New, centrally acting dopaminergic agents with an improved oral bioavailability
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Chapter 5

Dopamine D\(_2\) activity of R-(−)-apomorphine and selected analogues: a microdialysis study*

Abstract

In the present study, R-(−)-apomorphine and three of its analogues were studied for their potency in decreasing the release of dopamine in the striatum after subcutaneous administration and for their oral bioavailability using the microdialysis technique in freely moving rats.

The analogues R-(−)-N-n-propylnorapomorphine and R-(−)-11-hydroxy-N-n-propylnoraporphine displayed a higher potency than R-(−)-apomorphine in decreasing the release of dopamine in the striatum. A high dose of R-(−)-11-hydroxyaporphine, a dopamine D\(_2\) receptor partial agonist, had a small effect on the release of dopamine in the striatum. The catechols R-(−)-N-n-propylnorapomorphine and R-(−)-apomorphine displayed a comparable oral bioavailability (1%), while the monohydroxy analogue R-(−)-11-hydroxy-N-n-propylnoraporphine displayed a slightly higher oral bioavailability (3%).

In conclusion, R-(−)-N-n-propylnorapomorphine and R-(−)-11-hydroxy-N-n-propylnoraporphine did not show a substantial improvement in bioavailability. However, due to the clear difference in their efficacy in decreasing dopamine release, in spite of the similar agonist binding affinity for the dopamine D\(_2\) receptor of the two analogues compared to R-(−)-apomorphine, they could be useful alternatives for apomorphine in the treatment of Parkinson’s disease.

5.1 Introduction

Parkinson’s disease is a progressive neurodegenerative disorder of the basal ganglia, which most often becomes apparent after the age of 55. It is a prototypic hypokinetic disorder, with akinesia, bradykinesia, rigidity and tremor as the most prominent features. Depression and a general slowing of intellectual processes also occur, but are less well-defined. The neurological and psychiatric symptoms usually worsen with time (for review see ref. 118). The neuropathology of Parkinson’s disease reveals a striking loss of the dopaminergic neurons of the nigrostriatal pathway.119,120

As Parkinson’s disease is associated with a loss of dopamine, it is commonly treated with drugs which replace dopamine. Since dopamine itself cannot pass the blood-brain barrier, the most commonly used therapy is levodopa (L-DOPA), a precursor of dopamine. A complication of long-term treatment with L-DOPA, however, is the development of rapid fluctuations in clinical state where the patient switches suddenly between mobility and immobility; this phenomenon is known as the ’on-off’ effect.135,136

An alternative approach to the treatment with L-DOPA is the use of drugs that mimic the action of dopamine. Treatment with dopamine receptor agonists has some advantages over treatment with L-DOPA. Dopamine receptor agonists are effective in patients in the advanced stages of Parkinson’s disease unlike L-DOPA, because their action at postsynaptic receptors is unaffected by the lack of dopamine producing nerve cells. Furthermore, there is an increasing interest in the potential of dopamine receptor agonists to provide a neuroprotective effect. Theoretically, such a protective effect might result from (a) a decrease in L-DOPA application, as L-DOPA may cause oxidative stress,144 (b) stimulation of dopamine autoreceptors resulting in decreased dopamine synthesis, release, and turnover, as dopamine metabolism leads to reactive oxygen species,145 and (c) direct anti-oxidant effects.141-143

The dopamine D1/D2 receptor agonist R-(–)-apomorphine has proven to be very effective in Parkinson’s disease. Subcutaneously administered R-(–)-apomorphine in combination with L-DOPA rapidly and consistently reverses the ‘off’ period motor deficits.253-256 Beside its action as a dopamine D1/D2 receptor agonist, R-(–)-apomorphine can also act as a radical scavenger257 and, therefore, may have neuroprotective properties. One of the major limitations of the clinical use of R-(–)-apomorphine, a catechol-aporphine, however, is its low oral activity.258-260
With respect to the low bioavailability of R-(–)-apomorphine we initiated a study of three analogues (79, 80, 12) of R-(–)-apomorphine (11). The selected analogues all possess affinity for the dopamine D1 and D2 receptors comparable to R-(–)-apomorphine. It was postulated that the monohydroxy compounds would have a higher oral bioavailability, as compared to the catechols, because they are likely to be less sensitive to metabolic degradation. Although a great deal has already been reported on the in vitro and in vivo pharmacology of apomorphine (11) and selected analogues (79, 80, 12), no study has been undertaken to examine this series of compounds with respect to their oral bioavailabilities in vivo. We have now examined these compounds with respect to their potencies and relative bioavailabilities, using the microdialysis technique in freely moving rats. By measuring dopamine release in the striatum, information on the degree of dopamine D2 autoreceptor stimulation can be obtained. Dopamine D1 receptor stimulation was not investigated in this study. Comparisons were made after subcutaneous (s.c.) and per oral (p.o.) administration in an attempt to estimate the importance of the first-pass effect for this series of apomorphine analogues.

5.2 Materials and Methods

5.2.1 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. 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.
5.2.2 Drug treatment

The drugs were dissolved in degassed ultra pure water with approximately 0.5 mg/ml ascorbic acid to prevent oxidation of the compounds and stocked in a concentration of 300 nmol/ml for subcutaneous administration and 10 µmol/2 ml for oral administration and diluted, if necessary, with degassed ultra pure water before administration. To dissolve R-(−)-11-hydroxy-aporphine a drop of glacial acetic acid was added. Drugs used were R-(−)-apomorphine.HCl (11), R-(−)-11-hydroxyaporphine (79), R-(−)-N-n-propyl norapomorphine.HCl (80) and R-(−)-11-hydroxy-N-n-propynoraporphine.HBr (12). R-(−)-apomorphine.HCl was purchased from RBI, compounds 80 and 12 were provided by Prof. J.L. Neumeyer (Harvard Medical School, MA), R-(−)-11-hydroxy-aporphine (79) was synthesised in Groningen.

5.2.3 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 cannula 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 co-ordinates 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 phosphatine 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). 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 probe.

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 Na-acetate (Merck), 85 mg/l octane sulphonic acid (Aldrich), 50 mg/l EDTA (Merck), 8.5 % methanol (Labscan) and ultra pure water (pH=4.1 with glacial acetic acid).

5.2.4 Data analysis

Data were converted into percentage of basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. During 180 min after administration of the compound the dopamine release was measured. This time course was chosen to be able to compare the effects and Areas Under the Curve (AUC) of the different compounds and routes of administration. The AUC was determined using GraphPad Prism for Windows (GraphPad Inc.). To determine the AUC the mean of the first 4 samples were taken as baseline and then the AUC was calculated from t=0 min to t=180 min. At t=180 min the program draws an imaginary vertical line and left from this line the AUC is calculated. The experiments were terminated after 180 minutes to be able to compare the AUCs. The relative oral bioavailabilities were determined by comparing the curves 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. Microdialysis data were compared using one-way analysis of variance (ANOVA) for repeated measurements, followed by Dunnett’s Method post-hoc test. A significance level of 0.05 was applied. Statistical analysis of the AUCs was performed by a t-test. For comparison with R-(−)-apomorphine (11) 30 nmol/kg equal variance test failed and than Rank Sum Test followed by Mann-Whitney test was performed.

5.3 Results

The basal dialysate concentrations in the striatum for the experiments were 11.9 ± 0.7 (n = 79) fmol/min.

Figures 5.1A-D show that s.c. administration of R-(−)-apomorphine (11), R-(−)-N-n-propylnorapomorphine (80), and R-(−)-11-hydroxy-N-n-propylnoraporphine (12), but not R-(−)-11-hydroxyaporphine (79), induced a dose-dependent decrease in the release of dopamine in the striatum. R-(−)-apomorphine (11) induced a significant decrease in the release of dopamine in the striatum in a dose-range from 0.1 to 1 µmol/kg s.c. In a dose-range from 0.003 to 0.3 µmol/kg s.c., R-(−)-N-n-propylnorapomorphine (80) induced a significant decrease in the release of dopamine in the striatum. R-(−)-11-hydroxy-N-n-propylnoraporphine (12) induced a significant decrease in the release of dopamine in the striatum in a dose-range from 0.03 to 0.3 µmol/kg s.c. Although R-(−)-11-hydroxyaporphine (79) displays affinity for the dopamine D₂
receptor, it only induced a small significant decrease in the release of dopamine in the striatum in a dose of 1 µmol/kg s.c.

**Figure 5.1** Effects 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. (A) R-(−)-apomorphine (11); changes are significant (p < 0.05) from t = 30 min to t = 90 min for 0.1, and 0.3 µmol/kg s.c., and from t = 30 min to t = 120 min for 1 µmol/kg s.c. (B) R-(−)-N-n-propylnorapomorphine (80); changes are significant (p < 0.05) from t = 30 min to t = 105 min for 0.003 and 0.01 µmol/kg s.c., from t = 15 min to t = 180 min for 0.03, 0.1, and 0.3 µmol/kg s.c.
Figure 5.1 Effects 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. (C) R-(−)-11-hydroxy-N-n-propylnoraporphine (12); changes are significant (p < 0.05) from t = 30 min to t = 180 min for 0.03 and 0.1 µmol/kg s.c., from t = 15 min to t = 180 min for 0.3 µmol/kg s.c. (D) R-(−)-11-hydroxyaporphine (79); changes are significant (p < 0.05) from t = 45 min to t = 75 min for 1 µmol/kg s.c.

The dose-response relationships of the test compounds are given in Figure 5.2. The response of the compounds is given as the AUC. To compare the AUCs, the experiments were stopped after 180 min. The rank order in the potency upon s.c. administration of the compounds is: R-(−)-N-n-propynorapomorphine (80) > R-(−)-11-hydroxy-N-n-propylnoraporphine (12) > R-(−)-apomorphine (11).
The relative oral bioavailabilities of R-(–)-apomorphine (11) and two of its analogues 80 and 12 can be found from Figures 5.3A-C. The relative oral bioavailability was determined by comparing the curves and the AUC after s.c. and p.o. administration. When the AUCs were not significantly different, the relative oral bioavailability was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. It was known that R-(–)-apomorphine (11) possessed a low oral bioavailability. It was expected that the oral bioavailability of the three compounds would be between 1 % and 10 %. Based on this assumption the oral doses were chosen. With this method, both the catechols R-(–)-apomorphine (11) and R-(–)-N-n-propylnorapomorphine (80) possess a relative oral bioavailability of about 1 %. The mono-hydroxy compound R-(–)-11-hydroxy-N-n-propylnoraporphine (12) possesses a relative oral bioavailability of about 3 %.

When the courses of the curves after s.c. administration of compounds 11, 80, and 12, respectively, are compared it can be seen that the duration of action of the N-n-propyl analogues is longer than that of R-(–)-apomorphine.

Compound 79 does not induce an effect on the release of dopamine from the striatum (Figure 5.1D). The binding data together with literature data suggest that this compound is a partial dopamine D₂ receptor agonist and a dopamine D₁ receptor antagonist. Therefore, the present pharmacodynamic method was not suitable to determine the relative oral bioavailability.
Microdialysis study of R-(−)-apomorphine and analogues

Figure 5.3  Effects on striatal dopamine release in freely moving rats after s.c. and p.o. administration. The results are the mean ± S.E.M. of data obtained from 4 rats. (A) R-(−)-apomorphine (11); changes are significant (p < 0.05) from t = 30 min to t = 90 min for 0.1 µmol/kg s.c. and 10 µmol/kg p.o. (B) R-(−)-N-n-propylnorapomorphine (80); changes are significant (p < 0.05) from t = 30 min to t = 105 min for 0.01 µmol/kg s.c. and for t = 15 min to t = 180 min for and 1 µmol/kg p.o.
Figure 5.3  Effects on striatal dopamine release in freely moving rats after s.c. and p.o. administration. The results are the mean ± S.E.M. of data obtained from 4 rats. (C) R-(−)-11-hydroxy-N-n-noraporphine (12); changes are significant (p < 0.05) from t = 30 min to t = 180 min for 0.1 µmol/kg s.c., from t = 15 min to t = 180 min for 3 µmol/kg p.o.

5.4 Discussion

R-(−)-apomorphine (11) is a catechol and is known to have a low oral bioavailability. However, the drug is very useful in the treatment of Parkinson’s disease when L-DOPA treatment gives ‘on-off’ fluctuations. An analogue which also displays dopamine D₁ and D₂ receptor agonistic properties, but possessing a higher oral bioavailability, would be beneficial as an alternative treatment in Parkinson’s disease. The analogues tested (R-(−)-11-hydroxyaporphine (79), R-(−)-N-n-propynorapomorphine (80) and R-(−)-11-hydroxy-N-n-propynoraporphine (12)) possess affinity for the dopamine D₁ and D₂ receptors comparable to R-(−)-apomorphine (Table 5.1). In our experiments we monitored the dopamine D₂ receptor agonistic properties of the compounds, as the release of dopamine is under the control of dopamine D₂ autoreceptors.

Figures 5.1A-C show that compounds 11, 80, and 12 act as dopamine D₂ receptor agonists, because they all induce a decrease in the release of dopamine in the striatum. R-(−)-11-hydroxyaporphine (79) (Figure 5.1D) induces, in a dose of 1 µmol/kg, a small significant decrease in the release of dopamine in the striatum. This lack of biochemical activity of compound 79 was not expected from a structure-activity point of view. However, Schaus et al. already published that R-(−)-11-hydroxyaporphine (79) acts as a partial agonist at the dopamine D₂ receptor. This would explain our findings that this compound has a very weak effect on the dopamine D₂ autoreceptor.
microdialysis study of R-(–)-apomorphine and analogues

Table 5.1 Affinities of R-(–)-apomorphine (11) and its selected analogues (79, 80, 12).

<table>
<thead>
<tr>
<th>compound</th>
<th>$K_i$ (nM)</th>
<th>$[3^H]$-SCH23390 (D$_1$-antagonist)</th>
<th>$[3^H]$-spiperone (D$_2$-antagonist)</th>
<th>$[3^H]$-ADTN (D$_2$-agonist)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-(–)-apomorphine (11)$^a$</td>
<td>240</td>
<td>11.1</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>R-(–)-11-hydroxyaporphine (79)$^b$</td>
<td>107$^c$</td>
<td>58$^c$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>R-(–)-N-$n$-propylnoraporphine (80)$^a$</td>
<td>340</td>
<td>0.8</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>R(–)-11-hydroxy-N-$n$-propylnoraporphine (12)$^d$</td>
<td>434</td>
<td>0.9</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>

Footnotes: Affinity of R-(–)-apomorphine (11) and its selected analogues (79, 80, 12) as measured by their ability to displace in vitro $[3^H]$-SCH23390 (D$_1$-antagonist), $[3^H]$-spiperone (D$_2$-antagonist), and $[3^H]$-ADTN (D$_2$-agonist) from membrane preparations of rat brain corpus striatum tissue in order to measure the affinity for dopamine D$_1$ and D$_2$ receptors, respectively. $^a$ Values are taken from ref. 263; $^b$ values are taken from ref. 261; $^c$ IC50 in nM; $^d$ values are taken from ref. 264.

The dose-AUC relationships upon s.c. administration (Figure 5.2) clearly show that the N-$n$-propyl analogues 80 and 12 are more efficacious in the microdialysis experiments than the N-methyl analogue (R-(–)-apomorphine (11)). An explanation could be the presence of a propyl moiety on the nitrogen for R-(–)-N-$n$-propylnoraporphine (80) because a propyl moiety has a better fit into the receptor than a methyl. This, however, should have been shown in differences in binding affinities, which are not observed. Although there is a clear distinction in efficiency in the microdialysis experiments between the three compounds, this difference cannot be explained by the affinity for the dopamine D$_2$ receptor (Table 5.1). The relevant dopamine D$_2$ receptor agonist binding is comparable for the three compounds.

Figures 5.3A and B show that both of the catechol-containing aporphines (R-(–)-apomorphine (11) and R-(–)-N-$n$-propylnoraporphine (80)) possess a relative oral bioavailability of about 1%. The greater s.c. potency of R-(–)-N-$n$-propylnoraporphine (80) over R-(–)-apomorphine (11) is not likely to be the result from different rates of metabolism in the periphery because the main route of metabolism for both compounds is glucuronidation of the catecho moiety. The greater potency of the mono-hydroxy analogue R-(–)-11-hydroxy-N-$n$-propylnoraporphine (12) (Figure 5.3) could possibly be explained from differences in metabolism, R-(–)-11-hydroxy-N-$n$-propylnoraporphine (12) possesses a relative oral bioavailability of 3%. Although this represents an increase, it is still not an optimal oral bioavailability for a therapeutic agent. The improvement of the relative oral bioavailability of the mono-hydroxy analogue 12 may be explained from the fact that a mono-hydroxy analogue is less sensitive to metabolic degradation than a catechol moiety. Beside glucuronidation, the catechol moiety is also sensitive to oxidative degradation, as well as to degradation by catechol-O-methyl transferase (COMT), which will predominantly result in 10-methoxy-11-
hydroxyaporphine, an inactive compound.\textsuperscript{267,268} The catechol moiety is clearly not necessary for high affinity at the dopamine D\textsubscript{2} receptors. The presence of a free 11-hydroxy moiety is enough to confer dopamine D\textsubscript{2} receptor agonist-like activity, similar to that of 10,11-dihydroxyaporphines.\textsuperscript{266}

An explanation could be differences in intrinsic efficacy and differences in the ability to pass the blood-brain barrier. For R-(–)-N-n-propylapomorphine (80) this difference in ability to pass the blood-brain barrier compared to R-(–)-apomorphine has previously been published.\textsuperscript{265,269,270}

Beside comparable effects on the release of dopamine in the striatum, R-(–)-N-n-propylapomorphine (80) and R-(–)-11-hydroxy-N-n-propylaporphine (12) could also possess the same neuroprotective effects as R-(–)-apomorphine. This neuroprotective effect resides in the phenolic moiety, which can act as a radical scavenger.\textsuperscript{141-143} Both compounds possess this moiety.

Based on our results, R-(–)-N-n-propylapomorphine (80) and R-(–)-11-hydroxy-N-n-propylaporphine (12) could be good targets for treatment of Parkinson’s disease. Although their oral bioavailabilities are low, their greater efficacy results in the possibility of administering lower doses. R-(–)-N-n-propylapomorphine (80) has proven to be a useful adjunct in the long-term management of patients with unsatisfactory response to L-DOPA and produced a significant therapeutic benefit at doses much lower than the dose at which side-effects occur.\textsuperscript{271}

In conclusion, this microdialysis study shows that R-(–)-N-n-propylapomorphine (80) and R-(–)-11-hydroxy-N-n-propylaporphine (12) are more potent than R-(–)-apomorphine (11) in inhibiting dopamine release in the striatum. This difference in potency on the presynaptic receptors resembles the difference in potency of these analogues on supersensitive postsynaptic receptors as described by Kelly et al.\textsuperscript{269}