(−)-2-F-NPA	1300	0.071	18300
3	(*R*)-(−)-2-OH-NPA	1720	0.320	5.380
6	(*R*)-(−)-2-Br-NPA	970	0.890	1090
4	(*R*)-(−)-2-OCH_3-NPA	3340	1.02	3270
5	(*R*)-(−)-2-NH_2-NPA	>10000	5.50	>1800
12	(*R*)-(−)-2-F-MDO-NPA	3100	97.6	31
11	(*R*)-(−)-2-NH_2-MDO-NPA	>10000	704	>14

[a] Radioreceptor assays were carried out with a membrane preparation of corpus striatum tissue from rat brain, with the radioligands [^{3}H]SCH-23390 (D_1 agent, 300 pM) or [^{3}H]spiperone (D_2 agent, 0.15 nM test concentration, observed K_d = 0.03 nM), and four to six concentrations of each test agent.[13] IC_{50} values ± SEM were determined by computer-assisted curve fitting.[8] For simplicity, SEM are not shown, but averaged <±10% of mean IC_{50} below 1000 nM.

Results and Discussion

The D_1 affinity of 2-substituted congeners of NPA was less than that of unsubstituted NPA in the following rank order: $NH_2 < OCH_3 < OH \leq F \leq Br \leq H$ (Table I). In contrast affinity at D_2 receptors was increased, in some cases strikingly so, in the following rank order: F > OH > Br ≥ OCH_3 ≥ H ≥ NH_2, and D_2 vs D_1 selectivity increased as: F > OH ≥ OCH_3 ≥ NH_2 > Br > H (Table I). *R*-(−)-2-F-NPA showed low D_1 affinity (IC_{50} = 1.3 μM equivalent to K_i = 690 nM, derived[8] from K_d = 340 pM and D_1 ligand concentration of 300 pM) but the highest D_2 affinity of the analogues tested (IC_{50} = 71 pM, equivalent to K_i = 12 pM derived from K_d = 30 pM and D_2 ligand concentration of 150 pM), as well as the highest D_2 vs D_1 selectivity (18 300 based on IC_{50} ratio and 690/0.012 = 57 500 based on K_i ratio).

It appears that 2-substitution of *N*-*n*-propyl analogues of apomorphine (the present NPA series) exerts an important effect on the interactions of these agents with DA receptors in the mammalian brain. Changes in affinity to DA receptors in rat basal ganglia, and corresponding changes in selectivity for D_2 vs D_1 receptors, found with NPA derivatives (Table I) generally resemble recent observations with apomorphine derivatives (*N*-methylaporphines).[3,4] In both *N*-alkylaporphine series, there was a trend toward a decrease in D_1 affinity and an increase

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in D_2 affinity, although these varied significantly with the nature of the *N*-alkyl substituent. Thus, with 2-fluoro substitution, there was an important gain in D_2 affinity (68 times) and selectivity (138 times) for 2-F-NPA over that of NPA (Table I), whereas 2-fluoroapomorphine was only ca. 50% more potent than apomorphine itself in competing in a radioreceptor binding assay at D_2 sites;[4] 2-hydroxy substitution of apomorphine and NPA had a more similar D_2 affinity enhancing effect, although this was somewhat greater with apomorphine (29-fold[3]) than with NPA (15-fold; Table I). It is not clear whether lipophilicity or bulk of the *N*-alkyl substituent in the B ring contributes critically to the effect of substituting an electronegative group in the 2-position of aporphine A ring, but it does appear that the nature of the *N*-alkyl substituent contributes to the effects obtained with some 2-substituents.

Additional information concerning the structure–activity relations involving 2-substitution of NPA includes the much smaller effect of adding a Br than a F atom on increasing D_2 affinity and selectivity (which were more than an order of magnitude lower with 2-Br-NPA than with 2-F-NPA), as well as a somewhat smaller effect on decreasing D_1 affinity (Table I). While the differences between halogen-substituted NPAs may reflect the greater bulk of the Br vs F atom (possibly leading to a less favorable steric interaction at DA receptor surfaces), an alternative possibility is that Br may participate less well than F in hydrogen bonding with the receptor surface. An altogether different effect was found with 2-NH_2 substitution, which markedly diminished D_1 affinity of NPA and tended also to reduce D_2 affinity somewhat with NPA (Table I) and apomorphine[4] by an uncertain mechanism. Regarding D_1 sites, all 2-substituents tested consistently decreased D_1 affinity, possibly reflecting steric interference at the D_1 receptor surface, although this effect usually was relatively small except with 2-NH_2-NPA, as mentioned above (Table I). As was predicted by earlier studies of aporphines with occluded or missing hydroxy groups in the D ring, occlusion of the catechol moiety of 2-F- and 2-NH_2-NPA with a 10,11-methylenedioxy (MDO) bridge, markedly reduced D_2 receptor affinity while having little apparent additional effect on D_1 affinity (Table I); this observation confirms the importance of a free hydroxy group, especially in the 11-position on the aporphine D ring analogous to the *m*-OH in DA, for high D_2 affinity in aporphines.[9,10,11]

The present results, based on the preparation and DA-receptor affinity testing of a series of novel 2-substituted *N*-*n*-propylnorapomorphine (NPA) derivatives, indicate that affinity at D_1 sites was reduced, but only moderately and without a clear relationship on the type of substituent, except that a 2-NH_2 substituent markedly reduced D_1 affinity. More importantly, however, D_2 affinity usually was enhanced by 2-substitution of NPAs, and this effect was particularly striking with a 2-F substituent. Comparison of these results with *N*-*n*-propylaporphines (NPAs) to previous results with 2-*N*-methylaporphines (apomorphines) indicated, further, that the enhancement of D_2 affinity was influenced appreciably, though somewhat inconsistently, by the *N*-alkyl side chain.

A particularly important conclusion is that *R*-(−)-2-F-NPA had the highest D_2 binding affinity (IC_{50} = 71 pM; K_i = 12 pM) and D_2 selectivity (nearly 60 000 by D_1/D_2 ratio of K_i values) of any ligand yet described (Table I), including a series of aminotetralines, ergolines, and phenethylamines which were evaluated in another report.[12] The high affinity of 2-F-NPA led to the prediction that it would have high potency in a behavioral test of central DA agonist activity (induction of stereotyped gnawing in the rat), and it was found to be about ten-times more potent than NPA.[12] In addition to the potential experimental or medicinal interest in such a potent and selective, centrally neuropharmacologically active D_2 agonist as R-(−)-2-F-NPA, it should also be pointed out that this congener, R-(−)-2-NH_2-NPA, could serve as a precursor for the preparation of ^{18}F-labeled R-(−)-2-F-NPA, a potential imaging agent for positron emission tomography (PET) studies of agonist-labeled DA receptors in vivo.

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Expedient Synthesis and Biochemical Properties of an [^{125}I]-Labeled Analogue of Glyburide, a Radioligand for ATP-Inhibited Potassium Channels

Potassium (K) channels are ubiquitous and play critical and complex roles in the control of membrane potential in most excitable cells. As a consequence, a variety of physiological processes such as neurotransmitter release, electrical conduction in the heart, and insulin secretion are

