Dopamine Receptors Labelled by PHNO

PHILIP SEEMAN, CARLA ULPIAN, ROBERT D. LARSEN, AND PAUL S. ANDERSON
Department of Pharmacology (P.S., C.U.), Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8; Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486 (P.S.A.); and Rahway, New Jersey 07065 (R.D.L.)

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ABSTRACT Since the high-affinity state of dopamine D2 receptors may be abnormal in psychomotor diseases, it is desirable to develop a radioactive agonist to label this high-affinity site for possible clinical diagnostic use. (+)PHNO is a selective D2 agonist used to treat Parkinson's disease. We prepared [3H](+)PHNO from allyl-des-propyl- (+)PHNO. In binding to dopamine receptors in homogenates of canine brain striata, [3H](+)PHNO had a dissociation constant of 0.35 nM in the absence of NaCl, and 0.56 nM in the presence of NaCl. Dopamine agonists and antagonists inhibited the binding of [3H](+)PHNO at drug concentrations similar to those inhibiting other [3H]ligands at D2 receptors, but not similar to those acting at D4 receptors. Approximately 90% of the total [3H](+)PHNO binding was specific. Guanilylimidodiphosphate markedly inhibited [3H](+)PHNO binding, suggesting that [3H](+)PHNO was binding primarily to the high-affinity state of dopamine D2 receptors rather than to D3 receptors. The density of the [3H](+)PHNO binding sites was equal to that of [3H]Emonapride (or [3H]YM-09151-2), both densities of which were 1.5- to 2-fold higher than that of [3H]Sipiperone, compatible with the idea that [3H](+)PHNO binds to monomers of D2, while [3H]Sipiperone binds to dimers of D2. Although [3H](+)PHNO has good selectivity and affinity for the high-affinity state of D2, the [3H]ligand was sensitive to endogenous dopamine, since washing the tissue lowered the dissociation constant. For future in vivo labelling of D2 by an agonist, therefore, it will be essential to search for a related [3H]ligand with an even lower dissociation constant.

INTRODUCTION

For studying brain dopamine diseases clinically it will be useful to label the high-affinity state of dopamine receptors, because this is the functional state of these receptors (George et al., 1985) which may be abnormal in various diseases (Seeman and Niznik, 1990). From a clinical point of view, for example, in accounting for signs and symptoms in either Parkinson's disease or psychosis, the number of dopamine receptors in the high-affinity state may be an important factor.

In order to label the high-affinity state of the dopamine D2 receptor, for example, it is necessary to have an agonist which is selective for that receptor and which tightly binds to the dopamine D2 receptor with a dissociation constant below 1 nM. Despite the many dopamine agonists available, very few are selective for the dopamine D2 receptor (Seeman and Niznik, 1988). For example, apomorphine recognizes the high-affinity state of the dopamine D2 receptor at the same concentration (0.7 nM apomorphine) which recognizes the high-affinity state of the dopamine D1 receptor (0.7 nM apomorphine). The same holds for various aminotetra-lins (Seeman and Niznik, 1988).

Quinpirole is a dopamine agonist which is often used as a selective agonist for dopamine D2 receptors. Although quinpirole has a 250-fold selectivity for dopamine D2 receptors over dopamine D1 receptors (Seeman and Schaus, 1991), its high dissociation constant of 5 nM makes it vulnerable to inhibition by endogenous dopamine (Seeman et al., 1989a). Moreover, the high nonspecific binding of [3H]Quinpirole indicates that this compound binds to many other unidentified sites (Seeman and Schaus, 1991).

Another highly selective agonist for dopamine D2 receptors is PHNO [(±)-4-propyl-9-hydroxy-2,3,4a, 5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine HCl], prepared as the racemate (N-0500; Dykstra et al., 1985; Hazeldoff et al., 1986; Horn et al., 1984) or as the active (+) enantiomer (MK 458 or L-647 339 or naxagolide;...
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**Scheme**

Fig. 1. Scheme 1. (a) 25% NaOH/MeOH, reflux; (b) chloroacetyl chloride/ethyl acetate/aqueous sodium carbonate; (c) NaH/THF/DMF, −15°C; (d) NaAlH4/OCH2CH2OCH3/toluene, 80°C; (e) 7 N ethanolic hydrogen chloride/ethyl ether; (f) pyridine-HCl, 200°C.

Jones et al., 1984; Martin et al., 1985; Pettibone et al., 1987). (+)PHNO is effective in alleviating Parkinson's disease (Grandas Perez et al., 1986; Lieberman et al., 1988; Stoessel et al., 1985).

Although the long-term use of (+)PHNO may lead to a loss of clinical efficacy (Cedarbaum et al., 1990), the short-term use of tracer doses of radioactive (+)PHNO in positron emission tomography may be of diagnostic and therapeutic importance. For example, whether or not a Parkinson patient is or is not sensitive to L-DOPA or another dopamine agonist may depend on the number of high-affinity states that can be induced by these medications.

In addition to the high selectivity of (+)PHNO for dopamine D2 receptors, the dissociation constant of (+)PHNO for dopamine D2 receptors is much lower than that of quinpirole (Seeman and Niznik, 1988). A lower dissociation constant suggests that (+)PHNO may bind more tightly to the dopamine D2 receptor and, therefore, to be less sensitive to the endogenous dopamine which tends to interfere with the binding of any ligand to the dopamine D2 receptor. Hence, we prepared [³H](+)PHNO and examined its properties of binding to dopamine D2 receptors.

**MATERIALS AND METHODS**

**Preparation of [³H](+)PHNO**

[propyl-2,3-³H](+)PHNO was prepared by tritiumating the allyl congener of (+)PHNO, which in turn was prepared by the addition of an allyl group to the nitrogen of des-propyl-(+)PHNO (1). The latter (1), prepared by the scheme in Figure 1, had originally been synthesized by Jones et al. (1984), and later prepared chiral by enantioselective synthesis (Mellilo et al., 1987). The starting material for the synthesis of 1 was the propionamide 2a. The preparation of 1 began with the saponification of the chiral amide 2a to provide the amino alcohol 3; the carbamate derivative 2b, an earlier intermediate in the chiral synthesis of MK 458, can also be used. The amino alcohol was not isolated but was acylated directly with chloroacetyl chloride. The chloroacetamide 4 was then converted to the oxazine 5 with sodium hydride in tetrahydrofurandimethylformamide. The amide group of the oxazine was reduced to the amine to provide the oxazine ring of 6. The final demethylation was carried out with pyridine hydrochloride.

N-[1R,2R]-1,2,3,4-Tetrahydro-1-hydroxy-7-methoxy-2-naphthalenyl]-chloroacetamide (4)

The propanamide 2a (3.5 g, 14.06 mmol) was dissolved in methanol (75 mL) and under a nitrogen atmosphere a 25% aqueous sodium hydroxide solution (24 ml) was added dropwise. The reaction was then heated at reflux for 28 h. The mixture was cooled and the volatiles were removed under vacuum. The residue was taken up in water (200 ml) and the pH was adjusted to 6. Sodium carbonate (3.5 g, 33.0 mmol) and ethyl acetate (200 ml) were added to provide a two-phase mixture of the amino alcohol 3. Chloroacetyl chloride (1.34 ml, 16.9 mmol) was added at room temperature over 15 min. The mixture was stirred for 15 min. The layers were separated and the aqueous phase was washed with ethyl acetate (50 ml). The combined ethyl acetate
layers were washed with brine (50 ml) and dried (magnesium sulfate). Evaporation of the filtered mixture provided the chloroacetamide 4: 4.0 g, quantitative yield.

(4aR,10bR)-3,4,4a,5,6,10b-Hexahydro-9-methoxy-2H-naphth[1,2-b]-1,4-oxazin-3-one (5)

Sodium hydride (0.87 g of an 80% oil dispersion, 28.91 mmol) was suspended in tetrahydrofuran (93 ml) and the slurry was cooled to –30 to –20°C. The chloroacetamide 4 (2.6 g, 9.64 mmol) in tetrahydrofurandimethylformamide (9:1; 185 ml) was added to the sodium hydride slurry over 2 h maintaining the temperature at –20°C. After the addition the solution was allowed to warm to –15 to –10°C. At this temperature the cyclization took place over 3 h. The excess sodium hydride was quenched with water (50 ml). The volatiles were evaporated under vacuum to provide an aqueous slurry of the product. The oxazinone 5 was filtered, washed well with water and suction-dried: 1.76 g, 79% yield.

(4aR,10bR)-3,4,4a,5,6,10b-Hexahydro-9-methoxy-2H-naphth[1,2-b]-1,4-oxazine hydrochloride (6)

The oxazinone 5 was suspended in toluene (30 ml) and sodium bis(2-methoxyethoxy)aluminum hydride (3.6 mL of a 3.4 M solution in toluene, 12.3 mmol) was added at room temperature over 20 min. The mixture was heated at 80°C for 2 h. The mixture was cooled to room temperature and the excess hydride was quenched by the careful addition of water (5 ml) over 5 min. Aqueous sodium hydroxide (5%, 30 ml) was added and the two-phase mixture was well stirred for 15 min. The layers were separated and the aqueous layer was washed with toluene (15 ml). The combined toluene layers were washed with brine (20 ml) and dried (sodium sulfate). Evaporation of the filtered mixture provided the oxazine as the free base: 1.46 g, 97% yield. The oxazine was dissolved in ethyl alcohol (30 ml) and a 17 N ethanolic hydrochloric acid (2 ml) was added. The slurry of the oxazine hydrochloride 6 was cooled at 0–5°C for 1 h. The solid was filtered, washed with cold ethyl ether, and vacuum dried: 1.47 g, 84% yield.

(4aR,10bR)-3,4,4a,5,6,10b-Hexahydro-2H-naphth[1,2-b]-1,4-oxain-9-ol hydrochloride (1)

The oxazine O-methyl ether 6 (1.33 g, 5.2 mmol) and a pyridine-hydrochloride (2.1 g, 18.2 mmol) were mixed and heated at 200°C for 2 h. The brown residue was cooled to room temperature and taken up in water (30 ml). Concentrated ammonium hydroxide (4 ml) was added dropwise until pH 9. The slurry was cooled in an ice bath and the light purple solid was filtered and washed with water. The crude product 1 as the free base was dissolved in ethanol (10 ml) and the solution was decolorized with Darco. Ethyl ether (15 ml) was added to the filtrate and the mixture was cooled at 5°C overnight. The solid was collected by filtration. The mother liquors were concentrated to a brown residue, which produced a solid upon trituration with ethanol. The solid was filtered and combined with the above solid. The majority of the product was extracted from the aqueous filtrate with chloroform (5 × 25 ml). The combined chloroform layers were concentrated and the residue was taken up in ethanol (5 ml) and 7 N ethanoic hydrogen chloride (1 ml). The solution was concentrated to dryness and solids were combined with those from the ethanol solution, as well as the solids obtained from a small scale run (0.1 g, 0.391 mmol), and the whole was heated at 80°C under vacuum to remove pyridine hydrochloride by sublimation. The solids were then slurried in water and treated with Darco. The mixture was filtered to provide a water-white solution. The water was evaporated and the white solid was dissolved in ethanol (10 ml) and water (0.7 ml). The solution was cooled to room temperature, whereupon crystals formed. Ethyl ether (10 ml) was added dropwise and the mixture was stirred for 16 h. The slurry of (R,R)-9-hydroxynaphthoxazine hydrochloride (1) was then cooled in an ice bath for 1 h. The solid was filtered, washed with cold ethanol-ethyl ether, and then ethyl ether, and suction dried: 600 mg, 44% yield; m.p. 315–320°C; lit (Jones et al., 1984) 300–303°C.

The tritiation of allyl-des-propyl-MK 458 was done by New England Nuclear, E.I. du Pont de Nemours & Co., Inc. (Boston, MA). After purification on thin layer chromatography (at Merck Sharp and Dohme), the final specific activity of [3H]+[+]PHNO was 32.98 Ci/mmol.

Other [3H]ligands

[3H]lemonapride (or YM-09151-2; 71–75 Ci/mmol; 2,627–2,775 GBq/mmol) was purchased from New England Nuclear, E.I. du Pont de Nemours & Co., Inc. (Boston, MA). Two types of radioactive spiperone were used: [benzene ring-3H]spiperone (20–40 Ci/mmol; 740 GBq/mmol–1.48 TBq/mmol) from New England Nuclear (Boston, MA), and [3H]spiperone (60–100 Ci/mmol; 2.2–3.7 TBq/mmol) from Amersham Life Sciences, Oakville, Ontario, Canada.

Tissue

The binding of [3H]+[+]PHNO was tested on canine striatum. The tissues were shipped frozen from Pel-Freez (Rogers, AR) and stored at –70°C.

Measurement of receptor density

The dopamine receptor density was determined by minor modifications of a centrifugation method, previously described (Seeman et al., 1987). The frozen tissue, from which major myelin tracts were trimmed off, was lightly blotted and weighed frozen. Buffer was added to yield 4 mg tissue per ml suspension. The buffer con-
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50 mM Tris-HCl (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl₂, 4 mM MgCl₂, and 120 mM NaCl. Since high concentrations of NaCl reduce the proportion of dopamine receptors existing in the high-affinity state (Grigoriadis and Seeman, 1985), the NaCl was omitted in some experiments (later specified) in order to maintain the dopamine receptors in their high-affinity state. The suspension was homogenized with an A.H. Thomas glass homogenizer with Teflon piston (rotating at 650 rpm; ten up-down strokes). Although washing the homogenate generally causes a loss of receptors (Seeman et al., 1984), we found it necessary to wash the homogenate twice by centrifugation (48,000 g for 15 min at 4°C) and resuspension in buffer (see Results).

The receptor density was measured by saturating the tissue with increasing amounts of [³H](+)-PHNO, [³H]emonapride, or [³H]spiperone, as follows. Polypropylene microcentrifuge tubes (1.8 ml with screw cap; Sarstedt Co., Montreal) received aliquots in the following order: 0.25 ml buffer, 0.25 ml of [³H]ligand (12 final concentrations, ranging from 10 pM to 4,000 pM for [³H]emonapride and [³H]spiperone, and from 0.05 to 5 nM for [³H](+)-PHNO), and 0.5 ml of the tissue homogenate. In order to determine nonspecific binding in this D2 receptor assay, the 0.25 ml buffer aliquot (for half the tubes) contained 40 μM S(-)-sulpiride (Ravizza, Milan), such that the final concentration was 10 μM. The tubes were screw-capped, vortexed, and left to incubate at room temperature (20°C) for 2 h, at which time equilibrium had been achieved. The tubes were centrifuged in the horizontal position at 11,000 g for 6 min in a Beckman 12 microfuge at room temperature. These conditions were sufficient to recover all the D2 receptors, as monitored by [³H]spiperone, since centrifugation at 28,000 g for 15 min (Sorvall RC2B) or at 11,000 g for 15 min did not increase the D2 density in the pellets of tissues that had been homogenized by the glass-Teflon homogenizer. The supernatants were gently removed by aspiration, using a Pasteur pipette and a water-faucet vacuum. The pellets were not rinsed, since this lowered the D2 density by 5%. Using an electrically heated wire, the pellet-containing tip of each tube was cut off and put into a scintillation minivial (Packard Instruments, Chicago, IL). The minivials received 4 ml each of scintillant (Ready Solve, Beckman Co., CA) and were monitored 6 h later for tritium in a Packard 4660 scintillation spectrometer at 55% efficiency. The values for the density of [³H]ligand binding sites (Bmax) and the dissociation constants, KD, were obtained by Scatchard analysis.

Drug dissociation constants for inhibiting the binding of [³H](+)-PHNO

The potencies of various drugs for inhibiting the binding of 1 nM [³H](+)-PHNO were determined by the standard filtration method, using a Skatron cell harvester. Each incubation tube received 0.5 ml aliquots of buffer with or without drug, 3 nM [³H](+)-PHNO (final concentration of 1 nM), and tissue homogenate. After the 2 h incubation period, the incubates were filtered, using a 12-well cell harvester (Tittertek, Skatron, Lie, Norway) and buffer-presoaked glass fiber filter mats (No. 7034, Skatron, Sterling, VA). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pressed out and placed in scintillation minivials (Packard) and monitored for tritium, as above. Nonspecific binding was defined as that binding which occurred in the presence of 10 μM S-sulpiride. The drug association constants (Ki values) were derived using the nonlinear least-squares data-fitting program LIGAND (details in Grigoriadis and Seeman, 1985; Seeman et al., 1985) or were calculated from the concentration which inhibited binding by 50% (the IC50 value), using Ks = IC50/(1 + C/KD), where C* was the final concentration of competing [³H](+)-PHNO (between 0.7 and 1.5 nM), and Ks was the dissociation constant of [³H](+)-PHNO (independently measured in either the presence or absence of NaCl).

RESULTS

Time-course of [³H](+)-PHNO binding

The specific binding of [³H](+)-PHNO (between 1 and 4 nM) was 50% equilibrated in 10 min, and 90% equilibrated in 40 min, with no further increase in binding occurring after 1.5 h (with or without NaCl; data not shown). Although nonspecific binding was defined as that in the presence of 10 μM S-sulpiride, identical nonspecific binding occurred in the presence of 1 μM (+)-butaclamol or 1 μM apomorphine. Hence, a standard incubation period of 2 h was used, with nonspecific binding defined by 10 μM S-sulpiride.

Density of [³H](+)-PHNO sites: Effect of washing on dissociation constant

Using washed homogenate, the density of [³H](+)-PHNO binding sites was 30.1 ± 2 pmol/g (N = 12 independent experiments, each in duplicate). The dissociation constant, KD, was 0.56 ± 0.08 nM in the presence of NaCl, and was 0.35 ± 0.03 nM in the absence of NaCl. If the tissue was not washed, the dissociation constant was four-fold to six-fold higher, consistent with the principle that endogenous dopamine interferes with the binding of such [³H]ligands (see Introduction).

A typical result is shown in Figure 2, where the density of [³H](+)-PHNO sites is also compared to the densities of [³H]emonapride and [³H]spiperone in the same tissue homogenate. The density of the [³H](+)-PHNO sites in the canine striatum homogenate was consistently the same as that for [³H]emonapride, but approximately 1.5- to 2-fold higher than that for [³H]spiperone.
Agonist inhibition of $[^3]H$($\pm$)PHNO binding

Figure 3 and Table I lists the potencies of various dopamine-like agonists for inhibiting the binding of $[^3]H$($\pm$)PHNO. As mentioned in Materials and Methods, the agonists were generally more potent in the absence of NaCl. The rank order of the neurotransmitter agonist potencies (dopamine > noradrenaline > serotonin) indicates that $[^3]H$($\pm$)PHNO binds to dopamine receptor sites. ($\pm$)PHNO was almost five orders of magnitude more potent than ($-$)PHNO.

Antagonist inhibition of $[^3]H$($\pm$)PHNO binding

The potencies of various antagonists for the inhibition of $[^3]H$($\pm$)PHNO binding are given in Table I and Figure 4. Since the benzamide potencies are known to be very much reduced in the absence of NaCl (Niznik et
TABLE I. Drug dissociation constants (Kᵢ values) for inhibiting
[^3]H[^+]PHNO binding

<table>
<thead>
<tr>
<th>Drug agonists</th>
<th>Kᵢ, nM</th>
<th>-NaCl</th>
<th>+NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Adrenaline</td>
<td>82</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Apomorphine</td>
<td>1.5</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>6</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>Not done</td>
<td>10,300</td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>4.2</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>8-OH-dipropylaminotetralin</td>
<td>42</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>(-)-Noradrenaline</td>
<td>33</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>(-)-N-propyl-apomorphine</td>
<td>Not done</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>(+)-PHNO</td>
<td>Not done</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>(-)-PHNO</td>
<td>Not done</td>
<td>4.400</td>
<td></td>
</tr>
<tr>
<td>Des-propyl- (+)-PHNO</td>
<td>Not done</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Des-propyl- (+)-PHNO-methyl</td>
<td>Not done</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>565</td>
<td>2,400</td>
<td></td>
</tr>
<tr>
<td>SKF 28393</td>
<td>206</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

**Antagonists**

| Bepiperidol    | Not done | 0.6 |       |
| BRL 25594¹     | Not done | 0.043 |       |
| (+)-Butaclamol | Not done | 0.6 |       |
| Chlorpromazine | 4.1     | 3.1  |       |
| Clebopride     | Not done | 0.9 |       |
| Clozapine      | Not done | 508 |       |
| Emanopride (YM-09151-2) | Not done | 0.03 |       |
| Eticlopride    | Not done | 0.04 |       |
| Haloperidol    | 1.4     | 2.9  |       |
| Molindone      | Not done | 28  |       |
| Pentaozencine  | 30,000  |       |       |
| Promazine      | Not done | 94  |       |
| (-)-Propranolol| 4,400   | 4,000 |       |
| Raclopride     | Not done | 1.9 |       |
| Remoxipride    | Not done | 304 |       |
| SCH 23939      | 91      | 447  |       |
| Spiperone      | High affinity: | 0.033 | 0.635 |
|               | Low affinity:  | 0.61 | 0.35  |
| S-sulpiride    | Not done | 12.8 |       |
| Thiopentaline  | Not done | 3.8  |       |
| Trifluoperazine| Not done | 5.5  |       |
| Trifluoperidol | Not done | 1.6  |       |

¹BRL 25594 is identical to clebopride, except with a tropone bridge (+C-C-) on the piperidine ring (Hadley, 1982).

al., 1985), these compounds were only tested in the presence of NaCl.

Although all the antagonists inhibited the binding of
[^3]H[^+]PHNO in a single phase with one dissociation constant, spiperone inhibited the binding of[^3]H[^+]PHNO in two phases, as shown in Figure 4.

Effect of guanaine nucleotide

The co-incubation of 160 μM guanylimidodiphosphate or Gpp(NH)p reduced the density of[^3]H[^+]PHNO binding sites by 50% (data not shown). The concentration-dependence of this effect is shown in Figure 5, where it is seen that the guanaine nucleotide inhibited the binding of[^3]H[^+]PHNO in two concentration phases.

**DISCUSSION**

**Nature of the[^3]H[^+]PHNO binding site**

The data for agonists and antagonists indicate that[^3]H[^+]PHNO binds to dopamine D2 receptors, according to the following considerations.

The rank order of potencies for the three neurotransmitters (dopamine > noradrenaline > serotonin; Fig. 3) indicates that[^3]H[^+]PHNO binds to dopamine receptors. The antagonists, furthermore, inhibited the binding of[^3]H[^+]PHNO at drug concentrations identical to those which inhibit the binding of other[^3]Hligands to the dopamine D2 receptors (Seeman and Niznik, 1988).

More specifically,[^3]H[^+]PHNO primarily labels dopamine D2 receptors, rather than dopamine D3 or D4 receptors, in accordance with the considerations in the following three sentences. The 50% reduction in[^3]H[^+]PHNO binding by guanylimidodiphosphate suggests that[^3]H[^+]PHNO binds primarily to dopamine D2 receptors, since dopamine D3 receptors are relatively insensitive to the effects of this guanine nucleotide (Sokoloff et al., 1990). Moreover, the[^3]H[^+]PHNO binding sites are unlikely to be dopamine D4 receptors, because clozapine inhibited the binding of[^3]H[^+]PHNO at 508 nM (Table I), in contrast to clozapine's dissociation constant of 10 nM for the dopamine D4 receptor (Van Tol et al., 1991). This interpretation is further supported by the fact that raclopride inhibited[^3]H[^+]PHNO binding at 1.9 nM, in contrast to the value of 240 nM for raclopride at the dopamine D4 receptor (Van Tol et al., 1991).

Approximately 90% of the[^3]H[^+]PHNO binding is to dopamine receptors, with only about 10–15% bound to other receptors or to nonspecific sites. The receptor next closest in affinity for[^3]H[^+]PHNO would be the alpha₂-adrenoceptor, for which[^3]H[^+]PHNO has about 3-fold lower affinity than the dopamine D2 receptor (Martin et al., 1985).

**D2 dimer and[^3]H[^+]PHNO binding**

As discussed in the section just above, the data indicate that[^3]H[^+]PHNO binds to dopamine D2 receptors. However, the data in Figure 2 indicated that the density of[^3]H[^+]PHNO sites exceeded the density for those of[^3]Hspiperone by 1.5- to 2-fold. This inequality does not argue against the conclusion that[^3]H[^+]PHNO labels dopamine D2 receptors, because we have recently shown that cloned dopamine D2 receptors can reveal two different densities for two different[^3]Hligands:[^3]Hemonapride and[^3]Hspiperone. Therein (Seeman et al., 1992), we concluded that[^3]Hspiperone may bind to a dimer of the dopamine D2 receptor, while[^3]Hemonapride may bind to a monomer of the dopamine receptor, accounting for the two different densities of the two ligands.

Here, too, therefore, it may be that[^3]H[^+]PHNO binds to the monomer of the dopamine D2 receptor, since the density of[^3]H[^+]PHNO sites matches the density of[^3]Hemonapride sites (Fig. 2).
Positron emission tomography and $[^3]H(+)$PHNO

Hence, the data indicate that $[^3]H(+)$PHNO labels dopamine D2 receptors, and that the high density of these sites (higher than $[^3]H$spiperone) suggests that $[^3]H(+)$PHNO binds to the dopamine D2 monomer.

With these characteristics for $[^3]H(+)$PHNO, therefore, it appears that $[^3]H(+)$PHNO has several advantageous features for labelling the high-affinity state of the D2 receptor in vivo, using positron emission tomography. First, the $K_D$ value is low, approximately 10-fold lower than the 5 nM value for $[^3]H$quinpirole (Grigoriadis et al., 1992; Seeman and Schaus, 1991). The low dissociation constant of $[^3]H(+)$PHNO thus makes it less sensitive to endogenous dopamine. Second, $[^3]H(+)$PHNO labels the high-affinity state of D2, since the binding was effectively reduced by guanilylimidodiphosphate. Third, the specific binding was of the order of 90% of the total binding, with only 10–15% bound to non-dopamine sites. This high amount of specificity should assist in obtaining a reasonable striatum/
cerebellum ratio in vivo. The clinical value of radioactive (+)PHNO for positron emission tomography must await the synthesis and testing of [11C] (+)PHNO.

Role of endogenous dopamine

As mentioned above, the positive features of [3H](+)PHNO are that it selectively labels dopamine D2 receptors, it apparently labels the monomers of these receptors, and it has a low dissociation constant of 0.45 nM.

However, a major drawback of [3H](+)PHNO is that, despite the low dissociation constant of 0.45 nM, the ligand is sensitive to endogenous dopamine. This is apparent from the observation that the Kp dropped about 5-fold after washing the homogenate (see Results). As outlined previously (Seeman et al., 1989a,b, 1990; Seeman, 1992a,b; see also Dewey et al., 1991; Innis et al., 1992; Logan et al., 1991; Ross and Jackson, 1989; Wong et al., 1991; Young et al., 1991), [3H]ligands with dissociation constants higher than 0.2 nM would be expected to be sensitive to endogenous dopamine.

Therefore, despite the improved features of [3H](+)PHNO, compared to [3H]quinpirole, the dissociation constant of [3H](+)PHNO is still too high to make this an ideal ligand for monitoring the high-affinity state of dopamine D2 receptors. For this purpose, a [3H]agonist is needed with its Kp in the pM concentration range.

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