Neuropharmacological evaluation of a new dopaminergic prodrug with anti-parkinsonian potential

Abstract

In this study, a prodrug of hydroxylated 2-aminotetralins was tested against the prototypic dopamine receptor agonist S-(-)-5-OH-DPAT. The active S-enantiomer of the prodrug (DD9812) was able to decrease the release of dopamine in the striatum, but with a lower potency than S-(-)-5-OH-DPAT after s.c. administration. The R-enantiomer of the prodrug (DD9813) only had a limited effect on the release of dopamine in the striatum. The prodrug, however, showed an improved relative oral bioavailability, as compared to S-(-)-5-OH-DPAT.

6.1 Introduction

Parkinson’s disease is a neurodegenerative disease that is characterised by progressive damage of predominantly dopaminergic neurons in the substantia nigra. Damage to neurons in the substantia nigra causes a dopamine deficiency in the striatum, resulting in disturbed motor functioning. Only when approximately 70-80% of these cells have degenerated, symptoms of Parkinson’s disease arise.

Current drug-based therapies for Parkinson’s disease are palliative therapies, i.e. they relieve the symptoms, but do not cure the disease. One of the current and expanding therapies is treatment with dopamine D_2/D_3 receptor agonists. There are a number of such therapeutics, e.g. bromocriptine, pergolide, apomorphine, ropinirole, and pramipexole.

A class of compounds with a high affinity for e.g. dopamine D_2 receptors are the hydroxylated 2-aminotetralins, e.g. 5-OH-DPAT (9), N-0437 (31), 7-OH-DPAT (10) and 5,6-di-OH-DPAT (81). Preclinical data show that these compounds display limited activity upon oral administration. A major disadvantage of the hydroxylated 2-aminotetralins and other phenolic compounds is that they undergo considerable inactivation by glucuronidation in the gut and the liver. One of the strategies to circumvent the problem of the low oral bioavailability of the hydroxylated 2-aminotetralins is to search for suitable prodrugs. Frequently investigated prodrugs of phenols are esters and carbamates.

We have now synthesised 6-(N,N-di-n-propylamino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (PD148903), which is a dihydro analogue of 5-OH-DPAT and which may possibly act as a prodrug or a pharmacophore equivalent of 5-OH-DPAT. The individual enantiomers of racemic PD148903 were prepared in our laboratory (to be published). Johnson et al. published that the in vitro biochemistry of PD148903 showed that the compound itself did not have any affinity for the dopamine D_2 receptor. However, in vivo experiments showed that it has potent dopamine receptor agonist-like activity. These two observations suggest that in vivo the prodrug is, at least partly, converted to hydroxylated derivatives. Therefore, we have pharmacologically evaluated the S-(−)-enantiomer (DD9812, 83) and the R-(+)-enantiomer (DD9813, 82) of the potential prodrug PD148903 against the active enantiomer of the prototypic dopamine receptor agonist S-(−)-5-OH-DPAT. The exact mechanism of conversion and metabolism is still under investigation.
Neuropharmacological evaluation of a new dopaminergic prodrug

![Chemical structures of S-(–)-5-hydroxy-2-(N,N-di-n-propylamino)tetralin (S-(–)-5-OH-DPAT, S-(–)-9), S-(–)-5-hydroxy-2-(N-n-propyl-N-2-thienylethylamino)tetralin (S-(–)-N-0437, S-(–)-31), R-(+)-7-hydroxy-2-(N,N-di-n-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)10), 5,6-dihydroxy-2-(N,N-di-n-propylamino)tetralin (5,6-di-OH-DPAT, 81) and R- and S-6-(N,N-di-n-propylamino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (R-enantiomer, DD9813, 82; S-enantiomer, DD9812, 83).]

6.2 Material and methods

6.2.1 Animals

Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments. The rats were housed in Plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery and use in the experiments. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

6.2.2 Drug treatment

The drugs were dissolved in saline and stocked in a concentration of 1 mg/ml for subcutaneous (s.c.) and 1 mg/2 ml for oral (p.o.) administration and diluted, if necessary, with saline before administration. A volume of 1 ml/kg was administered per s.c. injection and 2
ml/kg per p.o. injection. Drugs used were DD9812, DD9813 and S-(−)-5-OH-DPAT and were synthesised at the Department of Medicinal Chemistry in Groningen. The amount of compound was recalculated to an amount of S-(−)-5-OH-DPAT, i.e. 1 mg/ml indicates an amount of compound equal to 1 mg/ml S-(−)-5-OH-DPAT.

6.2.3 Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described. In brief, the rats were anaesthetised with midazolam (5 mg/kg s.c.), atropine nitrate (0.1 mg/kg s.c.), ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.); 10% lidocaine was locally applied. The rats were then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontal. The skull was exposed and burr holes were drilled. A Y-shaped dialysis probe was used for the experiments, with an exposed tip length of 3 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following coordinates were used according to the atlas of Paxinos and Watson: AP + 1.0, LM ± 3.0 relative to bregma, and VD − 6.0 below dura. Before insertion into the brain the dialysis probe was perfused successively with ultra pure water, methanol, ultra pure water and Ringer solution (1.2 mM Ca²⁺). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received buprenorphine (0.1 mg/kg i.m.), an analgesic agent. The rats were housed solitary.

The experiments were performed in conscious rats 17-48 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l CaCl₂, 1.1 mmol/l MgCl₂) at 2 µl/min (CMA/102 microdialysis pump, Sweden).

Dopamine was quantitated by high-performance liquid chromatography (HPLC) with electrochemical detection with a detection limit of approximately 5 fmol/sample. An HPLC pump (LKB, Pharmacia) was used in conjunction with an electrochemical detector (Antec, Leiden) working at 625 mV versus an Ag/AgCl reference electrode. The analytical column was a Supelco Supelcosil LC-18 Column (3 µm particle size). The mobile phase consisted of a mixture of 4.1 g/l sodium acetate (Merck), 85 mg/l octane sulphonic acid (Aldrich), 50 mg/l EDTA (Merck), 1 mM tetramethylammonium chloride (ACROS), 8.5 % methanol (Labscan) and ultra pure water (pH=4.1 with glacial acetic acid).

After the experiments the rats were sacrificed and the brains were removed. After removal the brains were kept in 4% paraformaldehyde solution until they were sectioned to control the location of the dialysis probes.
6.2.4 Statistics

Data of the microdialysis experiments were converted into percentage of the basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. Dopamine release was measured for 180 min to be able to compare the curves and the Areas Under the Curve (AUCs). Microdialysis data were compared using one-way ANOVA for repeated measurements, followed by Dunnett’s Method post-hoc test. The relative oral bioavailabilities were determined by comparing the AUCs after p.o. and s.c. administration. When there was no significant difference between the effects on dopamine release, the s.c. dose was divided by the p.o. dose and multiplied by 100 to give a percentage representing the relative oral bioavailability. For determining the AUC the mean of the first four samples (t = – 45 to 0 minutes) was taken as baseline and then the AUC was calculated. At the end of the curve, whether basal levels were reached again or not, the program draws an imaginary vertical line and left from this line the AUC is calculated. To be able to compare the AUCs the AUC was calculated using the same time course for the different doses. Statistical analysis of the AUC was performed by a t-test. In all cases a significance level of 0.05 was applied.

6.3 Results

The control dialysate concentrations in the striatum for the experiments were 12.0 ± 0.9 (n = 51) fmol/min.

The results of the microdialysis experiments of the compounds DD9812, DD9813 and S-(−)-5-OH-DPAT are shown in the figures 6.1-6.3. S.c. administration of DD9812 and S-(−)-5-OH-DPAT, but not DD9813 induced a dose-dependent, and significant, decrease in the release of dopamine in the striatum. Also after p.o. administration DD9812 and S-(−)-5-OH-DPAT induced a significant decrease in the release of dopamine in the striatum. The effect of DD9813 was not studied upon p.o. administration.
Figure 6.1  Effect of DD9812 on striatal dopamine release in freely moving rats after s.c. (A) and p.o. (B) administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* p<0.05).

DD9812 induced a significant decrease in dopamine release by s.c. administration of a dose of 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg and 0.1 mg/kg with a maximum decrease of 25 %, 45 %, 50 % and 80 % of control values, respectively (Figure 6.1A). Figure 6.1B shows that the maximum significant decrease after p.o. administration of DD9812 in a dose of 0.1 mg/kg and 0.3 mg/kg was 50 % and 70 % of control values, respectively. For both s.c. and p.o. administration the duration of action of the compound was longer with a higher dose.
Only in a high dose (1 mg/kg s.c.) DD9813 has a limited, significant effect on the release of dopamine in the striatum (Figure 6.2).

S-(–)-5-OH-DPAT, upon s.c. administration, induced a significant decrease in dopamine release of maximally 35 % and 70 % of control values after doses of 0.001, and 0.003 mg/kg, respectively (Figure 6.3A). The significant effect of administration of 0.1 mg/kg p.o. and 0.3 mg/kg p.o. of S-(–)-5-OH-DPAT lasted from t = 15 min to t = 180 min with a maximum decrease of 55 % and from t = 30 min to t = 180 min with a maximum decrease of 70 % of control values, respectively (Figure 6.3B).

Figure 6.2  Effect of DD9813 on striatal dopamine release in freely moving rats after s.c. administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* p<0.05).
Figure 6.3  Effect of (–)-5-OH-DPAT on striatal dopamine release in freely moving rats after s.c. (A) and p.o. (B) administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* p<0.05).

The relative oral bioavailabilities, as determined by comparing the AUCs after s.c. and p.o. administration, of DD9812 and S-(–)-5-OH-DPAT were calculated from data shown in Figures 6.1 and 6.3 and are presented in Table 6.1. The AUCs were calculated by taking the mean of the first four points of the basal level and than determining the area beneath this baseline until the end of the curve. To be able to compare the AUCs after different doses the same time courses of 180 min were used. When the AUCs were not significantly different, the relative oral bioavailability, as expressed in per cent, was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. For DD9812 the relative oral bioavailability was 10-100 %, while for the reference compound S-(–)-5-OH-DPAT it was 1-3 %.
Table 6.1 AUCs of the microdialysis experiments of DD9812 and S(-)-5-OH-DPAT after p.o. and s.c. administration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Subcutaneous administration</th>
<th>Oral administration</th>
<th>Relative bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg)</td>
<td>AUC (± S.E.M.)</td>
<td>Dose (mg/kg)</td>
</tr>
<tr>
<td>DD9812</td>
<td>0.003</td>
<td>2650 ± 800\textsuperscript{a}</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>7430 ± 880</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>9980 ± 1280</td>
<td>0.1</td>
</tr>
<tr>
<td>S(-)-5-OH-DPAT</td>
<td>0.0003</td>
<td>1915 ± 420\textsuperscript{b}</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>9750 ± 1630</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Footnotes: \textsuperscript{a} Experiment lasted 165 min. \textsuperscript{b} Experiment lasted 150 min. All other experiments lasted 180 min. All the AUCs of s.c. and p.o. doses of each compound were compared, but only the doses that were not significantly different were put in line in the table.

Statistical analysis of the AUCs of the microdialysis experiments of DD9812 and S(-)-5-OH-DPAT after p.o. administration showed that there is no significant difference between the effects of the two compounds using the same dose.

6.4 Discussion

The enantiomers of the putative prodrug PD148903 were tested for their pharmacological effects on dopamine autoreceptors in vivo, by measuring dopamine release in the striatum using on-line microdialysis in freely moving rats. Stimulation of dopamine autoreceptors by dopamine D\textsubscript{2} receptor agonists cause a decrease in the release of dopamine.\textsuperscript{189}

DD9812, which is the S(-)-enantiomer of the prodrug, induces a dose-dependent decrease in the release of dopamine in the striatum after both s.c. and p.o. administration. This indicates that DD9812 acts as a dopamine receptor agonist in vivo. During the microdialysis experiments it was observed that in the highest doses administered (0.1 mg/kg s.c. and 0.3 mg/kg s.c.) DD9812 induced dopaminergic stereotyped behaviour, i.e. sniffing and rearing. DD9813, the R(+)-enantiomer, induced only a very limited effect on the release of dopamine in the striatum, i.e. only in a high dose (1 mg/kg s.c.) there was a significant but limited decrease in the release
of dopamine. It is uncertain whether this decrease is caused by the R-(+)-enantiomer itself or by a contamination of the S-(−)-enantiomer. The enantiomeric purity of the (+)-enantiomer is >99.8%. If the impurity of the S-(−)-enantiomer is 0.2% this would correspond to 0.002 mg/kg of DD9812, which may be an active dose. Because of the limited activity of DD9813 we did not determine its relative oral bioavailability. S-(−)-5-OH-DPAT induced a dose-dependent decrease in the release of dopamine in the striatum after both s.c. and p.o. administration.

Compared to S-(−)-5-OH-DPAT the prodrug DD9812 displayed a lower potency in decreasing dopamine release in the striatum after s.c. administration. This difference in potency might be due to the time course of DD9812 uptake and/or bioactivation, which may influence the responses to the prodrug. On the other hand, the prodrug may also be sensitive to metabolism into inactive metabolites, before its conversion into the active metabolite. It is known from the metabolism of the N,N-dipropylated 2-aminotetralins that they are N-dealkylated. For DD9812 this is also one of the possible routes of metabolism. The prodrug is compared with the very potent dopamine receptor agonist S-(−)-5-OH-DPAT, but this might not be correct since it has not been proven yet that bioactivation leads to this 2-aminotetralin. It is possible that DD9812 is converted into another active hydroxylated 2-aminotetralin. When the p.o. doses are compared the effects are similar, i.e. there are no significant differences in the AUCs for DD9812 and S-(−)-5-OH-DPAT at the same dose administered.

The relative oral bioavailabilities were calculated by measuring the pharmacological effect of the compounds. This is called the pharmacodynamic method of determining pharmacokinetic parameters. Only relative oral bioavailabilities can be determined in this manner. In order to be able to determine the absolute oral bioavailability pharmacokinetic experiments are necessary. DD9812 showed a relative oral bioavailability of 10-100%. (−)-5-OH-DPAT possessed a low relative oral bioavailability of 1-3%, which can be explained by the considerable inactivation via glucuronidation in the gut and the liver.

Thus, DD9812 seems to be an active prodrug of a dopamine receptor agonist with a better relative oral bioavailability than the prototypic dopamine receptor agonist S-(−)-5-OH-DPAT. However, the prodrug has a lower potency than S-(−)-5-OH-DPAT in decreasing the release of dopamine in the striatum after s.c. administration. To find out whether DD9812 is an useful prodrug and an alternative for hydroxylated 2-aminotetralins, metabolism studies are in progress to elucidate the mechanism of bioactivation and to find the active species.