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Continuous Dopaminergic Stimulation by Pramipexole Is Effective to Treat Early Morning Akinesia in Animal Models of Parkinson's Disease: A Pharmacokinetic-Pharmacodynamic Study Using in Vivo Microdialysis in Rats

BORIS FERGER,* KERSTIN BUCK, MAKOTO SHIMASAKI, ELIZA KOROS, PATRIZIA VOEHRINGER, AND ERICH BUERGER

CNS Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Strasse 65, 88397 Biberach, Germany

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ABSTRACT Short-acting dopamine (DA) agonists are usually administered several times a day resulting in fluctuating plasma and brain levels. DA agonists providing continuous dopaminergic stimulation may achieve higher therapeutic benefit for example by alleviating nocturnal disturbances as well as early morning akinesia. In the present study continuous release (CR) of pramipexole (PPX) was maintained by subcutaneous implantation of Alzet® minipumps, whereas subcutaneous PPX injections were used to mimic PPX immediate release (IR) in male Wistar rats. In the catalepsy bar test, PPX-CR (1 mg/kg/day) reversed the haloperidol-induced motor impairment in the morning and over the whole observation period of 12h. In contrast, PPX-IR (tid 1 mg/kg, pre-treatment the day before) was not effective in the morning but catalepsy was reduced for 6 h after PPX-IR (1 mg/kg) injection. In the reserpine model, early morning akinesia indicated by the first motor activity measurement in the morning was significantly reversed by PPX-CR (2 mg/kg/day). Again, PPX-IR (tid 0.3 mg/kg, pre-treatment the day before) was not able to antagonise early morning akinesia. These results are in agreement with in vivo microdialysis measurements showing a continuous decrease of extracellular DA levels and a continuous PPX exposure in the PPX-CR (1 mg/kg/day) group. In contrast, PPX-IR (0.3 mg/kg) produced a transient decrease of extracellular DA levels over 6 h and showed maximum PPX levels 2 h after dosing which decreased over the following 6–8 h. The present study demonstrates that PPX-CR may offer a higher therapeutic benefit than PPX-IR on early morning akinesia and confirms earlier reports that PPX-IR reverses motor impairment for several hours. **Synapse 64:533–541, 2010.**

INTRODUCTION

After discovery of L-3,4-dihydroxyphenylalanine (L-DOPA) and various dopamine (DA) receptor agonists, a new area of research started for the pharmacotherapy of Parkinson's disease (PD). Indeed, not only the affinity, potency, and selectivity for the distinct DA receptors of antiparkinsonian drugs were regarded as important to improve the pharmacotherapy, but also the knowledge about the impact of pulsatile or continuous dopaminergic stimulation (CDS) received more and more attention. DA agonists or L-DOPA formulations resulting in long plasma half-lives are aiming to achieve CDS which eventually should prevent

*Correspondence to: PD Dr. Boris Ferger, CNS Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Strasse 65, 88397 Biberach, Germany. E-mail: boris.ferger@boehringer-ingelheim.com

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unwanted effects related to fluctuations in brain and plasma drug levels.

The concept of CDS (Bezard et al., 2001; Jenner, 2008; Nutt et al., 2000; Olanow et al., 2006) postulates that it is desirable to avoid the nonphysiological pulsatile DA receptor stimulation. This concept could translate into prolonged therapeutic efficacy and on top would result in lower propensity to develop motor fluctuations, dyskinesia and less nocturnal disturbances as well as early morning akinesia. Pramipexole (PPX) may be a candidate for CDS because of its good tolerability and favorable pharmacokinetic properties (high oral bioavailablity, no significant interaction with hepatic cytochrome P450 enzymes, long half-life) in humans (Kvernmo et al., 2006). PPX is a nonergoline full DA receptor agonist with selectivity for the DA D_3 receptor (Mierau et al., 1995; Mierau and Schingnitz, 1992; Piercey et al., 1996; Piercey, 1998) and has been approved by the FDA and other regulatory authorities in 1997 for the treatment of early and advanced stages of PD. Since this time, PPX has become one of the most widely used DA receptor agonists and is in use in monotherapy as well as in combination with other antiparkinsonian medication such as L-DOPA for the treatment of PD. More recently, PPX is also registered for treatment of patients suffering from restless legs syndrome.

Preclinical data on CDS by PPX in animal models of PD are sparse. To date only one study using CDS by PPX has been published which aimed to investigate the neuroprotective effects of CDS against lipopolysaccharide (LPS)-induced dopaminergic cell death in vivo. Iravani et al. (2008) demonstrated in this study that continuous subcutaneous infusion of PPX over four weeks preserved tyrosine hydroxylase positive neurons in the nigrostriatal pathway against dopaminergic cell death induced by the supranigrally administered LPS in rats. This effect was absent in rats acutely injected with PPX arguing for the beneficial effects on neuroprotection of CDS over acute treatment.

This study focuses on CDS by PPX targeting symptomatic effects in two animal models of PD. In addition, for the first time brain PPX pharmacokinetics was simultaneously investigated with levels of the biomarker DA after PPX immediate (PPX-IR) and continuous release (PPX-CR).

MATERIALS AND METHODS

Animals

This study was conducted in male Wistar rats (RjHan:WI, Janvier, Le Genest St Isle, France) weighing 250–300 g and used only once. The animals were housed under a 12 h light/dark cycle (lights on 06:00–18:00) in temperature (23 ± 2°C) and humidity (55 ± 5%) controlled rooms with free access to food (GLP Vitamin fortified, Provimi Kliba AG, Kaiseraugst, Switzerland) and water throughout the experiment.

All in vivo studies were approved by the appropriate institutional governmental agency (Regierungspraesidium Tuebingen, Germany) and performed in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)—accredited facility in accordance with the European Convention for Animal Care and Use of Laboratory Animals.

Haloperidol-induced catalepsy

Haloperidol-induced catalepsy is used as an animal model of extrapyramidal side-effects (Hoffman and Donovan, 1995) and for screening antiparkinsonian drugs (Lorenc-Koci et al., 1996). Cataleptic immobility is regarded as an animal equivalent of akinesia and is demonstrated by an animal allowing its body to be placed in and maintain abnormal or unusual postures (Sanberg et al., 1988; Schmidt et al., 1992).

Catalepsy was induced by treatment of rats with haloperidol (0.5 mg/kg, i.p.) and maintained for 12 h by administration of haloperidol (0.1 mg/kg, i.p.) every 4 h.

Catalepsy was measured by means of the bar test by an observer blind to the treatment. The rats were gently placed with their forelimbs on a horizontal bar elevated 6 cm from the floor. The time (s) during which the rats retained in this unusual position was recorded up to 60 s (cut-off period 60 s). In fact, when the rat moved one or both of its forelimbs the catalepsy measurement was finished. Three treatment groups were chosen. In the PPX-CR group (n = 9), ALZET® osmotic minipumps (model 2004 or 1007D, DURECT Corporation, Cupertino, CA) filled with PPX solution were implanted subcutaneously under isoflurane anesthesia the day before the catalepsy experiment. PPX was delivered continuously at the dose of 1 mg/kg/day. The PPX-IR group (n = 9) was treated with PPX (1 mg/kg, s.c.) three times (morning, midday, evening) on the day before the catalepsy experiment. On the day of the experiment, the first measurement of catalepsy was performed 2 h after the bolus injection of haloperidol. Subsequently, the PPX-IR and vehicle group (n = 9) were treated with PPX (1 mg/kg, s.c.) and vehicle, respectively. Catalepsy was measured 2, 4, 6, 8, 10, and 12 h later.

Reserpine-induced akinesia

Reserpine-induced akinesia was measured in the open field system Actimot™ (TSE Systems GmbH, Bad Homburg, Germany) for 1 h in the morning. Rats were placed individually in the center of the activity box (46.5 cm × 46.5 cm) and horizontal motor activity (m) was determined in 10 min intervals by infrared

sensor pairs (interspace 1.4 cm) with a sampling rate of 100 Hz.

Three treatment groups were chosen. In the PPX-CR group (n = 7), ALZET® osmotic minipumps (model 1007D, DURECT Corporation, Cupertino, CA) filled with PPX solution were implanted subcutaneously under isoflurane anesthesia three days before the measurement of akinesia. PPX was delivered continuously at the dose of 2 mg/kg/day. The PPX-IR (n = 6) group was treated with PPX (0.3 mg/kg, s.c.) three times (morning, midday, evening) on the day before the akinesia measurement. All rats were treated with reserpine (1 mg/kg, s.c.) in the afternoon the day before the experiment. Reserpine was first dissolved in 100% acetic acid and in a subsequent step diluted with water to a final concentration of 1% acetic acid. Seventeen hours later, motor activity was measured in the open field system for 60 min (early morning akinesia).

In vivo microdialysis surgery

A new series of rats were anesthetized with a mixture of ketamine (70 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) and mounted in a stereotaxic frame (David Kopf, Tujunga, CA) on a flat-skull position. Anesthesia was maintained by using 0.2–2% isoflurane in N_2O/O_2 (70:30). An intracerebral guide cannula was implanted aiming at the striatum (MAB 4.9.IC, Microbiotech, Stockholm, Sweden) at the following coordinates relative to the bregma: AP: +0.7 mm, ML: +3.0 mm, DV: −3.0 mm (from skull), according to the rat brain atlas of Paxinos and Watson (1998). A hole was drilled for the placement of the guide cannula, which was fixed to the skull with two stainless steel screws and dental cement (PermaCem, DMG Chemisch-Pharmazeutische Fabrik GmbH, Hamburg, Germany). Subsequently, the ALZET® osmotic minipump (model 1007D, DURECT Corporation, Cupertino, CA) filled with PPX solution was implanted subcutaneously in rats of the PPX-CR group. PPX was delivered continuously at a dose of 1 mg/kg/day. Following surgery, rats were housed individually in perspex cages and allowed to recover for two days before performing the in vivo microdialysis procedure.

In vivo microdialysis procedure

On the day of the experiment, concentric microdialysis probes (MAB 4.9.4.Cu, 4 mm cuprophane membrane length, Microbiotech, Sweden) were introduced into the guide cannulae and the rats were placed into a microdialysis system for freely moving animals consisting of a counter-balanced lever arm (15 cm) with a low-torque dual channel quartz-lined swivel (Instech Laboratories, Inc., USA) and a clear animal container (diameter 30 cm, height 37 cm). The bottom of the container was covered with bedding material. The probes were perfused with artificial cerebrospinal fluid (aCSF) containing 147 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 0.85 mM $MgCl_2$ and 1 mM Na_2HPO_4, pH 7.0–7.4, at a constant flow rate of 2 μl/min. After an equilibration period of 2 h, dialysis samples were collected every 30 min into a vial containing 10 μl of hydrochloric acid (0.1 M). During the night, the sampling interval was prolonged to 60 min (20 μl of hydrochloric acid). Fractions 1 to 4 (0–2 h) were used for calculation of the basal levels. After 2 h, the PPX-IR and PPX-CR group were treated with PPX [0.3 mg/kg, s.c. (n = 4)] and vehicle [saline, s.c. (n = 4)], respectively. The sampling was then continued for 17.5 h up to the next morning. The reported data were not corrected for the in vitro recovery, which was 12–14% for DA and 8% for PPX. After the experiments the localization of the probes was verified and only the rats with appropriate probe placement were included in the experiment.

High-performance liquid chromatography analysis of microdialysis samples

Microdialysis samples were split for high performance liquid chromatography (HPLC) using electrochemical detection (ECD) (60 μl) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (10 μl) analysis. Samples were analyzed for DA using HPLC-ECD under isocratic conditions. The HPLC system consisted of an ASI-100T autosampler and P680 ISO isocratic pump system (Dionex, Idstein, Germany). The detector potential was set at +650 mV using a glassy carbon electrode and an ISAAC Ag/AgCl reference electrode (Antec VT-03, Leyden, The Netherlands). Chromatographic separation was achieved using a reversed-phase column (Grom-Sil 120 ODS-4 HE, 250 × 4.0 mm i.d., 5 μm particles, Grace Davison Discovery Sciences, Deerfield, IL) at 35°C. The mobile phase consisted of 1.85 mM 1-octanesulfonic acid sodium salt, 0.13 mM $Na_2EDTA \times 2 H_2O$, 8.00 mM NaCl, 57.51 mM NaH_2PO_4, adjusted to pH 2.50 with H_3PO_4, filtered through a 0.22 μm filter, mixed up with 5% acetonitrile and was delivered at a flow rate of 1 ml/min. Aliquots were injected by an autosampler with a cooling module set at 4°C. Data were calculated using an external five-point standard calibration.

LC-MS/MS analysis of microdialysis samples

Microdialysis samples were analyzed for PPX using LC-MS/MS. The LC-MS/MS system comprised an HTS PAL autosampler (CTC Analytics AG, Zwingen, Switzerland), an Agilent 1200 Binary Pump, an Agilent 1200 Micro Vacuum Degasser and an Agilent 1200 Thermostatted Column Compartment (Agilent Technologies, Morges, Switzerland). Mobile phase "A" and "B" consisted of 0.1% formic acid in LC-MS grade

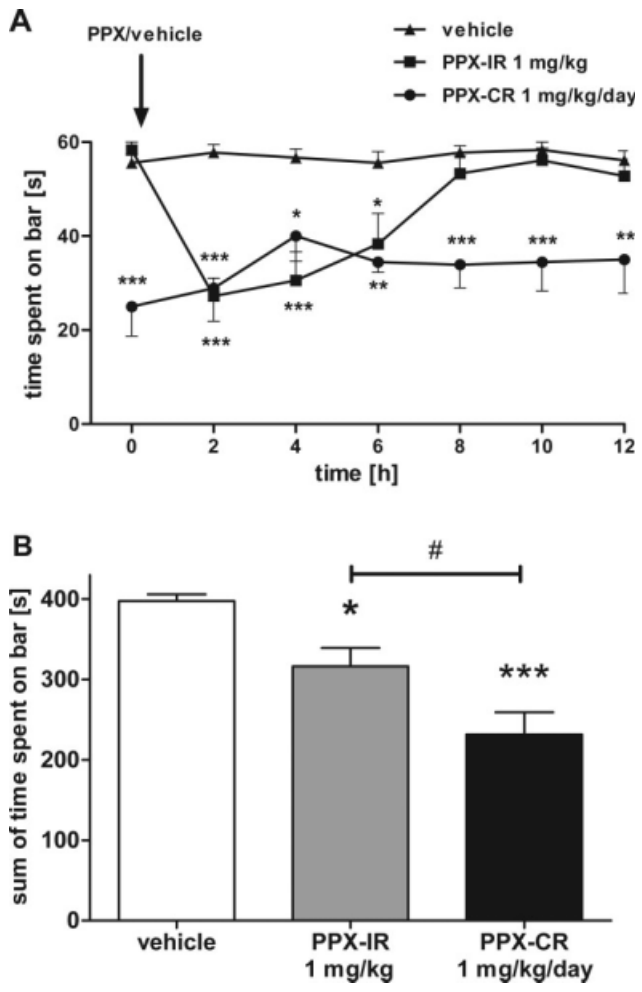

Fig. 1. Effects of PPX-IR (1 mg/kg, s.c., $n = 9$), PPX-CR (1 mg/kg/day, s.c., $n = 9$) and vehicle ($n = 9$, s.c.) on the time course (**A**) and cumulative data (**B**) of haloperidol-induced catalepsy. The PPX-IR group was treated with PPX three times on the day before the catalepsy experiment. Haloperidol (0.5 mg/kg, i.p.) was injected 2 h prior to the first catalepsy measurement. Catalepsy was maintained for 12 h by administration of haloperidol (0.1 mg/kg, i.p.). Data are expressed as mean ± SEM. The time course was analyzed by a two-way ANOVA followed by a Bonferroni post hoc test (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ vs. vehicle). Cumulative data was analyzed using one-way ANOVA followed by a Bonferroni post hoc test (***$P < 0.001$, *$P < 0.05$ vs. vehicle, #$P < 0.05$ PPX-IR vs. PPX-CR).

water and acetonitrile, respectively. The gradient was chosen as follows: 0.00 min: 100% A, 1.40 min 100% A, 1.41 min 0% A, 2.00 min 0% A, 2.10 min 100% A, 2.50 min 100% A and delivered at 0.5 ml/min onto a reversed-phase column (Synergi Polar-RP 80 A, 150 × 2.0 mm i. d., 5 μm particles, Phenomenex, Inc., Aschaffenburg, Germany) at 20°C. The column switching valve was set at 0.00 min to the waste, at 0.75 min to the mass spectrometer and at 2.00 min to the waste again.

Eluates were detected using an API 4000™ triple quadrupole LC/MS/MS mass spectrometer (MDS Sciex, Ontario, Canada) in the positive electrospray ionisation mode. The ion spray voltage was set at 4500 V and the source temperature at 500°C. Three transitions were chosen: 212-153 (declustering potential (DP) 56 V, collision energy (CE) 21 V, cell exit potential (CXP) 10 V), 212-111 (DP 56 V, CE 37 V, CXP 8 V), 212-126 (DP 56 V, CE 39 V, CXP 8 V) and transition 212-153 was used for the quantification of PPX. As internal standard [D_7]-PPX was analyzed using transition 219-153 (DP 86 V, CE 21 V, CXP 10 V).

Materials

All drugs were calculated as free base. PPX dihydrochloride as well as [D_7]-PPX dihydrochloride was synthesized at Boehringer Ingelheim Pharma GmbH & Co. KG. HPLC and LC-MS/MS chemicals of the highest available purity were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 5.01 for Windows (GraphPad software, La Jolla, CA). All values are expressed as mean ± SEM. $P < 0.05$ was considered as statistically significant.

The time course of haloperidol-induced catalepsy test as well as reserpine-induced akinesia was analyzed by a two-way analysis of variance (ANOVA) with treatment as independent and time as dependent factor followed by a Bonferroni post hoc test. Statistical analysis of the cumulative data of the haloperidol-induced catalepsy and the reserpine-induced akinesia test was carried out using one-way ANOVA with treatment as independent factor followed by a Bonferroni post hoc test. For comparison of basal DA and PPX levels an unpaired *t*-test was performed.

RESULTS

Haloperidol-induced catalepsy

Figure 1 shows the effect of PPX on haloperidol-induced catalepsy. Statistical analysis yielded a significant interaction of time x treatment ($F(12;144) = 6.388$; $P < 0.001$) as well as significant effects on time ($F(6;144) = 4.786$; $P < 0.001$) and treatment ($F(2;144) = 15.33$; $P < 0.001$) (Fig. 1A). Time spent on the bar of the PPX-CR group was significantly decreased in comparison to the vehicle group during the whole experiment (0 h, 2 h, 8 h, 10 h: $P < 0.001$; 4 h: $P < 0.05$; 6 h, 12 h: $P < 0.01$). The PPX-IR and vehicle group did not display a significant difference 2 h after haloperidol injection (time point 0; pretest before PPX/vehicle injection) indicating that pre-treatment with PPX the day before did not show an effect on haloperidol-induced catalepsy the next morning. Following injection with PPX, the time spent on the bar

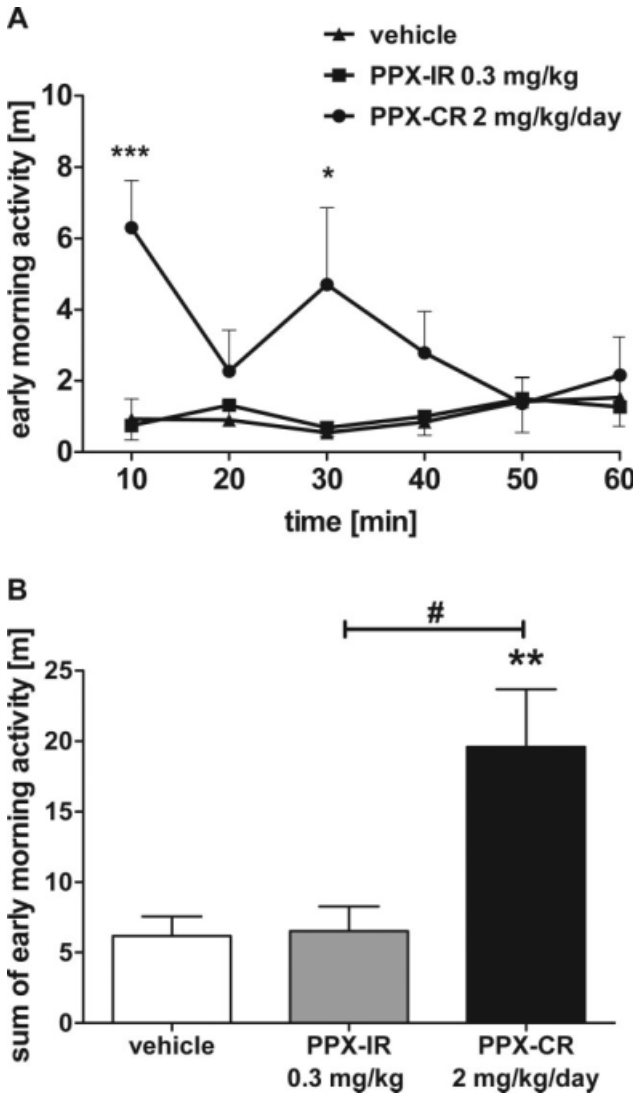

Fig. 2. Effects of PPX-IR (2 mg/kg, s.c., $n = 7$), PPX-CR (2 mg/kg/day, s.c., $n = 6$) and vehicle ($n = 6$, s.c.) on the time course (**A**) and cumulative data (**B**) of reserpine-induced akinesia. The PPX-IR group was treated with PPX three times on the day before the akinesia measurement. Reserpine (1 mg/kg, s.c.) was injected 17 h prior to the experiment. Data are expressed as mean ± SEM. The time course was analyzed by a two-way ANOVA followed by a Bonferroni post hoc test (***$P < 0.001$, *$P < 0.05$ vs. vehicle). The cumulative data was analyzed using one-way ANOVA followed by a Bonferroni post hoc test (**$P < 0.01$ vs. vehicle, #$P < 0.05$ PPX-IR vs. PPX-CR).

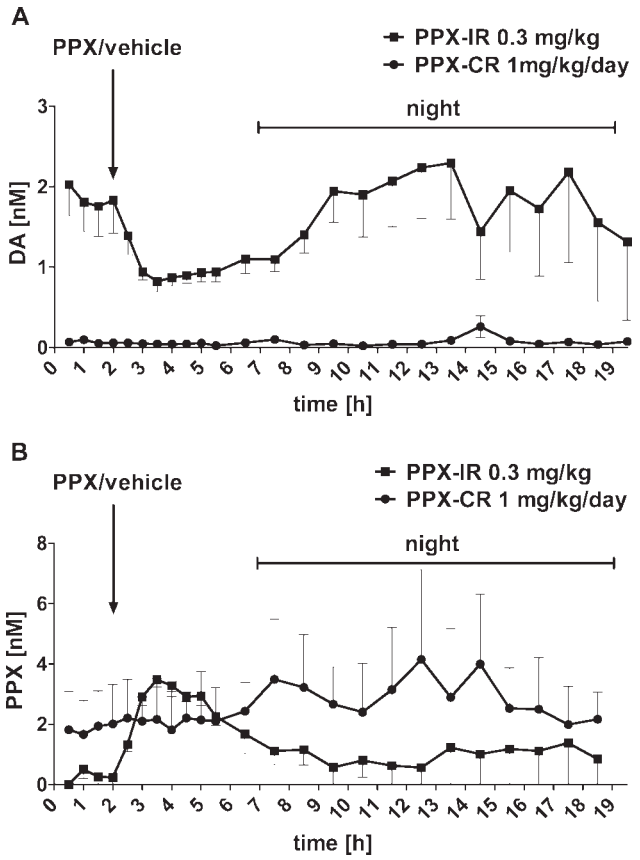

Fig. 3. In vivo microdialysis. Effects of PPX-IR (0.3 mg/kg, s.c., $n = 4$) and PPX-CR (1 mg/kg/day, s.c., $n = 4$) on extracellular levels of DA (**A**) and PPX (**B**) in the striatum. Data are expressed as mean ± SEM.

decreased significantly in the PPX-IR group at time points 2 h and 4 h ($P < 0.001$) as well as 6 h ($P < 0.05$). Regarding the cumulative data (Fig. 1B), PPX-CR ($P < 0.001$) as well as PPX-IR ($P < 0.05$) showed an improvement of haloperidol-induced catalepsy, while PPX-CR revealed a significant higher effect in comparison to PPX-IR ($P < 0.05$).

Reserpine-induced akinesia

The effects of PPX on reserpine-induced early morning akinesia are shown in Figure 2. Statistical analysis revealed a significant treatment effect ($F(2;18) = 7.266$; $P < 0.01$). No significant differences were observed between the vehicle and the PPX-IR group indicating that pre-treatment with PPX the day before does not alter early morning akinesia. In contrast, akinesia was improved by treatment with PPX-CR at 10 min ($P < 0.001$) and 30 min ($P < 0.05$) (Fig. 2A) as well as considering the whole experiment over 60 min ($P < 0.01$) (Fig. 2B).

Measurement of extracellular DA levels

Effects of PPX on extracellular DA levels in the striatum are displayed in Figure 3A. Pre-dose basal levels of DA in the PPX-IR were found to be 1.86 nM. In the PPX-CR group no pre-dose values could be measured because the microdialysis surgery and the implantation of the pump were carried out at the same time. At the time of DA measurement in the PPX-CR stable levels of ~0.07 nM were obtained which did not vary over time implicating steady state conditions. Statistical analysis revealed significantly lower DA levels in the PPX-CR group (96.2%) in com-

parison to the pre-dose values of the PPX-IR group ($P < 0.01$). The maximum effect in the PPX-IR group was observed 90 min after PPX treatment (44.4% in comparison to basal DA levels).

Measurement of microdialysate PPX levels

Extracellular PPX levels in the striatum are displayed in Figure 3B. Pre-dose levels of PPX in the PPX-IR were found to be 0.25 nM. As mentioned before, no pre-dose levels in the PPX-CR were obtained and basal PPX levels of the PPX-CR were found to be 1.86 nM. Injection of PPX in the PPX-IR group led to an increase in PPX levels, which was maximum 90 min following PPX injection (3.48 nM).

DISCUSSION

The present neuropharmacological study demonstrated the effects of acute and continuous exposure of the DA D_3/D_2 agonist PPX in two symptomatic animal models of PD including in vivo microdialysis measurements of PPX and DA.

We found in both animal models of PD that the effects of PPX are dependent on the PPX exposure in the brain. In particular, the day following acute PPX pre-treatment the symptomatic effects of PPX were no longer present which resulted in early morning akinesia. In contrast, continuous PPX exposure using subcutaneously implanted Alzet® minipumps prevented early morning akinesia. Additionally, continuous PPX exposure produced significantly lower extracellular DA levels than the peak decrease obtained after acute PPX administration, although the PPX exposure was lower in the PPX-CR group.

Neither haloperidol- nor reserpine-induced behavioral effects are associated with neurodegeneration indicated by no loss of dopaminergic neurons in the nigrostriatal pathway. Thus, these animal models of PD can only be considered to study symptomatic effects of drugs with predictive validity investigating DA agonists. The advantage of these symptomatic models are the robustness, clear behavioral readout, and simple procedure which do not require stereotaxic surgery as for non brain penetrating dopaminergic neurotoxins or safety constrictions as for MPTP which needs to be handled with extremely care not to be harmful for the experimenter. Haloperidol is able to induce parkinsonian-like symptoms such as muscle rigidity (Lorenc-Koci et al., 1996) and catalepsy (De Ryck and Teitelbaum, 1983; Fischer et al., 2002). Haloperidol-induced catalepsy is considered as an animal model of parkinsonian akinesia which reflects the impairment of postural stability and the inability to actively initiate phasic movements (De Ryck et al., 1980). Haloperidol-induced akinesia is a result of the blockade of DA D_2 receptors in the corpus striatum (Ellenbroek et al., 1985). We found a pronounced effect of PPX-IR which was reversible, declined after 6 h and was absent at 8 h. Because the effect of a single haloperidol injection led only to significant catalepsy within 6 h, we adapted the catalepsy model to maintain catalepsy over a longer period of time by multiple haloperidol injections using low haloperidol doses. This allowed us to study the effects of PPX-IR and PPX-CR over a long observation period of 12 h. Only PPX-CR antagonised the haloperidol-induced catalepsy over the whole observation period which is in agreement with PPX exposure measurements (please see above). In terms of maximum efficacy the PPX-IR and PPX-CR groups did not differ. The duration of the anticataleptic effect was longer in the PPX-CR group. The anticataleptic effects are in line with a previous study on haloperidol-induced catalepsy in which a single subcutaneous injection of PPX (1 and 3 mg/kg) led to a 2.5–3 h lasting relieve of catalepsy (Maj et al., 1997). In contrast, higher doses of PPX (3 and 5 mg/kg) were necessary to antagonise the haloperidol-induced muscle rigidity (Lorenc-Koci and Wolfarth, 1999).

In the second symptomatic animal model of PD, reserpine was used to investigate the effects of PPX-IR and PPX-CR on akinesia. In comparison to haloperidol which has a high affinity for DA D_2-like receptors (D_2, D_3, D_4: K_i value 1.2, 7, 2.3 nM, respectively) (Seeman and Van Tol, 1994) reserpine acts presynaptically by blocking the uptake of monoamines by the vesicular monoamine transporter (VMAT-2). This inhibition unselectively affects the storage of monoamine neurotransmitters such as adrenaline, noradrenaline, DA, histamine and 5-HT in the CNS and also in the periphery. Although not specific to a single neurotransmitter pathway and without involvement of neurodegenerative events the reserpine model is still a valuable tool to investigate symptomatic effects of DA receptor agonists in PD (Maj et al., 1997) as well as to study nondopaminergic mechanisms in PD (Kreitzer and Malenka, 2007; Niswender et al., 2008). We optimized the typical reserpine model by reducing the dose of reserpine to 1 mg/kg.

As seen in the haloperidol-induced catalepsy model, PPX-CR antagonised the motor impairment. PPX-CR was effective over the whole observation period including the first measurement on early morning akinesia. Using a higher dose of reserpine (5 mg/kg) in combination with α-methyl-p-tyrosine (α-MT) (250 mg/kg, i.p.) to additionally block DA biosynthesis, (Maj et al., 1997) showed that a single subcutaneous injection of PPX (0.3 and 1 mg/kg) increased locomotor activity. The effect of PPX was even higher than obtained in the vehicle+reserpine/α-MT group as well as in the vehicle+vehicle control group. Under our conditions we did not observe any hyperactivity neither in the PPX-IR nor in the PPX-CR group.

In this study only a single dose of PPX-IR and PPX-CR was selected after pilot dose finding studies. PPX-IR has been repeatedly tested over the last years serving as positive control in the haloperidol and reserpine rat model. We found reproducible effects in a dose range from 1 to 3 mg/kg s.c. in the haloperidol model and using a slightly different dose range of 0.3–3 mg/kg s.c. in the reserpine model (Ferger, unpublished data). The doses for PPX-CR were selected by measurements of PPX exposure to be in a similar range as for PPX-IR treatment. According to our behavioral data higher doses of PPX-CR are not necessary. However, lower doses may be sufficient to be effective, which has to be shown in follow-up experiments.

Unbound PPX brain tissue levels, cerebrospinal fluid (CSF) or microdialysate levels have not been published so far. Most of the pharmacokinetic data on PPX exposure relied on studies measuring PPX plasma levels. For example in healthy volunteers the plasma concentrations of PPX were proportional to dose under steady-state conditions (elimination half-life $t_{1/2}$ 8–12 h, t_{max} 1–3 h, c_{max} 0.375–4.5 ng/ml) (Kvernmo et al., 2006; Wright et al., 1997). Recently, we reported that PPX accumulated in the brain indicated by a brain plasma ratio of 6.7 obtained in mice after oral administration (Danzeisen et al., 2006), which is in accordance with the proposed active transport of PPX through the blood-brain barrier by an organic cation-sensitive transporter (Okura et al., 2007). In vivo microdialysis is the method of choice to analyze both the drug as well as the biomarker at the target side (i.e., the striatum in PD) in the same sample. We took advantage of the DA measurement using HPLC-ECD which is very sensitive and additionally established a method to analyze PPX in microdialysates using LC-MS/MS. The PPX exposure in the rat striatum was maximum at 90 min (3.48 nM) following injection in the PPX-IR group and declined over a period of 3 h. Animals continuously treated with PPX showed lower maximum PPX levels and revealed an almost constant striatal PPX exposure of 2.46 nM over the whole experiment.

In this study PPX-IR and PPX-CR affected extracellular DA levels. The reduction of extracellular DA levels can be explained by stimulation of presynaptic DA receptors in dopaminergic nerve terminals. This effect is characteristic for DA receptor agonists including PPX and reflects both the involvement on regulation of DA synthesis and on Ca^{2+}-dependent exocytotic DA release by the DA autoreceptor mediated feedback inhibition (Wolf and Roth, 1987). PPX binds preferentially to DA D_3 receptors followed by DA D_2 receptors (Mierau et al., 1995; Piercey et al., 1996), which fits to the role of presynaptic DA D_3 receptors on DA release (Gainetdinov et al., 1996) and DA synthesis (Wolf and Roth, 1990). Additionally, it was demonstrated that DA D_3 preferring compounds modulate the DA uptake in vitro and in vivo suggesting that the DA D_3 receptor activation increases DA uptake by modulating the DA transporter activity (Zapata and Shippenberg, 2002).

Indeed, systemic PPX administration caused a long-lasting reduction of extracellular DA and DA metabolite levels in rat striatum (Carter and Muller, 1991; Robertson et al., 1993). This effect was reversed by the D_2 DA receptor antagonist sulpiride but not by the D_1 DA receptor antagonist SCH 23390 (Carter and Muller, 1991). Local PPX administration reduced the 6-OHDA-induced increase of extracellular DA in rat striatum (Ferger et al., 2000) and reduced the L-type Ca^{2+} channel activator (BAY K 8644)-induced rise in extracellular DA levels (Maruya et al., 2003). Cumulative evidence underlines that extracelluar DA levels measured by in vivo microdialysis is a suitable biomarker to indicate DA D_2/D_3 receptor stimulation and therefore can be used to compare the effects of PPX-IR and PPX-CR. Although slightly higher peak PPX levels were obtained after PPX-IR, the extracellular DA levels were consistently lower in the PPX-CR group which speaks against desensitization and a tolerance effect on regulation of extracellular DA levels after CDS by PPX. Chernoloz et al. (2009) performed an electrophysiological experiment in anesthetized rats which were subcutaneously implanted with osmotic minipumps delivering PPX at a dose of 1 mg/kg per day for 2 or 14 days. They demonstrated a decrease in the spontaneous firing of dopaminergic neurons by 40% after 2 days of treatment, whereas after 14 days of PPX treatment the firing rate of DA neurons had recovered and implicated a desensitization of D_2/D_3 cell body autoreceptors after long-lasting continuous PPX treatment.

Continuous DA receptor stimulation using DA agonists delivered by miniosmotic pumps or by external infusion systems are useful in preclinical animal experiments but are impractical in most patients suffering from PD. In patients the most convenient route of administration is the oral one. Subcutaneous administration of DA receptor agonists as used in this study can be alternatively used when a rapid onset of action is needed. A continuous subcutaneous infusion is suitable to stabilize motor functions (Nyholm, 2006). A drawback of long-term subcutaneous infusion, however, could be adverse events such as skin reactions and nodules accompanied by variable drug absorption (Deleu et al., 2004). These disadvantages prompted the development to oral sustained or extended release formulations in several drug development programs. In case of low bioavailability of a compound, transdermal delivery of dopaminergic drugs can be a practical method to achieve CDS and

may be useful in patients with swallowing difficulties (Steiger, 2008).

Particularly at night and in the early morning hours constant plasma levels of short acting DA agonists cannot be maintained because of drug clearance. In fact, the therapeutic efficacy of the symptomatic effects of DA agonists including PPX is closely related to sufficient drug exposure levels. As a matter of fact cardinal PD symptoms appear if dopaminergic receptor stimulation cannot be maintained. Another issue beyond the prolongation of the symptomatic effects of CDS by DA agonists is the reduced risk to develop motor complications such as dyskinesia as demonstrated in parkinsonian cynomolgus monkeys (Bibbiani et al., 2005). Furthermore, the reversal of motor deficits without dyskinesia induction in MPTP-treated common marmosets argues for the concept of CDS (Stockwell et al., 2008).

In conclusion, this study highlights the potential benefit of CDS using PPX-CR and the advantage over PPX-IR in two symptomatic PD models. Currently, a once-daily PPX extended release formulation (tablet) undergoes regulatory review.

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