Demonstration of D1 dopamine receptor agonist actions of the antiparkinson drug rotigotine

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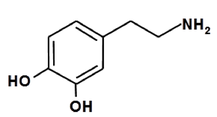
ABSTRACT

Parkinson's disease (PD) is a chronic, disabling neurological disorder that affects more than a million Americans, with the number still growing as the population ages. This project sought to investigate controversies surrounding rotigotine, an approved drug for PD. Rotigotine is thought to cause antiparkinson effects via activation of the D2 and D3 dopamine receptors, but this project was designed to test the hypothesis that rotigotine also had affinity for D1 receptors, and if so, what its functional properties were at the D1 receptor. We used competitive radioreceptor assays to compare the binding at ^{3}H-SCH23390-labeled D1 receptors of rotigotine, dihydrexidine (a known D1 full agonist) and SCH23390 (a known antagonist). The data demonstrate that rotigotine has modest, but potentially important D1 affinity. We then used the canonical functional assay (stimulation of cAMP synthesis) to demonstrate that rotigotine had high intrinsic activity at the cloned human D1 receptor. In parallel, experiments were conducted in the unilateral 6-OHDA rat model of PD. Rotigotine had activity in this model, and surprisingly, this activity was blocked by the selective D1 antagonist SCH23390, but not the selective D2 antagonist haloperidol. Together, these data indicate that rotigotine has potentially useful D1 agonist properties that are neither commonly appreciated nor used to advantage clinically.

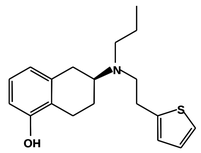
INTRODUCTION

Parkinson's disease is a progressive brain disorder marked by the death of dopamine neurons and subsequent loss of dopamine production. Dopamine is a neurotransmitter that plays an important role in motor control, various cognitive tasks, emotion, endocrine events, and reward. In Parkinson's disease (PD), the dopamine neurons that die are in the substantia nigra of the brain, but the target nerve cells that contain most of the dopamine receptors are largely unaffected. Thus, the disintegration of the nigrostriatal pathway causes dysfunction of motor control cause of insufficient dopamine to activate its receptors. The current gold standard therapy is levodopa (L-dopa) that is known to be converted in the brain into dopamine and thereby activate dopamine receptors. There are five dopamine receptors, and there has been controversy about which are most important to alleviate PD symptoms. Because of anatomical location, only D1, D2, and D3 receptors are likely to play a role. All of the approved antiparkinson drugs are selective for D2/D3 receptors, but are less efficacious than levodopa, suggesting the importance of the D1 receptor. Although no current drugs are D1 selective, a variety of evidence suggests that D1 agonists (drugs that activate receptors like dopamine) may have effects equal to levodopa. Thus, it would be useful to have an approved drug that could test this idea.

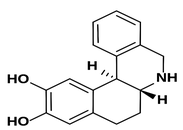
Recent evidence suggests that the drug rotigotine (marketed as Neupro), might be unique among approved drugs in having both D1 affinity and high D1 intrinsic activity. Since the literature also indicated that high intrinsic activity requires a structural feature (catechol group) not present in rotigotine, this observation became important to study. We therefore undertook to test the hypothesis that rotigotine had both affinity and functional activity at the dopamine D1 receptor.



dopamine

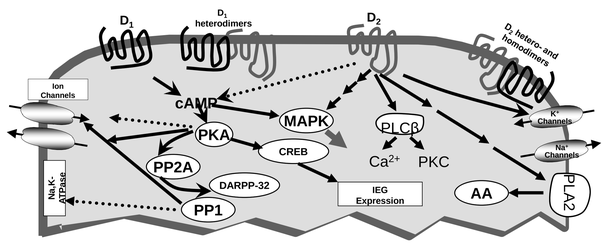


rotigotine



dihydrexidine

Dopamine Receptor Signaling



MATERIALS AND METHODS

Animal Model

The animal model used four unilaterally-lesioned 6-OHDA rats in which injection of 6-OHDA into the medial forebrain bundle had caused >90% death of substantia nigra dopamine neurons. This causes a "supersensitivity" to drugs that activate dopamine receptors on the side of the brain that was lesioned. Therefore, when a rat is challenged with a D1 or D2/D3 dopamine agonists, they will begin a right circling in the direction away from the lesion (contralateral). This circling is thought to be due to the fact that the lesioned side is "supersensitive" and produces a greater motor signal than does the unlesioned side. Four 6-OHDA rats were used in these studies. The rats were videotaped during this time period, and rotations quantified using the Colbourne Instruments system. All protocols were reviewed by the Institutional Animal Care and Use Committee of the Penn State Hershey Medical Center.

Competition Assay

Dilutions of dihydrexidine (DHX), rotigotine, and SCH23390 were made from 10mM stock solutions. The buffer used was 50 mM HEPES, 4 mM $MgCl_2$, 0.1% ascorbic acid, brought to pH 7.4 with 1 M KOH. Buffer, [^{3}H]SCH23390, test drug solution at appropriate dilution, and membranes from the striatum of Sprague-Dawley rat brain were placed in each well for a total volume of 500 µL. The plate was covered with Parafilm and vortexed using a multi-tube vortexer. Incubation of the plate occurred in a 37ºC water bath for 15 min. Following this, a PEN cell harvester was used to vacuum filter and wash (three-times) each well.. The filtermat was dried in a 50ºC oven for 60 min, and Micro-scintillation fluid (35 µL) was pipetted into each well. A PEN TopCount plate reader was used to quantify the radioactivity in each well.

Functional Assay

The D1-mediated stimulation of cAMP synthesis was measured using the GloSensor method. GloSensor transfected cells were seeded into white TC treated 96-well ½ area plates. These cells were grown overnight in a 37ºC incubator with 5% CO_2. The media was completely removed from all the wells and 50 µL of GloSensor loading buffer was added to each well. The plate was then incubated in the dark at room temperature for 2h. Dilutions of dopamine, dihydrexidine, and rotigotine were created from 10 mM stock solutions. They were diluted with an assay media containing Ham's F-12 media, HEPES, IBMX, and 0.1% ascorbic acid. After the incubation, the plate was pre-read, for background luminescence using the ultrasensitive chemiluminescent program on the Envision instrument. Once removed from the reader, the antagonists, drug dilutions, and agonists were added. The plate was mixed gently then read again after 15 min and 30 min of incubation in the dark at room temperature.

RESULTS

Behavioral studies

Initially, two rats (Group A) were administered 0.3 mg/kg haloperidol (a known D2/D3 antagonist) and two other rats (Group B) were treated with 1 mg/kg rotigotine. After 30 min, Group A rats received 1 mg/kg rotigotine and Group B rats received 0.3 mg/kg haloperidol.

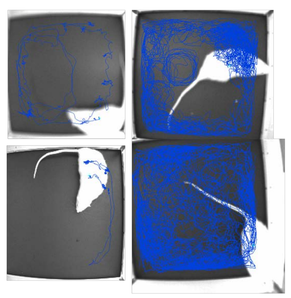
As expected, in Group A rats, haloperidol alone caused no rotation or other motor behavior. In Group B rats, rotigotine caused contralateral rotations that began ~5 min after drug injection. During the first 30 min, the Group B rats averaged 243 rotations consistent with activation of one or more dopamine receptors.

After the second set of injections were made at 30 min, Group A rats began to rotate and averaged 220 contralateral rotations. The Group B rats also continued to rotate with no apparent effect of the haloperidol, recording an average of 265 rotations during the same time period.

Administration of haloperidol in Group A
Administration of rotigotine in Group B

0.3 mg/kg haloperidol

1 mg/kg rotigotine



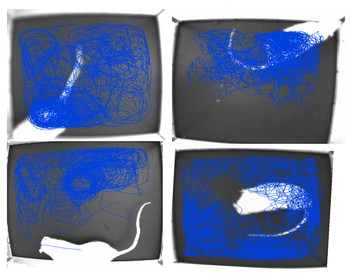
0 rotations in 30 min

243 rotations in 30 min

Administration of rotigotine in Group A
Administration of haloperidol in Group B

0.3 mg/kg haloperidol
+
1 mg/kg rotigotine

1 mg/kg rotigotine
+
0.3 mg/kg haloperidol



220 rotations in 30 min

265 rotations in 30 min

In vitro studies

The data from the D1 competition binding studies are shown in Figure 1. As can be seen visually, the rank order of affinity was SCH23390 > dihydrexidine > rotigotine, although rotigotine clearly had some affinity for the D1 receptor. Analysis of these data, using Prism 5, indicted that the $K_{0.5}$ of SCH23390 and dihydrexidine were 0.5 and 20 nM, consistent with published data. The $K_{0.5}$ of rotigotine was 240 nM also consistent with a recent report, but the competition curve appeared to have normal, not shallow steepness, possibly suggesting D1 antagonist properties. Since essentially identical data were obtained from two experiments showing D1 affinity for rotigotine, we then examined the functional properties of the drug.

As can be seen in Figure 2, both rotigotine and dopamine caused a dose-dependent stimulation of cAMP synthesis. The stimulation of high concentrations of both dopamine and rotigotine was also completely abolished by the D1 selective antagonist SCH23390. The potency of rotigotine is similar to that of dopamine.

RESULTS

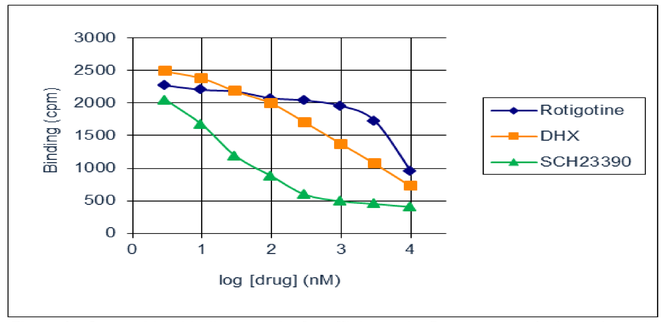


Figure 1 - Competition binding results for affinity to the D_1 receptors. It shows the rank order of affinity was SCH23390 > dihydrexidine > rotigotine, Only SCH23390 demonstrated a normal displacement curve.

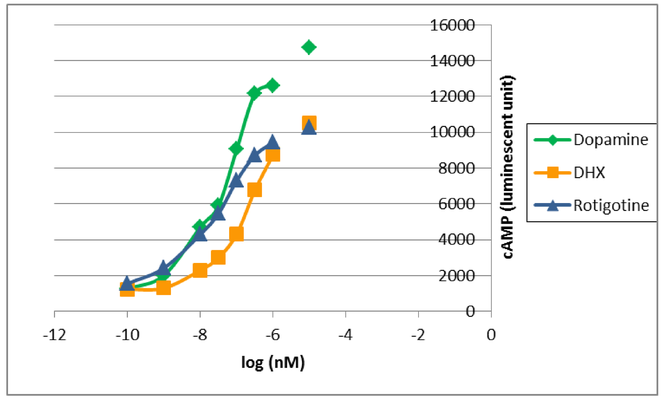


Figure 2 - cAMP production. Both rotigotine and dopamine caused a dose-dependent stimulation of cAMP synthesis.

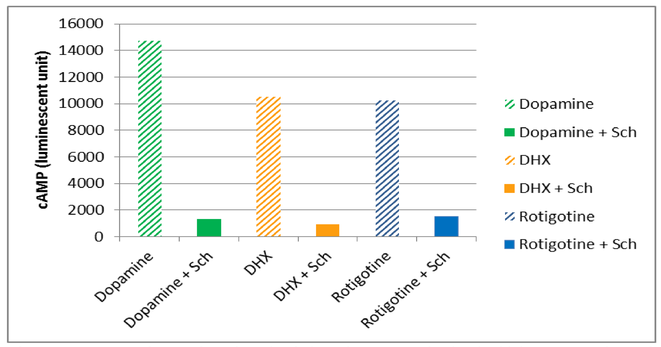


Figure 3 - Antagonist study. The stimulation of cAMP production by high concentrations of both dopamine and rotigotine was completely abolished by the D1 selective antagonist SCH23390.

CONCLUSIONS

- Rotigotine has moderate affinity for rat dopamine D1 receptors, and has very high intrinsic activity and moderate potency in the canonical D1 functional assay, activation of adenylate cyclase.
- Rotigotine causes marked rotations in the unilateral 6-OHDA rat, and surprisingly these rotations are abolished by the D1 antagonist SCH23390 but not the D2 antagonist haloperidol. This suggests D1 activity in this animal model of PD that is solely dependent on occupation of D1 receptors.
- This is the first demonstration that a non-catechol dopamine D1 receptor ligand has high intrinsic activity at the D1 receptor.
- If the hypothesis of Mailman and co-workers about the importance of D1 receptors is correct, then rotigotine may have clinical potential if it were used at higher doses to achieve D1, rather than just D2 occupancy. This may require co-treatment with a D2 antagonist like quetiapine, but seems worthy of clinical investigation.

ACKNOWLEDGEMENTS

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