Research report

The dopamine agonist pramipexole scavenges hydroxyl free radicals induced by striatal application of 6-hydroxydopamine in rats: an in vivo microdialysis study

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Abstract

Hydroxyl free radical production seems to play an important role in the pathogenesis of Parkinson’s disease. In the present study, we investigated the dopamine agonists pramipexole and pergolide as well as the nitrene compound S-PBN (N-tert-butyl-α-(2-sulfophenyl)nitri-}
reacts with iron ions to form hydroxyl radicals the most reactive oxygen species.

The ‘gold standard’ for treatment of Parkinson’s disease today is levodopa, the precursor of dopamine, in combination with a peripheral decarboxylase inhibitor. For many years the safety of levodopa and its long-term benefit have been topics of discussion. Levodopa significantly increased hydroxyl radical formation after systemic injection in combination with the peripheral decarboxylase inhibitor [37] and many in vitro studies reported toxic effects of levodopa (for review see [19]) and more recently the induction of apoptosis [41]. Furthermore, long-term levodopa therapy is accompanied with psychiatric and motor side effects (dyskinesias) and the efficacy of levodopa medication decreases after some years of treatment. Therefore, delaying the onset of levodopa therapy or adjunctive medication with dopamine agonists or MAO inhibitors may be beneficial in the early phase of PD.

Dopamine agonists act on dopamine receptors to mimic the effects of dopamine. In contrast to levodopa they do not need surviving presynaptic dopaminergic neurons for uptake and metabolism. Pramipexole is a nonergot dopamine agonist with an azepine structure and exerts full intrinsic activity on D2 subfamily receptors, especially the D3 receptor, with little interaction to adrenergic and serotonergic receptors [26, 30, 31]. The mechanism of D2 autoreceptor mediated reduction of extracellular dopamine levels was postulated to protect nigral neurons against associated oxidative stress [23].

Indeed, pramipexole reduced extracellular dopamine levels [4] and more recently neuroprotective effects of pramipexole by additional mechanisms such as antioxidant effects and induction of a trophic factor were claimed [5].

To study the effects of pramipexole on hydroxyl free radicals in vitro, we used a cell-free Fenton system. Under these conditions the potential antioxidant effects are directly related to the chemical structure of the compound and not to indirect effects such as interaction with biological antioxidant mechanisms such as induction of antioxidant enzymes or reduction of the radical generating dopamine metabolism.

In vivo, two routes of administration (systemic and local) of pramipexole were applied to investigate the potential effect on hydroxyl radical levels. In the first experiment, systemic application of pramipexole was compared with pergolide on the reduction of basal hydroxyl radical levels without any exogenous stimulation of hydroxyl radical formation. In the second experiment, systemic application of pramipexole was compared with pergolide on the reduction of hydroxyl radical levels induced by striatal reverse dialysis with 6-OHDA. In the third experiment, local application of pramipexole was compared with S-PBN (instead of pergolide, because of the poor solubility of pergolide in the perfusion fluid) on the reduction of hydroxyl radical levels again induced by striatal reverse dialysis with 6-OHDA. Furthermore, extracellular dopamine levels were measured in the third experiment.

2. Material and methods

2.1. In vitro Fenton system

Hydroxyl radicals were generated according to a previously published method [12, 39]. In brief, a mixture of 0.3 mM FeCl3, 0.3 mM Na2EDTA and 3 mM H2O2 in 5 ml of Tris-buffer adjusted to pH 7.4 in the presence of 0.5 mM salicylic acid was incubated for 15 min at 37°C. S-PBN, pramipexole or vehicle were co-incubated in this Fenton system in order to assess their possible radical scavenging effects using the salicylate hydroxylation assay.

2.2. Animals

Adult male albino Wistar rats (Hsd/Cpb:WU, Fa. Harlan-Winkelmann GmbH, Borchen, Germany) weighing about 300 g at the time of microdialysis probe implantation, were used in this study. An ambient room temperature was maintained at 23±2°C, the relative humidity at 55±5%. The animals were kept in a 12 h light–dark cycle (lights on at 07.00 a.m.–07.00 p.m.) and housed individually in Plexiglas cages (40×20×24 cm) with light metal covering and fed with standard food (Altromin®, Fa. Altromin, Lage, Germany) and tap water. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. The experimental protocols were approved by the appropriate institutional governmental agency (Regierungspresident Gießen, Germany).

2.3. In vivo microdialysis experiments

The microdialysis experiments were carried out as described earlier [13, 38]. A guide cannula was implanted under chloral hydrate anaesthesia (400 mg/kg i.p.) and aimed at the head of the caudate nucleus (coordinates from bregma: AP: +1.25, ML: +2.6, DV: −2.5 according to the atlas of Paxinos and Watson [29]). On the following day a microdialysis probe (CMA 12, membrane diameter 0.5 mm, membrane length 4 mm, Carnegie Medicin, Stockholm, Sweden) was introduced and the rats were placed in a microdialysis system with balance arm for freely moving animals. The perfusion fluid contained 5 mM salicylic acid dissolved in a modified Ringer solution (Na+ 147 mM, K+ 4 mM, Ca2+ 1.3 mM, Mg2+ 1 mM, Cl− 155.6 mM, flow rate: 2 μl/min). After an equilibration period of approximately 180 min a stable baseline was obtained and the intraperitoneal injection of pramipexole (0.05, 0.2, and 1 mg/kg), pergolide (0.05 mg/kg) or saline was carried out. The mean of three 20 min basal dialysate fractions were set as 100%. 
In experiments with exogenous hydroxyl radical stimulation the perfusion fluid was changed for 60 min to a fluid additionally containing 0.2 or 2 nmol/2 μl/min of 6-hydroxydopamine for reverse dialysis to deliver 6-OHDA into the surrounding tissue of the microdialysis probe. Then, the perfusion fluid was changed back again to the perfusion fluid of the baseline recording, which was without 6-OHDA. In control experiments modified Ringer solution was used instead of 6-OHDA.

Samples were collected every 20 min in vials containing 10 μl 0.4 M perchloric acid and were directly analyzed by HPLC or stored at −70°C until analysis.

2.4. HPLC-analysis

The HPLC system for determination of 2,3- and 2,5-DHBA and dopamine consisted of a solvent delivery system (Waters model 600S in combination with Waters model 616, Millipore, Milford, USA), an autoinjector with cooling module set at 4°C (Waters model 717 plus, Millipore, Milford, USA), a column thermostat set at 22°C (Gynkotek model STH 585, Germering, Germany), an online degasser (Knauer model A1050, Berlin, Germany), a two channel electrochemical detector (BAS LC 4C, Bioanalytical Systems, West Lafayette, USA) and a data collection and calculation unit controlled by Gynkosoft software (Gynkotek, Germering, Germany). The detector potentials were set at +750 mV using a glassy carbon electrode and an Ag/AgCl reference electrode with a range of 1–10 nA/V. The mobile phase contained 0.14 g octane sulfonic acid sodium salt as an ion-pair reagent, 0.1 g disodium EDTA, 6 ml triethylamine and 35 ml acetonitrile in 1 l of millipore Q® water adjusted to pH 2.95 with concentrated phosphoric acid. The eluent was delivered with a flow rate of 0.5 ml/min onto a reversed-phase column (125×3 mm, pre-column 5×3 mm filled with Nucleosil 120-3 C18 (Knauer, Berlin, Germany). Data were calculated by an external standard calibration.

The detection limit for 2,3-DHBA was approximately 0.1 nmol/l and its in vitro recovery (microdialysis probes CMA 12) was 15–20%. The values of 2,3-DHBA and dopamine are expressed as percentages of three predrug dialysates and were not corrected for the recovery of the probes.

3. Results

3.1. In vitro studies

Incubation of salicylic acid alone in the radical generating system resulted in a similar increase in 2,3-DHBA and 2,5-DHBA levels and to a lesser extent in an increase in catechol (data not shown), reflecting an enhanced hydroxyl radical formation. In control experiments without drug incubation 2,3-DHBA levels (mean±S.E.M.) of 4.4±0.12 μM were obtained and set as 100%. Increasing concentrations of pramipexole (0.5–5 mM) and S-PBN (0.5–5 mM) led to a significant decrease in 2,3-DHBA levels (Fig. 1). In each concentration tested, pramipexole was more effective than S-PBN (P<0.001). The highest concentration of pramipexole (5 mM) reduced the increase of 2,3-DHBA to about 3.5% of the control values, whereas S-PBN (5 mM) reduced the increase of 2,3-DHBA to about 35% of the control values.

3.2. Effects of saline, pramipexole and pergolide on basal 2,3-DHBA levels

The perfusion with 5 mM salicylic acid produced stable...
basal 2,3-DHBA levels, which were not significantly different between the experimental groups. After injection of saline, pramipexole (1 mg/kg) or pergolide (0.05 mg/kg) no significant changes in 2,3-DHBA levels were observed compared to the corresponding pre-injection basal values (mean ± S.E.M.) 53.44 ± 3.5, 55.64 ± 3.9 and 57.92 ± 2.2 nmol/l, respectively (Fig. 2).

3.3. Effects of saline, pramipexole and pergolide on 6-OHDA-induced increase in 2,3-DHBA levels

Before onset of hydroxyl free radical stimulation with 6-OHDA, 2,3-DHBA mean basal levels ± S.E.M. in the saline, pramipexole (0.05, 0.2 and 1 mg/kg i.p.) and pergolide (0.05 mg/kg i.p.) treated groups of 53.42 ± 6.3, 50.56 ± 3.0, 52.49 ± 5.8, 54.78 ± 5.3 nmol/l and 50.44 ± 6.0 nmol/l, respectively were obtained. Perfusion with 6-OHDA (0.2 nmol/2 μl/min) via the probe led to an about 14-fold increase in 2,3-DHBA levels in the saline treated group, to an about 12–13-fold increase in the pramipexole treated groups and to an about 17-fold increase in the pergolide treated group. There were no significant differences between any of the experimental groups (Fig. 3).

3.4. Effects of local application of saline, S-PBN and pramipexole on 6-OHDA-induced increase of 2,3-DHBA levels

Before onset of hydroxyl free radical stimulation with 6-OHDA the following 2,3-DHBA mean basal levels ± S.E.M. were obtained in the saline, S-PBN (2 nmol/2 μl/min) and pramipexole (2 and 10 nmol/2 μl/min) treated groups of 54.42 ± 5.1, 56.91 ± 2.9, 55.34 ± 2.8 and 55.96 ± 4.9 nmol/l, respectively. Perfusion with 6-OHDA (0.2 nmol/2 μl/min) via the probe led to an about 14-fold increase in 2,3-DHBA levels in the saline treated group and to an about 12-fold increase in the S-PBN group. This reduction was not statistically significant. In contrast, in the pramipexole (2 and 10 nmol/2 μl/min) treated groups the 6-OHDA-induced elevation in 2,3-DHBA levels was significantly attenuated to a 7.8- and 8.6-fold increase, respectively (Fig. 4A).

3.5. Effects of local application of saline, S-PBN and pramipexole on 6-OHDA-induced increase in dopamine levels

The following dopamine mean basal levels ± S.E.M. were obtained in the saline, S-PBN (2 nmol/2 μl/min) and pramipexole (2 and 10 nmol/2 μl/min) treated groups of 2.31 ± 0.3, 2.43 ± 0.2, 2.34 ± 0.5 and 2.21 ± 0.4 nmol/l, respectively. Perfusion with 6-OHDA (0.2 nmol/2 μl/min) led to an about 25.5-fold increase in dopamine levels in the saline treated group compared to mean basal levels. In the S-PBN treated group during 6-OHDA perfusion an attenuation to an 11-fold increase was observed. In contrast, pramipexole (2 and 10 nmol/2 μl/min) pretreatment significantly attenuated the 6-OHDA-induced increase in dopamine levels to a 6.9- and 4.6-fold elevation, respectively (Fig. 4B).

4. Discussion

In the present study, pramipexole showed a pronounced effect in reducing hydroxyl radical levels which were generated in a cell-free in vitro Fenton system. In vivo, local application of pramipexole decreased the 6-OHDA-
induced hydroxyl radical formation which was paralleled by an attenuation of the 6-OHDA-induced increase in extracellular dopamine concentration.

Pramipexole was designed as a novel D₂ receptor agonist [25]. Binding studies revealed a 5- to 10-fold higher selectivity for human D₂ receptors compared with D₂ like subtypes (D₂, D₂, D₄) [26,31]. The dopamine receptor agonistic properties of pramipexole may account for the antagonism of parkinsonian symptoms in MPTP pretreated monkeys [25], the improvement of parkinsonian symptoms in MPTP-induced hemiparkinsonian macaques monkeys [9] and the symptomatic benefit in parkinsonian patients. Besides its postsynaptic effects, pramipexole activates presynaptic dopamine autoreceptors and attenuates the extracellular dopamine concentration [4]. However, the basal hydroxyl radical levels were not reduced after systemic administration in the present study. We think that the autoreceptor regulated dopamine release seems not to play an important role to reduce hydroxyl radicals under basal conditions where only low nanomolar concentrations of dopamine were measured in striatal dialysates. Conversely, 6-OHDA produced a massive increase in the extracellular dopamine concentration (25.5-fold), which was significantly reduced by pretreatment with pramipexole. The role of excessive dopamine concentrations seems to be critical for hydroxyl radical formation. In a previous study with local application of malonate a massive overflow of dopamine was paralleled by an increase in hydroxyl radical formation. Furthermore, the lesion of the substantia nigra with 6-OHDA, which almost completely depleted striatal dopamine, prevented the malonate-induced hydroxyl radical formation [11]. Moreover, Lancelot et al. [22], reported that dopamine release contributes to an enhanced hydroxyl radical formation after local glutamate application.

The antioxidant activity of many dopamine agonists seems to contribute to their protective effects as demonstrated for piribedil [7], bromocriptine [27,40], pergolide [15,28], apomorphine [14,33], ropinirole [17] and pramipexole [6,16,42]. However, direct radical scavenging properties are difficult to observe under in vivo conditions.

We demonstrated that pramipexole was only effective to reduce hydroxyl radical levels when a local administration via the microdialysis probe was performed. The applied concentrations seem appropriate because the diffusion-controlled pretreatment of pramipexole via the probe was carried out before the onset of 6-OHDA perfusion and pramipexole was not present in the perfusion fluid during 6-OHDA delivery. This was done to avoid a direct interaction of 6-OHDA with pramipexole before brain entry.

The concept of a radical scavenger is to protect biomolecules such as lipids, proteins and nucleic acids from radical attacks. Indeed, in PD oxidative stress indicated by
enhanced lipid peroxidation [8], oxidative protein alterations [1] and oxidative DNA alterations [2,34] is evident. In respect of their radical scavenging activity, pramipexole and S-PBN have to compete with biomolecules in the reaction with hydroxyl radicals in order to reduce oxidative stress and protect against cell damage. Therefore, only the higher brain concentrations which were achieved by local application of pramipexole were able to reduce hydroxyl radical levels in vivo. In contrast, S-PBN was not able to reduce 2,3-DHBA levels which is in line with the results of the in vitro system in which pramipexole was more effective to decrease hydroxyl radical levels than S-PBN.

Although our study was not designed to measure neuroprotection by itself, our findings provide further evidence that pramipexole exerts neuroprotective effects which were reported by Hall et al. [16] in mice against...
methamphetamine toxicity and in gerbils in the model of forebrain ischemia with posts ischemic nigral degeneration. They postulated direct radical scavenging properties for pramipexole and an antioxidant capability indicated by a low oxidation potential. Inhibitory effects on MPTP-induced dopamine reduction in mice [20], methamphetamine toxicity in mice [21], protection against 3-acetyl-pyridine [35] and against local application of MPP+ [6] are further hints for additional effects of pramipexole not related to its D2-like agonistic effects. The protection of cultured mesencephalic dopaminergic neurons against levodopa neurotoxicity may be mediated by pramipexole-mediated induction of trophic activity of pramipexole [5].

Taken together, pramipexole was able to reduce hydroxyl radical levels induced by the Fenton reaction in vitro and by 6-OHDA after local application in vivo. This property of pramipexole may be beneficial under conditions of enhanced hydroxyl radical formation in parkinsonian brains and may add to its well known dopamine D2-like receptor agonistic effects.

References


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