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ORIGINAL ARTICLE

The in vitro receptor profile of rotigotine: a new agent for the treatment of Parkinson's disease

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Abstract Rotigotine (Neupro®) is a non-ergoline dopamine agonist developed for the once daily treatment of Parkinson's disease (PD) using a transdermal delivery system (patch) which provides patients with the drug continuously over 24 h. To fully understand the pharmacological actions of rotigotine, the present study determined its extended receptor profile. In standard binding assays, rotigotine demonstrated the highest affinity for dopamine receptors, particularly the dopamine D_3 receptor (K_i= 0.71 nM) with its affinities to other dopamine receptors being (K_i in nM): $D_{4.2}$ (3.9), $D_{4.7}$ (5.9), D_5 (5.4), D_2 (13.5), $D_{4.4}$ (15), and D_1 (83). Significant affinities were also demonstrated at α-adrenergic (α_{2B}, K_i=27 nM) and serotonin receptors (5-HT_{1A} K_i=30 nM). In newly developed reporter-gene assays for determination of functional activity, rotigotine behaved as a full agonist at dopamine receptors (rank order: D_3>D_{2L}>D_1=D_5>$D_{4.4}$) with potencies 2,600 and 53 times higher than dopamine at dopamine D_3 and D_{2L} receptors, respectively. At α-adrenergic sites, rotigotine acted as an antagonist on α_{2B} receptors. At serotonergic sites, rotigotine had a weak but significant agonistic activity at 5-HT_{1A} receptors and a minor or nonexistent activity at other serotonin receptors. Thus, in respect to PD, rotigotine can be characterized as a specific dopamine receptor agonist with a preference for the D_3 receptor over D_2 and D_1 receptors. In addition, it exhibits interaction with D_4 and D_5 receptors, the role of which in relation to PD is not clear yet. Among non-dopaminergic sites, rotigotine shows relevant affinity to only 5-HT_{1A} and α_{2B} receptors. Further studies are necessary to investigate the contribution of the different receptor subtypes to the efficacy of rotigotine in Parkinson's disease and possible other indications such as restless legs syndrome.

Keywords Rotigotine · Parkinson's disease · Restless legs · Dopamine receptor agonist · Serotonin receptor · Adrenaline receptor

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Introduction

Since the discovery that patients with Parkinson's disease (PD) exhibit a dopamine deficiency in the striatum, research has focused on the development of dopaminergic drugs for the treatment of the disease. Levodopa was the first of these drugs and is remarkably effective for reversing akinetic symptoms (Cotzias 1971; Sit 2000). This agent has been in use for over 40 years and continues to be the most widely prescribed drug for the disease (Sit 2000; Tan et al. 2005). However, despite its substantial efficacy over the

short-term, levodopa is not an ideal therapy. After long-term use, the response to levodopa diminishes and most patients experience extreme fluctuations in efficacy ("on–off" phenomenon) and disabling motor complications (dyskinesia; Fahn 1999).

In order to improve the therapy of PD, a number of dopamine agonists have been developed. Many of these were developed to replace levodopa with the additional goal of providing a more continuous dopaminergic stimulation. That treatment regimen is considered to avoid the motor complications which are believed to be associated with pulsatile administration of dopaminergic drugs (Fahn 1999; Maratos et al. 2003; Olanow and Obeso 2000). Clinical trials have confirmed that the treatment of patients with early PD with dopamine agonists is effective, potentially neuroprotective, and by delaying the use of, or reducing, levodopa therapy, can avoid the motor complications (Clarke and Guttman 2002; Jenner 2003; Rascol et al. 2002).

Rotigotine ([−]2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin, previously known as N-0923) is a new drug developed for the once daily treatment of idiopathic PD (Fig. 1). Due to its extensive gastrointestinal metabolism (Swart and de Zeeuw 1992), rotigotine is poorly suited for oral administration. However, its high lipid solubility and other physiochemical properties suggested its development for a transdermal administration (e.g., via a patch). Preclinical studies with rotigotine have demonstrated potent effects in rat and monkey models of PD (Belluzzi et al. 1994) even after transdermal administration as well as neuroprotective properties (Scheller et al. 2007). Clinical trials have shown that once-daily rotigotine patch application provides dose-related improvements in patients' motor function (Parkinson Study Group 2003; Watts et al. 2004). Moreover, a stable drug release profile was maintained throughout the 24-h period that the patch was in place resulting in stable plasma levels and providing the basis for a potential continuous dopaminergic stimulation (Metman et al. 2001).

In order to characterize its pharmacologic properties in detail, the interaction of rotigotine with a broad range of receptors, transporters, and ion channels was investigated. Cell-based functional assays were developed to characterize its functional properties regarding dopaminergic, serotonergic, and adrenergic receptors.

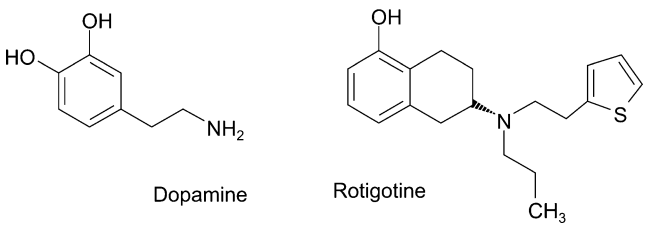

Fig. 1 Chemical structure of rotigotine compared with dopamine

Materials and methods

Radioligand-binding experiments

For each receptor tested, Table 1 lists the origin, the experimental conditions, and a bibliography documenting the general procedures for the binding assays.

Functional assays

Cell lines

Every recombinant receptor was expressed in Chinese hamster ovary cell lines (CHO-DUKX) except for the D_3 receptor, which was expressed in human neuroblastoma cell lines (SH-SY5Y). All human receptor cDNAs were cloned from human preparations by reverse-transcription polymerase chain reaction (RT-PCR) with sequence-specific primers covering the start and stop codons, respectively, using high-fidelity DNA polymerases (Pfu Turbo, Stratagene, La Jolla, CA, USA; Platinum Pfx, Invitrogen, San Diego, CA, USA). cDNA inserts were directionally subcloned into the expression vector pCIneo (Promega, Mannheim, Germany) and sequenced. The deduced amino acid sequences (including a Kozak sequence GCC A/G CCC ATG in front of the start codon) were in accordance with those published in GenBank (D_1: S58541, D_{2L}: M29066, D_3: U32499, $D_{4.4}$: L12398, and D_5: M67439). Expression plasmids were introduced into eukaryotic cells (CHO-DUKX-CRE, CHO-DUKX-SRE, or SH-SY5Y-SRE) harboring the luciferase reporter gene driven 5× CRE- or 2× SRE elements (corresponding to −357 to −276 from the c-fos gene in front of a minimal promoter driving the expression of the luciferase gene), as indicated by the name of the cell line. Transfections were performed in six-well plates using the Lipofectamine Plus reagent (Invitrogen) according to instructions of the manufacturer. Two days after transfection, cells were selected for G418 (0.4 mg/ml) resistance and grown for 10 days. Cells were seeded into 96-well plates in a limited dilution of 200 cells per plate. Two weeks later, single colonies were split into three wells and tested for agonist responsiveness. The clonal cell lines used exhibited the most robust signal and highest assay-specific increment and were pharmacologically further characterized. For CHO-DUKX-SRE-Luci-D2–17, Cho-DUKX-SRE-Luci-D4.4–69, and SH-SY5Y-SRE-Luci-D3–121e cells, receptor plasmids were co-transfected with pCMVSPORT-Galphaqo5-IRES-hygro in a ratio of 10:1 receptor plasmids. Co-transfection has been done only with Gαi-coupled receptors. The G-protein Gαq was amplified from human cerebellar cDNA using Pfu Turbo Polymerase and the upstream primer encoding the C-terminal five amino acids of the Gαq protein. The resulting PCR product was direc-

Table 1 Receptors, experimental conditions, and methodology used in radioligand binding experiments in which rotigotine showed significant binding

Receptor	Receptor Origin	Tracer Ligand	Nonspecific Ligand	Incubation Conditions	Bibliography
Adrenergic					
α_{1A}	Rat salivary glands	[^{3}H]Prazosin (0.06 nM)	Pentolamine (10 μM)	60 min/22°C	Michel et al. 1989
α_{1B}	Rat liver	[^{3}H]Prazosin (0.05 nM)	Pentolamine (10 μM)	60 min/22°C	Michel et al. 1989
α_{2A} (*h*)	Human Recombinant (Sf9 cells)	[^{3}H]RX821002 (1.5 nM)	(−)Epinephrine (100 μM)	60 min/22°C	Devedjian et al. 1994
α_{2B}	NG 108–15 cells	[^{3}H]RX821002 (2.5 nM)	(−)Epinephrine (100 μM)	25 min/22°C	Bylund et al. 1988
α_{2C} (*h*)	Human recombinant (Sf9 cells)	[^{3}H]RX821002 (5 nM)	(−)Epinephrine (100 μM)	60 min/22°C	Devedjian et al. 1994
Dopaminergic					
D_1 (*h*)	Human recombinant (L cells)	[^{3}H]SCH 23390 (0.3 nM)	SCH 23390 (1 μM)	60 min/22°C	Zhou et al. 1990
D_2 (*h*)	Human recombinant (CHO cells)	[^{3}H]Spiperone (0.3 nM)	(+)Butaclamol (10 μM)	60 min/22°C	Grandy et al. 1989
D_3 (*h*)	Human recombinant (CHO cells)	[^{3}H]Spiperone (0.3 nM)	(+)Butaclamol (10 μM)	60 min/22°C	MacKenzie et al. 1994
$D_{4.4}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]Spiperone (0.3 nM)	(+)Butaclamol (10 μM)	60 min/22°C	Van Tol et al. 1992
$D_{4.2}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]Spiperone (0.5 nM)	(+)Butaclamol (10 μM)	60 min/22°C	Van Tol et al. 1992
$D_{4.7}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]Spiperone (0.5 nM)	(+)Butaclamol (10 μM)	60 min/22°C	Van Tol et al. 1992
D_5 (*h*)	Human recombinant (GH4 cells)	[^{3}H]SCH 23390 (0.3 nM)	SCH 23390 (10 μM)	60 min/22°C	Sunahara et al. 1991
Histamine H_1					
Central	Guinea pig cerebellum	[^{3}H]Pyrilamine (0.5 nM)	Triprolidine (100 μM)	10 min/22°C	Dini et al. 1991
Peripheral	Guinea pig lung	[^{3}H]Pyrilamine (0.1 nM)	Triprolidine (100 μM)	15 min/22°C	
Muscarinic-acetylcholine					
M2 (*h*)	Human recombinant (CHO cells)	[^{3}H]AF-DX 384 (2 nM)	Atropine (1 μM)	60 min/22°C	Dorje et al. 1991
M4 (*h*)	Human recombinant (CHO cells)	[^{3}H]4-DAMP (0.2 nM)	Atropine (1 μM)	60 min/22°C	
Serotonergic					
5-HT_{1A} (*h*)	Human recombinant (CHO cells)	[^{3}H]8-OH-DPAT (0.3 nM)	[^{3}H]8-OH-DPAT (10 μM)	60 min/22°C	Mulheron et al. 1994
5-HT_{1D}	Bovine caudate	[^{3}H]Serotonin (2 nM)	Serotonin (10 μM)	30 min/22°C	Heuring and Peroutka 1987
5-HT_{2B} (*h*)	Human recombinant (cho cells)	[^{3}H]LSD (1.2 nM)	Serotonin (10 μM)	30 min/37°C	Bonhaus et al. 1995
5-HT_{5A} (*h*)	Human recombinant (hek 293 cells)	[^{3}H]LSD (1 nM)	Serotonin (100 μM)	30 min/37°C	Rees et al. 1994
5-HT_7 (*h*)	Human recombinant (CHO cells)	[^{3}H]LSD (4 nM)	Serotonin (10 μM)	120 min/22°C	Shen et al. 1993
Pertussin-reovirus attachment protein					
σ_1	Guinea pig cerebral cortex	[^{3}H](+)Pentazocine (2 nM)	Haloperidol (10 μM)	150 min/22°C	Bowen et al. 1993

Table 1 (continued)

Receptor	Receptor Origin	Tracer Ligand	Nonspecific Ligand	Incubation Conditions	Bibliography
σ_2	Rat cerebral cortex	[^{3}H]DTG (5 nM) (+300 nM (+)pentazocine)	Haloperidol (10 μM)	120 min/22°C	Bowen et al. 1993
Na^+ channel					
Site 1	Rat cerebral cortex	[^{3}H]Saxitoxin (2 nM)	Tetrodotoxin (1 μM)	30 min/22°C	Catterall 1979
Site 2	Rat cerebral cortex	[^{3}H]Batrachotoxinin (10 nM)	Veratridine (300 uM)	60 min/22°C	Brown 1986
Norepinephrine transporter (*h*)	Human recombinant (MDCK cells)	[^{3}H]Nisoxetine (0.3 nM)	Desipramine (1 μM)	60 min/4°C	Pacholczyk et al. 1991
Dopamine transporter (*h*)	Human recombinant (CHO cells)	[^{3}H]GBR12935 (0.5 nM)	BTCP (10 μM)	120 min/4°C	Andersen 1987
5-HT transporter (*h*)	Human recombinant (HEK 293 cells)	[^{3}H]Paroxetine (0.1 nM)	Imipramine (10 μM)	30 min/22°C	Tatsumi et al. 1997

tionally cloned into pCMVSPORT (Invitrogen) already harboring an EMCV-IRES-linked hygromycin resistance gene.

Stable transfected CHO-DUKX cells were cultivated in Dulbecco's modified Eagle medium (DMEM)/F12-Mix (Invitrogen) supplemented with 10% heat-activated fetal bovine serum (FBS; Invitrogen), HT supplement (Invitrogen), 0.2 mg/ml hygromycin B (Invitrogen), and 0.4 mg/ml G418 (Invitrogen). Cells were grown in a humidified chamber at 37°C and 5% CO_2. Stable transfected SH-SY5Y cells [SH-SY5Y-SRE-Luci-D3-121e were cultivated in DMEM (Invitrogen)] supplemented with 15% heat-activated FBS, 0.2 mg/ml hygromycin B, and 0.4 mg/ml G418. Cells were grown in a humidified chamber at 37°C and 8% CO_2.

Cyclic AMP accumulation assay

Cells were detached from the culture dish by treatment with Versene (3–5 min, Invitrogen) and seeded in 384-well microtiter plates (Packard Optiplate NEW, Packard BioScience, Meriden, CT, USA, Part No. 6007290) at a density of approximately 10,000 cells per well in stimulation buffer [Hank's balanced salt solution (HBSS) containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 0.1% bovine serum albumin (BSA)] already containing 75 μg/ml anti-cAMP acceptor bead solution. Five microliters of stimulation buffer was added to each well prior to the addition of 2.5 μl of agonist. Cells were incubated in the dark at room temperature for 30 min. After incubation, 15 μl biotinylated-cAMP/streptavidin donor beads detection mix was added and incubated for 1 to 4 h. Plates were read in a Fusion-α microplate analyzer. cAMP formation assays were performed using the AlphaScreen cAMP kit according to the instructions provided by the supplier (Packard BioScience). Data points were run in triplicate and concentration–response experiments were performed twice.

[^{35}S]GTPγS binding assay

For the preparation of cell membranes, cells were first cultured in 176 cm^2 Petri dishes. At 90% confluency, 5 mM butyrate was added to increase the receptor expression level, and the cells were incubated for an additional 24 h. The medium was removed and Petri dishes were washed once with 5 ml phosphate-buffered saline(PBS; 1.54 mM KH_2PO_4, 155.17 mM NaCl, 2.71 mM Na_2HPO_4–$7H_2O$), incubated in 4 ml Versene [0.2 g/l ethylenediaminetetraacetic acid (EDTA)–4Na in PBS) for 10 min at room temperature and detached. Cells were pelleted at 460×*g* and resuspended in 5 mM Tris–HCl buffer (containing 5 mM EDTA, 5 mM EGTA, 0.1 mM phenyl-methylsulphonyl fluoride and inhibitor cocktail 100 μg/ml AEBSF, 100 μg/ml bacitracin, 5 μg/ml leupeptin, 2 μg/ml pepstatin A; pH 7.6). Cells were homogenized, centrifuged for 15 min at 50,000×*g* and resuspended in 5 mM Tris–HCl buffer, frozen, and stored at −80°C in aliquots until used.

For the [^{35}S]GTPγS binding assay, cell membranes (10–25 μg) were incubated in a total volume of 200 μl containing binding buffer (50 mM Tris–HCl, 10 mM MgCl2, 100 mM NaCl, and 2–20 μM GDP; pH 7.6) and 0.2 nM [^{35}S]GTPγS. Following a 60-min incubation period at 30°C in the absence or presence of various concentrations of agonist, the assay mixture was rapidly filtered through UniFilter® GF/B filters using a FilterMate® filtration device (Perkin Elmer Life Sciences, Zaventem, Belgium). Filters were quickly washed with 1 ml of 50 mM Tris–HCl, 10 mM $MgCl_2$, and 100 mM NaCl at pH 7.6. Radioactivity retained on the filters was

determined by liquid scintillation counting. Each data point was performed in triplicate, and each assay was designed to fit into a single 96-well microtiter plate. Concentration response experiments were repeated two or four times in order to show reproducibility. Non-specific GTPγS binding was determined by incubation with a 50,000-fold excess of cold GTPγS.

Luciferase reporter-gene assays

Cells were seeded in 96-well microtiter plates at a density of approximately 30,000 cells per well in growth medium, supplemented with 0.2 mg/ml hygromycin and 0.4 mg/ml G418. After 24 h, the medium was replaced by 90 µl medium without supplements and serum. Cells were starved under these conditions for 15–18 h prior to stimulation by agonist. Subsequently, the corresponding endogenous agonist or rotigotine (dissolved in PBS containing 1 mg/ml BSA) at the concentrations indicated at the graphs, was added. The cells were kept for another 4 h in the incubator at 37°C, the medium was removed, 20 µl lysis buffer (Promega) was applied, and 30 µl of luciferase assay reagent (Promega) was added. After shaking, the luminescence of the solution was measured, integrative for 3 s with a Fluoroskan Ascent® FL (Labsystems, Helsinki, Finland). Concentration response curves using rotigotine as agonist or antagonist were performed twice (with n=3 replicates). When rotigotine behaved as an agonist, a receptor selective antagonist was used (when available) to confirm this receptor-dependent activation.

Monoamine uptake and release assays

Table 2 lists the origins for the synaptosomes used in the norepinephrine, dopamine, and 5-HT uptake/release assays, reference compounds tested, experimental conditions, and a bibliography documenting the methodology. Scintillation counting was used to detect the quantity of radioactive tracer ([^{3}H]dopamine, [^{3}H]norepinephrine, or [^{3}H]5-HT) incorporated into synaptosomes or released from synaptosomes.

Calculations

For radioligand binding and monoamine uptake/release experiments, IC_{50} and EC_{50} values were determined (via computer software) by nonlinear regression analysis of the competition curves using Hill equation curve fitting. In the functional assay experiments for all receptors tested, EC_{50} values were determined by sigmoidal curve fitting using ORIGIN (OriginLab, Northampton, MA, USA).

Inhibition constants (K_i) were calculated from the Cheng–Prusoff equation ($K_i = IC_{50}/(1 + L/K_D)$, where L=concentration of the radioligand in the assay, and K_D=affinity of the radioligand for the receptor.

Drugs

Rotigotine was provided by Schwarz Biosciences (Monheim, Germany). Dopamine, R(+)-SCH-23390, and L-745,870, were purchased from Sigma Chemical (St. Louis, MO, USA). L-741,626 and GR103691 were purchased from Tocris Cookson (Ballwin, MO, USA).

Results

Rotigotine binding/affinity assays

Using well-established techniques, the ability of rotigotine to inhibit the binding of typical radioligands (K_i) was tested in detail for 28 receptors, ion channels, and transporter molecules (Table 1). The selection of these 28 cell surface molecules was based on a previously performed screening for the potential binding of rotigotine at a fixed concentration of 1 µM with a broader range of 78 different receptors, channels, and transporters. These receptors were with the (h) indicating human origin: A_1 (h), A_{2A} (h), A_{2B} (h), A_3 (h), α_{1A}, α_{1B}, α_{2A} (h), α_{2B}, α_{2C}(h), β_1 (h), β_2 (h), β_3 (h), $BZD_{central}$, $BZD_{peripheral}$, B_1, B_2 (h), CB_1 (h), CB_2 (h), D_1 (h), D_2 (h), D_3 (h), $D_{4.2}$ (h), $D_{4.4}$ (h), $D_{4.7}$ (h), D_5 (h), $GABA_A$, $GABA_B$, AMPA, Kainate, NMDA, $Gly_{strychnine\text{-}sensitive}$,

Table 2 Synaptosome origins, experimental conditions, and methodology for monoamine uptake/release experiments

Assay	Synaptosome Origin	Reference Compound	Tracer	Incubation Conditions	Bibliography
Norepinephrine uptake	Rat hypothalamus	Protriptyline	[^{3}H]Norepinephrine (0.2 µCi/ml)	20 min/37°C	Perovic and Muller 1995
Dopamine uptake	Rat corpora striatum	GBR 12909	[^{3}H]Dopamine (0.2 µCi/ml)	15 min/37°C	Janowsky et al. 1986
5-HT uptake	Rat brain	Imipramine	[^{3}H]5-HT (0.2 µCi/ml)	15 min/37°C	Perovic and Muller 1995
Norepinephrine release	Rat hypothalamus	Amitriptyline	[^{3}H]Norepinephrine	15 min/37°C	Yamagushi et al. 1998
Dopamine release	Rat corpora striatum	Amphetamine	[^{3}H]Dopamine	20 min/37°C	Bondiolotti et al. 1995
5-HT release	Rat brain	Fenfluramine	[^{3}H]5-HT	20 min/37°C	Bonanno et al. 1994

Table 3 K_i values and Hill coefficients for rotigotine and reference compounds at various receptors and transporters

Receptor	Rotigotine		Reference Compounds		
	K_i (nM)	n_H	Compound	K_i (nM)	n_H
Adrenergic					
α_{1A}	176	1.5	WB 4101	0.12	1.0
α_{1B}	273	1.1	Spiperone	0.75	1.2
α_{2A} (*h*)	338	1.1	Yohimbine	4.3	1.2
α_{2B}	27	0.6	Yohimbine	2.8	1.2
α_{2C} (*h*)	135	0.8	Yohimbine	2.1	0.9
Dopaminergic					
D_1 (*h*)	83	0.9	SCH 23390	0.25	1.1
D_2 (*h*)					
1st test	17	0.8	(+)butaclamol	2.4	1.4
2nd test	10	0.9	(+)butaclamol	7.9	1.2
D_3 (*h*)	0.71	1.0	(+)butaclamol	3.4	1.4
$D_{4.4}$ (*h*)	15	0.9	Clozapine	37	0.9
$D_{4.2}$ (*h*)	3.9	0.7	Clozapine	43	0.9
$D_{4.7}$ (*h*)	5.9	0.7	Clozapine	40	0.8
D_5 (*h*)					
1st test	6.3	0.5	SCH 23390	0.21	0.9
2nd test	4.5	0.5	SCH 23390	0.26	0.7
Histamine H_1	330	0.9	Pyrilamine	0.76	1.1
Serotonergic	576	1.0	Methoctramine	21	1.0
5-HT_{1A} (*h*)	30	1.0	8-OH-DPAT	0.39	1.3
5-HT_{1D}	853	1.2	5-HT	1.5	1.1
5-HT_{2B} (*h*)	1950	1.2	5-HT	73	1.0
5-HT_7 (*h*)	86	1.0	5-HT	0.26	0.9
NE transporter (*h*)	2220	1.0	Protriptyline	10	1.1
DA transporter (*h*)	826	0.9	GBR 12909	3.6	3.1
5-HT transporter (*h*)	4810	1.3	Imipramine	2.3	1.0

h Human, *NE* norepinephrine, *DA* dopamine

$Gly_{strychnine\text{-}insensitive}$, TNF-α (h), $H_{1central}$, $H_{1peripheral}$, H_2, H_3, $I_{2central}$, $I_{2peripheral}$, M_1 (h), M_2 (h), M_3 (h), M_4 (h), M_5 (h), NK_1 (h), NK_2 (h), NK_3 (h), Y_1 (h), Y_2 (h), $N_{neuronal}$, α-$BGTX_{insensitive}$, δ (h), κ, μ (h), PCP, 5-HT_{1A} (h), 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A} (h), 5-HT_{2B} (h), 5-HT_{2C} (h), 5-HT_3 (h), 5-HT_4, 5-HT_{4e} (h), 5-HT_{5A} (h), 5-HT_6 (h), 5-HT_7 (h), and $\sigma_{nonselective}$, σ_1, σ_2; the transporters included were adenosine, norepinephrine, dopamine, GABA, choline, 5-HT transporter, and the ion channels were Ca^{++} (L-type, DHP site; L-type, diltiazem site, L-type, verapamil site and N-type), K^+ (ATP-dependent, voltage-dependent, and Ca^{++}-dependent), Na^+ (sites 1 and 2), and Cl^-. Table 3 displays the specific receptor radioligands which rotigotine effectively (>20% at 1 μM) competed with. The receptor affinity for these receptors was studied in detail.

Binding analysis revealed highest affinity of rotigotine at dopamine receptors, particularly at the D_3 receptor (K_i= 0.71 nM). The overall rank order for the binding affinities of rotigotine at dopamine receptors was: D_3>>$D_{4.2}$~D_5~$D_{4.7}$~D_2~$D_{4.4}$>D_1. The affinity to the D_3 receptor was 5.5 to 117 times higher than those of the other dopamine receptors. Rotigotine also demonstrated significant, but lower affinities for α-adrenergic receptors (α_{2B}, K_i=27 nM) and for 5-HT receptors (5-HT_{1A} and 5-HT_7 with K_i values of 30 or 86 nM, respectively), but only minor affinities to 5-HT_2 receptors (5-HT_{2B}, K_i=1,950 nM), which are probably insignificant in vivo. Affinities at muscarinic–acetylcholine and histamine receptors were low (K_i=330–576 nM). The binding studies have been repeated with the aid of another contract research organization which confirmed the aforementioned observations (data not shown, but are on file at Schwarz BioSciences). Rotigotine-mediated binding inhibition was also measured at norepinephrine, dopamine, and 5-HT transporters (Table 1). The affinity at dopamine transporters was the highest among the transporters tested (by 2.7–5.8 times) with K_i=826 nM. Its relevance for in vivo effects is also questionable. Plasma levels in humans are in the low nanomolar range (~*2.5* nmol/l).

Functional assays measuring the intrinsic activity of rotigotine at dopamine receptors

The D_1-like receptors, D_1 and D_5, are known to activate the cellular second messenger cyclic AMP (Missale et al. 1998). Thus, in order to assess the functional activity of

Table 4 EC_{50} values for rotigotine and dopamine at dopamine D_1, D_{2L}, D_3, $D_{4.4}$, and D_5 receptors in functional assays

Receptor classification	Receptor subtype	Cyclic AMP assay			[^{35}S]GTPγS assay			Reporter gene assay		
		DA EC_{50} (nM)	RTG EC_{50} (nM)	EC_{50} Ratio DA/RTG	DA EC_{50} (nM)	RTG EC_{50} (nM)	EC_{50} Ratio DA/RTG	DA EC_{50} (nM)	RTG EC_{50} (nM)	EC_{50} ratio DA/RTG
D_1-like	D_1	9.5±3.7	7.6±1.1	1.25	–	–	–	4.18	0.952	4.4
	D_5	6.2±2.9	26.2±5.5	0.24	–	–	–	2.00	1.39	1.4
D_2-like	D_{2L}	–	–	–	2416±261	12.5±4.8	193.28	19.10	2.35[a]	8.1
								19.10	0.36[b]	53.1
	D_3	–	–	–	24.1±10.0	1.9 ±1.4	12.68	535.8	0.21	2600
	$D_{4.4}$	–	–	–	111.4±5.4	38.2±4.9	2.92	12.89	4.10	3.1

Concentration response curves were performed twice (with n=3 replicates)
DA Dopamine, *RTG* rotigotine
[a] Rotigotine EC50 value calculated for maximal response, which was greater than that of dopamine.
[b] Rotigotine EC50 value calculated for first phase of the apparent biphasic response. The first phase of the response reaches the maximal response of dopamine and is assumed to result from activation of a high-affinity site on the dopamine D2L receptor. An EC50 value could not be calculated for the second phase.

rotigotine on these dopamine receptors, the relative ability of rotigotine and dopamine to induce production of cyclic AMP was measured in CHO cells. For the dopamine D_1 receptor, rotigotine was equipotent (EC_{50} values) to dopamine, but for the dopamine D_5 receptor, rotigotine exhibited a fourfold lower potency (Table 4). Interestingly, these results are in contrast to radioligand-binding experiments (K_i values), where rotigotine was 16-fold more selective for dopamine D_5 over dopamine D_1 receptors. Similarly, the reference compound SCH23390 has also shown substantially different profiles in binding experiments versus functional assays. SCH23390 was equally effective for D_1 and D_5 receptors in binding assays (K_i 471 and 418, respectively) but was seven times more potent at D_5 than D_1 receptors in functional (luciferase) assays (EC_{50} 38.9 and 277.7 pM, respectively). The maximal response to rotigotine was similar to the maximal response to dopamine after activation of either dopamine D_1 or D_5 receptors (Fig. 2). These results suggest that rotigotine is a full agonist at dopamine D_1 and D_5 receptors in this assay.

The D_2-like receptors (D_{2L}, D_3, and $D_{4.4}$) are prototypic G-protein coupled receptors, which inhibit adenyl cyclase and cyclic AMP production, and activate K^+ channels (Missale et al. 1998). To inhibit the production of cAMP, stimulation by forskolin is required. To avoid that requirement, the functional activities of rotigotine and dopamine were determined in CHO and SH-SY5Y human neuroblastoma cells as a function of their ability to induce GDP/[^{35}S]GTPγS exchange by receptor-associated G_α protein. Table 4 lists EC_{50} values for both agonists at dopamine D_{2L}, D_3, and $D_{4.4}$ receptors. At all of these receptors, rotigotine showed a significantly higher functional activity (EC_{50} values) in comparison to dopamine (3- to 193-fold). Based on GDP/[^{35}S]GTPγS exchange activity, rotigotine behaved as a full agonist at D_3 receptors and as a partial agonist at D_{2L} and $D_{4.4}$ receptors (E_{max}=92.3±9.2%, 68.0±13.9%, and 48.5±2.1%, respectively, graphs not shown).

In addition to the GTPγS assay, the intrinsic activity of rotigotine at dopamine receptor subtypes was also investigated in a more downstream reporter gene assay (luciferase assay; Table 4). In these studies, rotigotine behaved as an agonist at all tested human dopamine receptor subtypes as evidenced by concentration-related increases in luciferase enzyme activity (Fig. 3). EC_{50} values for rotigotine at

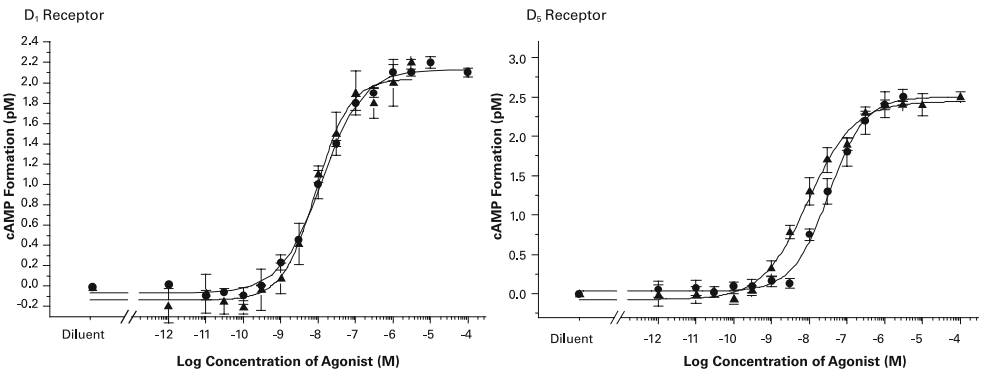

Fig. 2 Rotigotine- and dopamine-induced cAMP activity in Chinese hamster ovary cells expressing human dopamine D_1 and D_5 receptors. Cells were stimulated with increasing concentrations of either dopamine (*filled circles*) or rotigotine (*filled triangles*). Concentration response curves using rotigotine as agonist or antagonist were performed twice (with n=3 replicates)

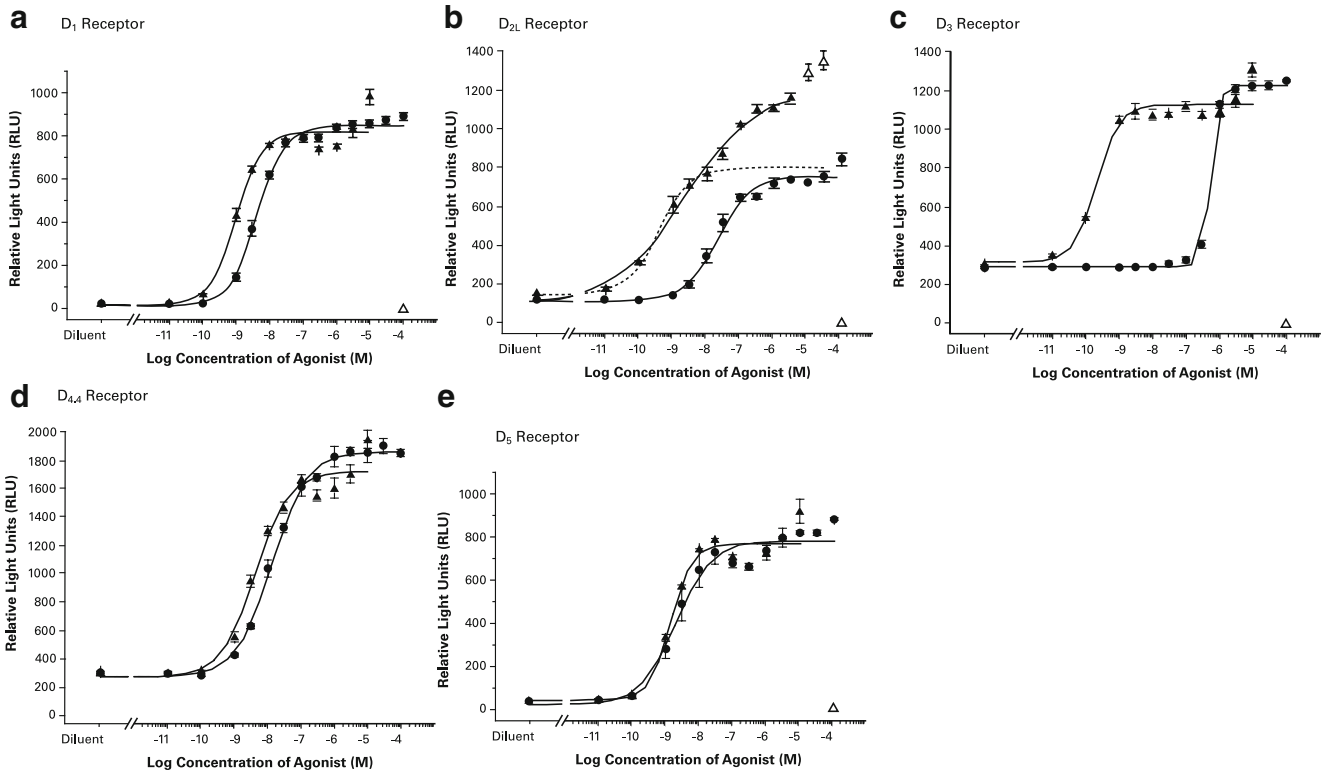

Fig. 3 Rotigotine- and dopamine-induced luciferase reporter-gene activity in Chinese hamster ovary cells expressing human dopamine D_1, D_{2L}, D_3, $D_{4.4}$, and D_5 receptors. Cells were stimulated with increasing concentrations of either dopamine (*filled circles*) or rotigotine (*filled triangles*). Concentration response curves using rotigotine as agonist or antagonist were performed twice (with $n=3$ replicates). For the D_{2L} response curve, the *dotted line* depicts the first phase of activation by rotigotine at an apparent high-affinity D_{2L} binding site. Higher concentrations of rotigotine caused nonspecific effects (*gray symbols*), and these data points were not included in the regression analysis

dopamine D_1, D_{2L}, $D_{4.4}$, and D_5 receptors were lower than those of dopamine (by as much as 53 times); however, at the D_3 receptor, the EC_{50} value for rotigotine was almost 2,600-fold less than that of dopamine (Table 4).

In luciferase-reporter assays for the D_1 and D_5 receptors, rotigotine behaved as a full agonist, eliciting a similar maximal response to that of dopamine (Fig. 3a,e). However, with the dopamine D_{2L} receptor luciferase assays, the maximal activation by rotigotine was higher ('supramaximal') than by dopamine and the concentration–response curve seemed to be biphasic (Fig. 3b). An artificial mathematical approximation suggests an EC_{50} of 0.36 nM for the first phase of the biphasic response curve—a value closer to that at the dopamine D_3 receptor (Table 4, Fig. 2b)—and a much higher EC_{50} value for the second phase of activation, which however, could not be calculated. Based on that approximation, the affinity of rotigotine for this apparent high-affinity site on the D_{2L} receptor was greater than its affinity for dopamine D_1, D_5, or $D_{4.4}$ receptors (2.6 to 11.4 times). While considering EC_{50} values including a calculated value for the high-affinity D_{2L} site, the rank order of potency for rotigotine at dopamine receptors is $D_3>D_{2L}>D_1=D_5>D_{4.4}$. The maximal activation by rotigotine at the dopamine D_3 and $D_{4.4}$ receptors was slightly (≈10%) less than that of dopamine (Fig. 3c,d), but it may be considered a full agonist at these receptors in this assay.

With each of the five dopamine receptor constructs, rotigotine-induced (10 nM) luciferase activity was inhibited by specific antagonists (1 μM) of the respective receptor subtypes, demonstrating the binding and activation by rotigotine. The inhibition of the reporter gene response for D_1 and D_5 (antagonist *R*(+)-SCH-23390) was 65% and 87%, respectively.

D_2-like receptors D_{2L}, D_3, $D_{4.4}$ (antagonists L-741,626; GR103691; L-745,870) responses were inhibited by 79%, 58%, and 86%, respectively. As described, the inhibition of the rotigotine-induced receptor activation by the antagonists was not complete. This is in accordance with investigations with the natural agonist dopamine. Partial antagonism (similar to inhibition of percent) could be demonstrated for the natural agonist dopamine except at the D_{2L} receptor, where L-741,626 was a full antagonist (data not shown). It might be possible that the antagonist concentrations were not sufficient, or the antagonists may not be full antagonists.

Obviously, the different functional assays yielded different results which may be due to the methods and assay specifics.

Table 5 Values associated with agonistic and antagonistic properties of rotigotine at α_{1A}-, α_{1B}-, α_{2A}-, α_{2B}-, and α_{2C}-adrenergic receptors, muscarinic-acetylcholine M_1 and M_2 receptors, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_7 receptors, and histamine H_1 receptors based on luciferase reporter gene assays

Receptor	Rotigotine EC_{50} (nM)	Natural Ligand[a] EC_{50} (nM)	Activation E_{max} at 10 μM[b] (%)	Reference Antagonist	Rotigotine IC_{50} (nM)	Rotigotine pK_i	Inhibition I_{max} at 10 μM[c] (%)
α_{1A}	26±4	17±1.4	45±2	Prazosin	740±252	7.4±0.2	28±3
α_{1B}	NA	37±1.4	–	Prazosin	2,700±690	6.5±0.1	77±3
α_{2A}	>1,000	3±0.5	56±2[d]	Yohimbine	NI	NI	NI
α_{2B}	>1,000	54±4.3	23±5[d]	Yohimbine	394±168	7.3±0.2	64±7
α_{2C}	NA	60±4.6	–	Yohimbine	588±116	7.2±0.1	63±13
M_1	NA	1107±483	–	Scopolamine	3,100±190	6.4±0.1	49±1
M_2	NA	62±13	–	Scopolamine	776±207	6.9±0.2	82±3
5-HT_{1A}	1040±220	15±1.4	71±7[d]	WAY100635	NI	NI	NI
5-HT_{1B}	>1,000	14±1.9	28±6[d]	Methiothepin	NI	NI	NI
5-HT_{1D}	31[e]	17±10.4	36±10[e]	Methiothepin	NI	NI	NI
5-HT_7	NA	25±1.7	–	Clozapine	NI	NI	NI
H_1	NA	3730±270	–	Pyrilamine	2,440±1,050	6.6±0.2	55±2

Concentration response curves were performed twice (with n=3 replicates)
NA No activity, *NI* no inhibition by rotigotine
[a] Natural ligands: for adrenergic receptors (α_{1A}, α_{1B}, α_{2A}, α_{2B}, α_{2C}) = epinephrine; for muscarinic-acetylcholine receptors (M_1, M_2) = acetylcholine; for 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_7) = 5-HT; for histamine receptors (H_1) = histamine.
[b] Maximal response to 10 μM rotigotine as a percentage of the maximal response to the natural ligand at the respective receptor. Due to limits of the assay, concentrations higher than 10 μM for rotigotine could not be tested.
[c] Inhibition of response to the natural ligand by 10 μM rotigotine as a percentage of the inhibition by the reference antagonist. Natural ligand concentration was set at EC_{80} for the respective receptor. Due to limits of the assay, concentrations higher than 10 μM for rotigotine could not be tested. Saturation levels of rotigotine may not have been reached at some receptors.
[d] Saturation levels were not reached with 10 μM rotigotine.
[e] Saturation could not be reproduced in the second experiment.

Intrinsic activity of rotigotine at non-dopaminergic receptors

In radioligand binding studies, rotigotine displayed moderate affinity for only a few, but potentially important non-dopamine receptors (adrenergic and serotonergic receptors). Therefore, rotigotine was further tested in luciferase-reporter assays to determine the functional interaction with these receptors compared to their natural ligands. Agonistic effects (EC_{50} and E_{max}) for rotigotine and control ligands are listed in Table 5. Regarding functional activity, rotigotine displayed the highest potency (EC_{50} values) at α_{1A}-adrenergic receptors where it was identified as a partial agonist (E_{max}=45%). This partial agonistic activity of rotigotine could be completely blocked by the selective alpha-1 antagonist prazosin (data not shown).

For the 5-HT_{1A} receptor, the EC_{50} value was found to be 1,040 nM. After extrapolation, the E_{max} at that receptor was 90±6%. Minor agonistic activations were also seen at α_{2A}-adrenergic, 5-HT_{1B}, and 5-HT_{1D} receptors (Table 5).

Activity of rotigotine at 5-HT_{1A}, α_{1A}-adrenergic, and 5-HT_{1D} receptors was abrogated in a concentration-dependent way by the specific antagonists WAY100635, yohimbine, and methiothepin. These investigations determined that the rank order for the agonistic activities of rotigotine (considering EC_{50} and E_{max} at 10 μM) at these receptors was α_{1A}-adrenergic>5-HT_{1A}>HT_{1B}=5-HT_{1D}.

Possible antagonistic activities of rotigotine at non-dopamine receptors were determined by evaluating its IC_{50} values (on reporter-gene activity induced by the natural ligand) at each receptor as listed in Table 5. Based on IC_{50} value and percent inhibition at 10 μM, we demonstrated that rotigotine inhibited the activity of the natural ligands of α_{2B}- and α_{2C}-adrenergic receptors at IC_{50} concentrations of ≈500 nM. Inhibition of reporter-gene activity also occurred at α_{1A}-, and M2 receptors (IC_{50} concentrations of ≈700 nM) and α_{1B}-, M_1, and H_1 receptors (IC_{50} concentrations of >2,000 nM; Table 5).

These results correlated fairly well with the results of radioligand binding experiments: rotigotine had the highest affinity for α_{2B}-, and α_{2C}-adrenergic receptors, with lesser affinity demonstrated for the others (α_{1A}>α_{1B}=H_1>M_2).

Due to the binding and functional activation of monoamine receptors by rotigotine, its effects on monoamine uptake and release in synaptosomes were also measured. Rotigotine inhibited the uptake of radiolabeled monoamines up to different degrees (norepinephrine IC_{50}=48 nM, dopamine IC_{50}=160 nM, and 5-HT IC_{50}= 710 nM, data not shown). Rotigotine had little effect on monoamine release from synaptosomes with an EC_{50} of

6,000 nM for dopamine and 15,000 nM for 5-HT. The EC_{50} could not be determined for norepinephrine as it was above the highest rotigotine concentration tested (30 μM; data not shown).

Discussion

The present study was undertaken to characterize the receptor profile of rotigotine using a broad spectrum of receptors. As has recently been shown (Millan et al. 2002), the clinically used dopamine agonists for the treatment of PD do not only interact with D_2 and D_3 receptors (although they might previously have been characterized as such) but also with other receptors (with different affinities). Therefore, it was of importance to determine the receptor profile of rotigotine in detail as the interaction with the various receptors may play a role not only concerning the motor effects in PD but also regarding disease progression, propensity to induce dyskinesia or potential other effects.

It was found that rotigotine acts as an agonist for all dopamine receptors with a moderate selectivity for the D_2-like subtypes, particularly the D_3 receptor. Rotigotine also was found to bind solely to the $5HT_{1A}$ and the α_{2B} receptor subtypes as non-dopamine receptors when taking into account the clinically relevant plasma concentrations. The interaction with these receptors may be considered as potentially relevant for its activity as anti-Parkinsonian agent.

To determine its functional activity, different assays based on different technologies were used. A cAMP assay was used to determine the intrinsic activity of rotigotine with respect to the D_1 and D_5 receptors which per se stimulate the production of cAMP and thus allow for a precise measurement. The [^{35}S]GTPγS assay was used with respect to the D_2-like receptors which pre se inhibit the production of cAMP and thus cause a decline of intracellular cAMP levels; the decline of cAMP cannot be directly measured without stimulation of cAMP production by forskolin which was to be omitted. In addition, a luciferase reporter gene assay was used which allowed for a direct comparison of the responses via the different dopamine (and non-dopamine) receptors. However, although the read-out is more downstream of the intracellular signaling cascade when compared to the cAMP or the [^{35}S]GTPγS assay, the validity of the results should be confirmed by using these assays. In addition, the luciferase reporter gene assay has a high sensitivity and has the advantage that the responses of G_i coupled D_2-like receptors could be measured without the use of forskolin by the SRE-based reporter gene assay (George et al. 1998; Fan et al. 2005; Al-Fulaij et al. 2007; Jiang et al. 2005). This should help to primarily identify and confirm the intrinsic activity of rotigotine; it was not intended to compare potencies or efficacies in the various assays and among the investigated receptors, which were to be expected to be different when using different methods (Vanhauwe et al. 1999). Indeed, some of the discrepancies in receptor signaling are attributable to the different assay conditions (temperature, buffer composition, membrane binding compared to cellular assay). However, the results were considered to be more conclusive than previous investigations which used membrane fractions of calf caudate nuclei (Van der Weide et al. 1987, 1988) or rat or mouse vas deferens (Friedman et al. 1992; Martin et al. 1993), which required more complex pharmacological procedures and were difficult to interpret due to the complexity of the organ incubations (see the efforts of (Friedman et al. 1992; Martin et al. 1993). In addition, only D_2 and D_1 receptors were known at that time, and thus, rotigotine could only be characterized regarding potential interaction with D_2 or D_1 dopamine receptors. In fact, the previous observations regarding the agonism of rotigotine on D_1 and D_2 receptors were confirmed by our investigations thus validating our approach.

The data obtained here show that rotigotine is a potent agonist at the dopamine receptors with K_i values in the nanomolar or even subnanomolar (for the D_3 receptor) range. In functional terms, rotigotine was about 2,600 times more potent than dopamine at the D_3 receptor, whereas it was more or less equally potent as dopamine at the other dopamine receptor subtypes. Thus, at therapeutic concentrations (~0.8 ng/ml or ~2.5 nM plasma concentration), rotigotine is to be expected to activate all five of these dopamine receptors (Poewe and Leussi 2005). However, it is theoretically possible that rotigotine may stabilize a distinct conformational state of the dopaminergic GPCR or may regulate signals via different G proteins that are coupled to the dopamine GCPRs "agonist-directed trafficking" in comparison to dopamine, thus acting as a "protean" agonist (Lane et al. 2007).

The utility of D_2 receptor activation in PD is well established. Most dopamine agonists with proven efficacy in Parkinson's bind to the D_2-like subtypes as opposed to the D_1-likes (Millan et al. 2002). The D_2 receptors not only are highly expressed in the striatum (Missale et al. 1998), but are also supersensitive in PD (PD; Rinne et al. 1993) and models of PD (Doudet et al. 2000). However, there is growing evidence for an important modulatory role of the dopamine D_3 receptors (Joyce 2001). In fact, the binding affinities of antiparkinsonian agents in clinical use are similar or even higher at D_3 than at the D_2 receptors (Gerlach et al. 2003; Piercey 1998; Millan et al. 2002). Although D_3 receptors are sparse relative to D_2 in the caudate–putamen, the ventral striatum is densely populated with D_3 receptors (Joyce 2001) having a modulatory role on the motor output as well as on the affective state.

Evidence suggests that in PD, the D_2 receptor number is enhanced, whereas the D_3 receptor density is decreased in the ventral striatum, particularly in later stages of the disease (Joyce 2001). Thus, not only D_2, but also D_3 receptor agonism may be of importance for the effective treatment of both motor and mood disturbances in PD. Additionally, recent data have suggested that D_3-preferring dopamine agonists have neuroprotective effects (Joyce and Millan 2007; Carvey et al. 2001; Hall et al. 1996).

Of the dopamine-receptor subtypes, the D_1 receptor is the most widely distributed in the central nervous system and, like the D_2 receptor, highly expressed in the striatum (Missale et al. 1998). Although agonists that selectively activate the D_1 receptor have not been developed for clinical use, activation of D_1 receptors has been shown to provide marked antiparkinsonian activity (Taylor et al. 1991). However, no D_1 agonist is currently being marketed. The reasons could be the limited bioavailability of the compounds synthesized to date (potentially due to the catechol moiety), the development of tolerance, and the risk of inducing epilepsy (Mailman et al. 2001; Corvol et al. 2006).

It is generally agreed that selective D_2 agonists are far less efficacious in the treatment of the Parkinsonian symptoms in humans than levodopa. A simple explanation could be that the D_1 receptors play a crucial role (Loschmann et al. 1992; Giardina and Williams 2001; Mailman et al. 2001; Mailman and Nichols 1998; Williams et al. 1997). Importantly, Rascol et al. (2001b, 1999) showed that ABT-431 (a full D_1 agonist similar to dihydrexidine; see Giardina and Williams 2001) was equieffective to levodopa, the only D_1 agonist to ever show effectiveness in humans. Together, this suggests an important role for D_1 receptors in the effective treatment of PD. In fact, the simultaneous activation of dopamine D_1 and D_2 receptors is known to produce greater locomotor stimulation than D_2 activation alone (Clark and White 1987). Rotigotine indeed shows affinity to the D_1 receptor which might contribute to its efficacy (Loschmann et al. 1992), although it is far less than to the D_2 and D_3 receptors. In contrast to that, ropinirole or pramipexole do not show D_1 activity (Gerlach et al. 2003; Millan et al. 2002), which might explain weaker activity in experimental models (Loschmann et al. 1992).

The possible role of D_4 or D_5 receptor activation in PD has not been established. Although most antiparkinsonian drugs have significant binding affinities at D_4 receptors (Millan et al. 2002), the range of affinity/efficacy seen for this receptor varies widely and its activation does not appear to impact clinical efficacy (Newman-Tancredi et al. 1997). This may be due to very low levels of expression of dopamine D_4 receptors in the striatum (Missale et al. 1998). Although dopamine D_5 receptors are more abundant in striatum (Missale et al. 1998), the binding affinities of anti-Parkinsonian agents at these receptors is generally low or even negligible ($pK_i<5$ for pramipexole and ropinirole; Millan et al. 2002), explaining perhaps why the role of D_5 receptor activation by anti-Parkinson drugs has not been taken into consideration so far. Interestingly, dopamine D_5 receptors are highly expressed in hippocampus where they may mediate learning and memory (Missale et al. 1998).

In summary and regarding the actual knowledge available, rotigotine may be considered as a dopamine agonist eliciting its efficacy in PD via $D_3/D_2/D_1$ receptors.

Although activation of dopamine receptors is considered a prerequisite for antiparkinsonian action, other monoaminergic receptors may play a role in the efficacy and side-effect profiles of agents used to treat PD (Millan et al. 2002). The activity of rotigotine at α_{2B}-adrenergic and 5-HT_{1A} receptors may be considered as of importance. Experimental studies have shown that the α_2-adrenergic antagonists idazoxan, rauwolscine, and yohimbine were active, e.g., reducing levodopa-induced dyskinesia in rat (Henry et al. 1999) or exhibiting neuroprotective properties (Srinivasan and Schmidt 2004). Idazoxan also was found to be active in monkeys (Grondin et al. 2000). Clinical studies, however, remain contradictory (Rascol et al. 2001a; Manson et al. 2000). The 5-HT_{1A} receptor is known to modulate dopaminergic activity and motor function in the basal ganglia and can reduce levodopa-induced dyskinesia without reducing, and potentially increasing, the therapeutic effects of levodopa (Nicholson and Brotchie 2002; Carta et al. 2007). In a monkey model of PD, the selective 5-HT_{1A} agonist sarizotan reduced levodopa-induced dyskinesia by more than 90% (Bibbiani et al. 2001). In patients with advanced PD, concomitant sarizotan and levodopa therapy was shown to reduce dyskinesias and prolong antiparkinsonian action (Bara-Jimenez et al. 2005). Furthermore, 5-HT_{1A} agonists also have been shown to inhibit excitotoxic mechanisms and thus impart neuroprotection in vitro (Madhavan et al. 2003) and in vivo (Mauler and Horvath 2005). The 5-HT_{1A} receptor also is supposed to mediate antidepressant activity (Blier and Abbott 2001). Thus, although these observations suggest a beneficial role of the α_{2B} and 5-HT_{1A} receptor, detailed investigations regarding the potential interactions of rotigotine with these receptors have not been performed. It is noteworthy that rotigotine lacks affinity for the 5-HT_{2B} receptor, which is involved in valvular diseases (Setola et al. 2003; Launay et al. 2002; Zanettini et al. 2007).

In summary, rotigotine is a non-ergolinic dopamine $D_3/D_2/D_1$ dopamine receptor agonist with therapeutic potency in PD; its potential interaction with D_4/D_5 receptors needs further evaluation in that respect. Although rotigotine shows specificity for the dopaminergic system, it also exhibits a characteristic interaction with 5-HT_{1A} and α_{2B} receptors which might contribute to its efficacy especially

with respect to dyskinesia or disease progression but also needs further investigations.

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