PRIORITY COMMUNICATION

High-Affinity States of Human Brain Dopamine D2/3 Receptors Imaged by the Agonist [^{11}C]-(+)-PHNO

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Background: *The high-affinity states of dopamine D2-receptors ($D2^{high}$) are postulated to be functionally responsible for signal transduction. At present, no useful in vivo method exists to selectively measure $D2^{high}$ in humans, as current D2 radioligands for positron emission tomography (PET) are either not D2-selective or do not differentiate between D2 high- and low-affinity states.*

Methods: *The D2-agonist (+)-PHNO [(+)4-propyl-9-hydroxynaphthoxazine] was labeled with carbon-11 and studied with PET. Eight [^{11}C]-(+)-PHNO scans were acquired in four healthy volunteers.*

Results: *We observed greatest [^{11}C]-(+)-PHNO accumulation in caudate, putamen, and globus pallidus [binding potentials (BPs): 3.00 ± .4, 3.10 ± .2, and 4.17 ± 1.2]. Small but detectable binding was identified in the substantia nigra/ventral tegmental area. Preliminary test-retest data in two subjects suggests BP-estimates to be reliable. Pre-treatment with haloperidol reduced BPs in regions showing specific binding with no detectable changes in cerebellum. Parallel imaging with [^{11}C]-raclopride showed substantial differences in the globus pallidus.*

Conclusions: *[^{11}C]-(+)-PHNO proved to be a D2/3-receptor agonist-radioligand with good brain uptake and favorable kinetics for PET in humans. [^{11}C]-(+)-PHNO delineated D2/3-receptor rich brain regions with high signal-to-noise ratio. This is the first demonstration of a viable agonist-radioligand for D2 receptors in humans and opens the door for investigating $D2^{high}$ in health and disease.*

Key Words: Dopamine, high affinity, agonist, PET, G protein, globus pallidus

The dopamine D2 receptor is believed to be involved in the pathogenesis of several psychiatric and neurological disorders, and it is a key target of many therapeutic agents such as antipsychotics in schizophrenia and dopamine agonists in Parkinson's disease. It is well established in vitro that dopamine D2 receptors exist in two interconvertible affinity states for its natural agonist dopamine (DA), the high-affinity state ($D2^{high}$; dissociation constant Kd for DA 1.5 ± .2 nM) and the low-affinity state ($D2^{low}$; Kd for DA in the micromolar range) (Sibley et al 1982). Under physiological conditions, DA is expected to bind predominantly to the $D2^{high}$ state, and since this binding mediates the activation of the second-messenger cascade, the high-affinity state is believed to be the functionally important one (George et al 1985; Leff 1995).

Over the last two decades, several positron emission tomography (PET) and single photon emission computer tomography (SPECT) imaging studies have investigated the human D2/3 receptor in vivo. These studies have provided important insight into the pathophysiology of psychiatric (Abi-Dargham et al 2000) and neurological (Bruecke et al 2000) disorders and into the mechanism of action of antipsychotic drugs (Kapur and Remington 2001). However, conventional D2/3 radioligands ([^{11}C]-raclopride, [^{11}C]-FLB 457, [^{18}F]-fallypride, ^{123}IBZM, etc.) are D2/3 receptor antagonists. Antagonist radiotracers bind to all D2 receptors, without distinguishing the functionally inert low-affinity from the functionally active high-affinity states of the receptor. In contrast, "agonist" radiotracers are expected to bind preferentially to the high-affinity state, so that they allow for differentiating between low- and high-affinity states. Since it is the high-affinity state that is thought to mediate signal transduction in postsynaptic neurons, imaging $D2^{high}$ can be expected to provide new information on the conditions under which dopaminergic signaling at D2/3 receptors occurs in the living human brain. Thus, several research groups have lately reported on animal experiments using newly developed D2/3 agonist radioligands (Cumming et al 2003; Hwang et al 2004; Shi et al 2004).

(+)4-Propyl-3,4,4a,5,6,10b-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol] ((+)-PHNO) was first synthesized in 1984 and reported as the most potent known D2 receptor agonist at that time (Jones et al 1984). Under various names (Dopazinol, Naxagolide, Nazagolide, L 647339, MK 458) this drug has been tested in Phase II clinical trials for treatment of Parkinson's disease. After initial enthusiasm, drug development was eventually discontinued since studies had suggested a progressive tolerance to its efficacy and superior therapeutic action of less selective DA agonists (Ahlskog et al 1991; Cedarbaum et al 1990; Muenter et al 1988; Weiner et al 1989). [^{3}H]-(+)-PHNO has since then successfully been used as a D2 agonist radioligand in in-vitro and autoradiography studies (Seeman et al 1993; Nobrega and Seeman 1994). [^{3}H]-(+)-PHNO binding in animal brain preparations conforms well to the anatomical localization of D2 receptors (Nobrega and Seeman 1994) and the nearly full inhibition of [^{3}H]-(+)-PHNO binding by Guanilylimidodiphosphate, a substance known to convert $D2^{high}$ into $D2^{low}$, indicates that [^{3}H]-(+)-PHNO selectively binds to D2 receptors in the high-affinity state (Nobrega and Seeman 1994; Seeman et al 1993). The Kd for [^{3}H]-(+)-PHNO in the canine striatum is .56 nM (in presence of 120 mM NaCl) or .35 nM in absence of NaCl (Seeman et al 1993). Although labeling of (+)-PHNO with carbon-11 has been previously reported, (Brown et al 1997), no details or use of the ligand are published.

We recently reported on the radiosynthesis and preclinical characterization of [^{11}C]-(+)-PHNO as an agonist radiotracer for in vivo measurement of D2/D3 receptors using PET (Wilson et al 2005). In brief, [^{11}C]-(+)-PHNO showed a robust specific/non-specific binding signal in rodent striatum, a brain region known to be rich in dopamine D2/3 receptors. This signal was almost

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fully (i.e. > 90%) displaceable by well characterized D2 antagonists but was insensitive to dopamine D1, norepinephrine, or serotonin receptor ligands. It showed marked and appropriate sensitivity to manipulations of endogenous DA levels both in a positive and negative fashion, and finally, no specific binding was measured in the cerebellum, a brain region virtually devoid of D2 receptors (Hall et al 1996) and, as such, frequently used as reference region in human PET imaging. In sum, [^{11}C]-(+)-PHNO showed all properties of a good candidate PET radioligand to study the D2 high-affinity state in humans.

The aim of this study was to introduce and validate [^{11}C]-(+)-PHNO as an agonist D2/3 radioligand for PET imaging in humans. We report on a total of eight [^{11}C]-(+)-PHNO PET scans acquired in four healthy volunteers. For a first sense of test-retest reliability of the [^{11}C]-(+)-PHNO PET signal, two subjects underwent two naïve [^{11}C]-(+)-PHNO PET scans with at least one week in between scans. To verify the specificity of [^{11}C]-(+)-PHNO binding to D2/3 receptors in humans, two subjects underwent an additional [^{11}C]-(+)-PHNO scan after pretreatment with haloperidol. For a first direct comparison with D2/3 antagonist radioligand binding, two subjects also underwent PET imaging with [^{11}C]-raclopride, a widely used and well characterized D2/3 antagonist radioligand.

Methods and Materials

Study Subjects and Safety Procedures

This study has been approved by the local Ethics Committee and the Canadian Ministry of Health, Therapeutic Products Research Department. Four healthy volunteers (2 males, 2 females; age range: 29–48 years) were recruited by advertisements or word of mouth. Written informed consent was obtained after full explanation of the study procedures and risks. Routine blood and urine tests, an electrocardiogram (ECG) and a physical exam were performed before study inclusion. Psychiatric disorders were excluded using the MINI-Plus structured interview (Sheehan et al 1998). Subjects with serious or unstable medical or neurological conditions or with axis one psychiatric diagnoses were not included into the study. Likewise, subjects with substance abuse other than caffeine or nicotine within six months prior to their baseline visit were not included. Participants were asked to consume no more than their usual amount of coffee (and, in smokers, cigarettes) the day of PET examinations, and to abstain from alcohol intake 24 hours before PET scans.

Standard urine tests for psychotropic substances were performed at inclusion and immediately before PET scans. Pregnancy was excluded using serum HCG analysis at inclusion and standard urine pregnancy tests before each scan. Repeated blood pressure measurements and continuous three-lead ECG monitoring were performed during all [^{11}C]-(+)-PHNO scans. Participants had a physical exam and standard ECG immediately after each [^{11}C]-(+)-PHNO scan. Another medical exam including physical examination, ECG, routine blood and urine analysis was performed the day after each [^{11}C]-(+)-PHNO PET scan.

[^{11}C]-(+)-PHNO Synthesis

The radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere (Wilson et al 2005). Briefly, [^{11}C]-propionyl chloride was reacted with 9-hydroxynaphthoxazine to generate a [^{11}C]-amide which was subsequently reduced by lithium aluminium hydride. Purification by high performance liquid chromatography (HPLC) and formulation gave radiochemically pure [^{11}C]-(+)-PHNO as a sterile, pyrogen-free solution suitable for human studies. [^{11}C]-raclopride was synthesized as previously described by methylation of the desmethyl precursor using [^{11}C]-methyl iodide (Ehrin et al 1985; Wilson et al 2000).

Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) System

Positron emission tomography studies were performed using a CPS-high resolution research tomograph (HRRT), neuro-PET camera system (CPS Inc. Knoxville, Tennessee) measuring radioactivity in 207 brain sections with a thickness of 1.2 mm each. This camera system consists of 8 panel detectors, each panel being composed of 117 phoswich detectors, arranged 13 axial by 9 radial. The phoswich detector is manufactured from LSO (lutetium oxyorthosilicate) crystal and a LYSO (lutetium yttrium oxyorthosilicate) crystal, each crystal element having a dimension of $2 \times 2 \times 10$ mm^3. The in-plane resolution of the scanner is approximately 2.8 mm full width at half-maximum (FWHM). MRI images of the head were acquired on a Signa 1.5 T whole body MRI scanner (General Electric Medical Systems, Milwaukee, Wisconsin).

Study Protocol

As outlined in Table 1, a total of eight [^{11}C]-(+)-PHNO PET scans were acquired in 4 healthy control volunteers. A first group of two subjects participated in a test-retest analysis of [^{11}C]-(+)-PHNO binding and were examined, at baseline conditions, in two [^{11}C]-(+)-PHNO PET scans performed at least one week apart. A second group of two subjects participated in two [^{11}C]-(+)-PHNO scans performed at least one week apart. In a

Table 1. Binding Potential (BP) Values Obtained in Healthy Control Volunteers at Either Baseline Conditions or Following Pretreatment with Haloperidol 2 mg with Either [^{11}C]-(+)-PHNO or [^{11}C]-raclopride

	[^{11}C]-(+)-PHNO						[^{11}C]-raclopride		
	Baseline			Haloperidol 2 mg			Baseline		
	Caudate	Putamen	Globus Pallidus	Caudate	Putamen	Globus Pallidus	Caudate	Putamen	Globus Pallidus
Subject A	3.02	3.17	3.80	—	—	—	2.78	3.79	1.26
Subject A	2.73	2.94	3.35	—	—	—	—	—	—
Subject B	3.33	3.31	5.36	—	—	—	—	—	—
Subject B	3.58	3.35	6.07	—	—	—	—	—	—
Subject C	2.83	2.77	3.34	1.71 (40%)	1.70 (39%)	1.71 (49%)	—	—	—
Subject D	2.51	3.08	3.13	1.95 (22%)	2.31 (25%)	2.69 (14%)	3.80	4.32	1.50
Mean ± SD	3.0 ± .4	3.1 ± .2	4.2 ± 1.2	1.8 ± .2 (31%)	2.0 ± .4 (32%)	2.2 ± .7 (31%)	3.3 ± .7	4.1 ± .4	1.4 ± .2

Numbers in parentheses indicate percent occupancy calculated as $[(BP_{baseline} - BP_{drug\ scan})/BP_{baseline} \times 100]$.