ELSEVIER

European Journal of Pharmacology 342 (1998) 153–161

ejp

Behavioral and neurochemical effects of the preferential dopamine D_3 receptor agonist *cis*-8-OH-PBZI

Anders Fink-Jensen *, Erik Bardrum Nielsen, Liselotte Hansen, Mark A. Scheideler

Health Care Discovery and Development, Novo Nordisk A / S, Novo Nordisk Park, DK-2760 Måløv, Denmark

Received 23 June 1997; revised 29 October 1997; accepted 31 October 1997

Abstract

In the present study we investigated the in vivo pharmacological profile of the benz[e]indole *cis*-8-hydroxy-3-(*n*-propyl)1,2,3a,4,5,9b-hexahydro-1H-benz[e]indole (*cis*-8-OH-PBZI), which has been described as a preferential dopamine D_3 receptor agonist in vitro. The compound inhibited spontaneous locomotor activity in mice, an effect which was antagonized by the dopamine D_3 receptor antagonist 5,6-dimethoxy-2-(di-u-propylamino) indan (U99194A). Moreover, *cis*-8-OH-PBZI inhibited conditioned avoidance responding in rats, a preclinical test indicative of antipsychotic efficacy, at doses which did not induce catalepsy. Doses of *cis*-8-OH-PBZI (6 and 12 mg/kg) that inhibited spontaneous locomotor activity in rats did not affect interstitial levels of dopamine and dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens or dorsolateral striatum. In contrast to the effect of the dopamine receptor agonist ±-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphythalene (7-OH-DPAT), *cis*-8-OH-PBZI did not induce locomotor activity in reserpinized mice. In conclusion, *cis*-8-OH-PBZI exhibits a pharmacological profile that suggests it has antipsychotic activity but lacks the motoric side effects often associated with antipsychotic medication. The data suggest a mechanism requiring the activation of postsynaptic dopamine D_3 receptors and support the hypothesis that these receptors mediate inhibitory behavioral effects. © 1998 Elsevier Science B.V.

Keywords: Dopamine D_3 receptor; Antipsychotic; Conditioned avoidance responding; Locomotion; Rotation; Microdialysis

1. Introduction

Many of the prototypical neuroleptics still used in the clinic have serious motor side effects (Baldessarini and Tarsy, 1980) that are attributable to their actions in motor regions of the brain. The recent molecular cloning of the major dopamine receptors established that the dopamine D_2 receptor subtype is widely expressed and likely involved in the motor side effects associated with neuroleptics (Sokoloff et al., 1990). Importantly, recent transgenic studies have shown that deletion of the structural gene for the dopamine D_2 receptor subtype results in Parkinsonian-like locomotor impairment (Balk et al., 1995). Consequently, the possibility of developing new antipsychotics which selectively target limbic areas of the brain has been proposed. In this respect, mRNA encoding the dopamine D_3 receptor subtype has been shown to be primarily localized to limbic brain structures including the nucleus accumbens (Sokoloff et al., 1990, Landwehrmeyer et al., 1993). In the accompanying paper *cis*-8-OH-PBZI was shown to be a preferential dopamine D_3 receptor agonist in vitro with a limbic site of action in vivo (Scheideler et al., 1997) In the present study we report on the in vivo effects of *cis*-8-OH-PBZI in models in which efficacy is regarded as indicative of antipsychotic potential, i.e. D-amphetamine-induced hyperactivity, spontaneous locomotor activity and conditioned avoidance responding, as well as in models investigating motor side effect potential, i.e. catalepsy. In addition, we investigated the in vivo selectivity and pre- versus postsynaptic mode of action of *cis*-8-OH-PBZI by using various behavioral and neurochemical models including contralateral rotations in 6-hydroxydopamine-lesioned rats, locomotor activity measurements in normal and reserpinized mice and quantification of interstitial levels of dopamine and its metabolite dihydroxyphenylacetic acid (DOPAC) in the rat forebrain.

* Correspondence author. Tel.: +45-4444-8888; fax: +45-4466-3939; e-mail: afj@novo.dk

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, Wistar rats or NMRI mice (Moellegaards Breeding Labs, Skensved), weighing 250 ± 20 g, 250 ± 20 g and 20 ± 2 g, respectively, were used. The animals were housed in temperature-controlled rooms (20–22°C) with a light/dark cycle (light from 06:00–18:00) and access to food and water ad libitum. The experimental protocols used were in accordance with the international accepted principles of the care and use of laboratory animals and have been approved by the Danish Commitee for Animal Research. Wistar rats were used in the conditioned avoidance experiments. In the other rat experiments, Sprague–Dawley rats were used.

2.2. Drugs

The hydrochloride salt of *cis*-8-hydroxy-3-(*n*-propyl)1,2,3a,4,5,9b-hexahydro-1H-benz[e]indole (*cis*-8-OH-PBZI) was synthesized as previously described by Cruse et al. (1993). The synthesis of 7-OH-DPAT ((±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene) was performed as previously described by Wikström et al. (1985). Haloperidol, D-amphetamine sulfate, (−)-apomorphine and 6-hydroxydopamine were purchased from Sigma Chemicals (St. Louis, MO). Haloperidol was subsequently converted to a hydrochloride salt at Novo Nordisk. Dopamine, PD 128907 (*S*(+)-(4a*R*,10b*R*)-3,4,4,a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1, 4-oxazin-9-ol) and U99194 (5,6-dimethoxy-2-(di-*n*-propylamino)indan) were purchased from RBI (Natick, MA). *Cis*-8-OH-PBZI, 7-OH-DPAT, PD 128907, D-amphetamine sulfate and U99194A were dissolved in sterile water and adjusted to pH 6.5–7.0 with 1 M NaOH. Haloperidol was dissolved in distilled water heated to 60°C. (−)-Apomorphine was dissolved in distilled water containing 0.2 mg/ml ascorbic acid. 6-Hydroxydopamine was dissolved in 0.9% (v/v) of saline solution containing 0.2 mg/ml ascorbic acid. All solutions were prepared immediately before experimental use.

2.3. Locomotor activity

Mice or rats were placed in acrylic glass chambers (width × length × height: 29 × 29 × 38 cm, respectively) situated within a frame of photocells (4 × 4) located 1 cm above the floor. Each photocell interruption was recorded on a computer. Locomotor activity was measured for 20 min after the animals were placed in the glass chambers, 20 min after administration of the test compounds. In the *cis*-8-OH-PBZI/U99194 interaction study, *cis*-8-OH-PBZI was administered 15 min prior to testing. In experiments measuring locomotor activity in dopamine-depleted animals, mice were given 5 mg/kg of reserpine (s.c.) 18–22 h before testing. The effect of *cis*-8-OH-PBZI on D-amphetamine-induced hyperactivity in mice was investigated 15 min after mice were injected with D-amphetamine (2 mg/kg, s.c.). Moreover, U99194A was tested in mice habituated for 2 h to the environment before recording locomotor activity and the compound was administered 20 min prior to the 20 min sampling period.

2.4. Conditioned avoidance responding

Rats were trained to perform a shuttle response in Coulbourn Instruments two-way shuttle boxes (model E99-36) in order to avoid a 1 mA electric shock (unconditioned stimulus) through the grid floor. Each shock was signaled by a 82 dB tone (conditioned stimulus) from a 'Sonalert' tone generator/speaker mounted in the wall of the experimental chamber. The specific experimental conditions for evaluation of effects on performance on, were as follows: intertrial interval, 25 s; conditioned stimulus–unconditioned stimulus interval, 10 s; maximum unconditioned stimulus duration, 1 min. Sessions were terminated after 45 min or after the completion of 40 trials. Avoidance responses were reinforced with 10 s of shock-free time added to the next intertrial-interval. The compound was injected subcutaneously 30 min prior to testing.

2.5. Catalepsy

The method was essentially similar to that described by Morelli and Di Chiara (1985). Briefly, rats were injected with the test compound and placed individually on an inclined (70°) wire-mesh screen (0.8 mm steel-wire, 7 mm mesh). The extremities of the animal were then gently abducted. The latency to move any extremity was used to define the intensity of catalepsy according to the following scale from 0 to 3: (0) latency < 15 s; (1) 15–29 s; (2) 30–59 s; (3) > 60 s. After injection of the test compound, the animals underwent catalepsy testing at 5, 15, 30, 90 and 120 min as described by Klemm (1985). *Cis*-8-OH-PBZI was tested at doses of 1, 3, 6, 12, 24 and 48 mg/kg (s.c.). A total of 8 animals were administered compound for each dose tested.

2.6. Rotation in unilaterally 6-hydroxydopamine-lesioned rats

Animals were injected with 15 mg/kg of desimipramine (i.p.) and anesthetized with 400 mg/kg of tribromoethanol (i.p.). Thirty minutes after administration of desimipramine, 9.7 μg of 6-hydroxydopamine (corresponding to 8 μg of the free base), dissolved in 0.9% (v/v) of saline solution containing 0.2 mg/ml ascorbic acid, was stereotaxically injected into the left nigrostriatal/mesolimbic pathway of the animals (4.8 mm caudal and 1.5 mm lateral to bregma, 8.1 mm below the skull surface), using an infusion pump (Carnegie Medicine) with

a flow rate of 1 ml/min. After two weeks, (−)-apomorphine was administered (0.25 mg/kg s.c.). Rats that turned through 360° more than 30 times over the next 20 min were then used for further experiments. Animals were placed in plexiglass bowls and attached to a swivel via a Velcro band strapped around the waist. Thirty minutes later test compounds were injected (s.c.) and rotation was measured for 90 min by computer detection of rotation-operated photocell interruptions.

2.7. Microdialysis procedure

Microdialysis probes obtained from CMA/Microdialysis (Stockholm) were of polycarbonate tubing of O.D. 0.5 mm and 2 mm in membrane length. The probes were constantly perfused during the experiments with a modified Ringer solution (in mM: 145.0 Na^+, 3.0 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, 152.4 Cl^-) using a CMA/100 microinfusion pump (CMA/ Microdialysis) with a flow rate of 1 ml/min. Samples were collected every 20 min in glass vials containing 4 μl of 2 mM perchloric acid (in order to prevent oxidation of the monoamines). The implantation of guide cannulas and microdialysis probes were performed as earlier described by Fink-Jensen et al. (1996). Following probe implantation the perfusion was stopped and started again 20–24 h later whereafter at least five perfusate samples (20 min each) were collected prior to drug administration. Measurements were continued for 3 h following drug administration. The animals were then killed, the probe was retracted, and the brains were removed and sectioned in 250 μm coronal slices. The placement of the probe was verified in each instance by using a stereomicro-

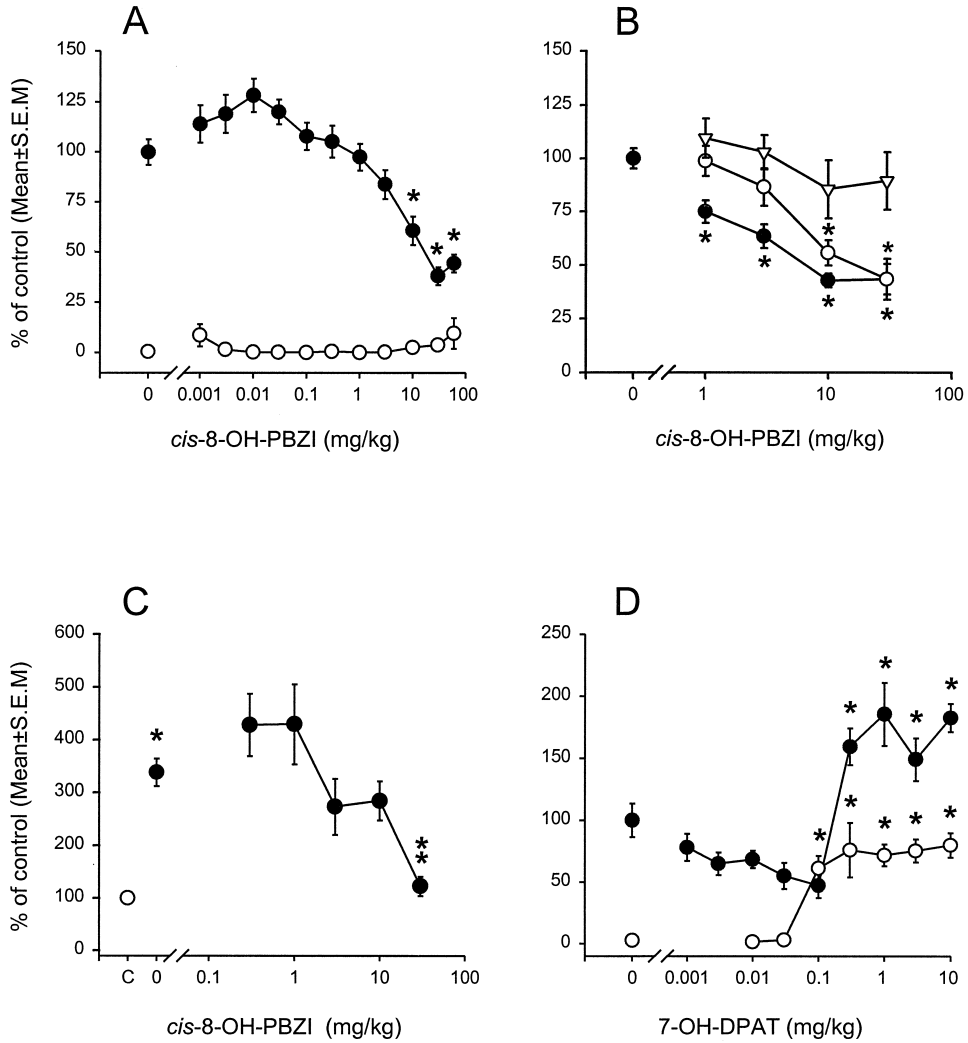

Fig. 1. Effects of (A) *cis*-8-OH-PBZI on spontaneous locomotor activity in normal mice (●) or in mice pretreated with 5 mg/kg (s.c.) of reserpine (○), (B) *cis*-8-OH-PBZI in combination with U99194A, (C) *cis*-8-OH-PBZI on D-amphetamine-induced hyperactivity and (D) 7-OH-DPAT on spontaneous locomotor activity in normal mice (●) or in mice pretreated with 5 mg/kg (s.c.) of reserpine (○). Test compounds were administered (s.c.) 20 min before start of activity measurements, except in the U99194A/*cis*-8-OH-PBZI interaction study, when, U99194A and *cis*-8-OH-PBZI were administered 20 min and 15 min before the start of measurements, respectively. Results are shown as means ± S.E.M., expressed as a percentage of the control value. Eight mice were used per dose except when *cis*-8-OH-PBZI was tested in normal mice ($n = 24$). $^{*}P < 0.05$, compared to the control (one-way analysis of variance followed by Student–Newman–Keuls post-hoc test). $^{**}P < 0.05$, compared to the D-amphetamine/saline group (C).

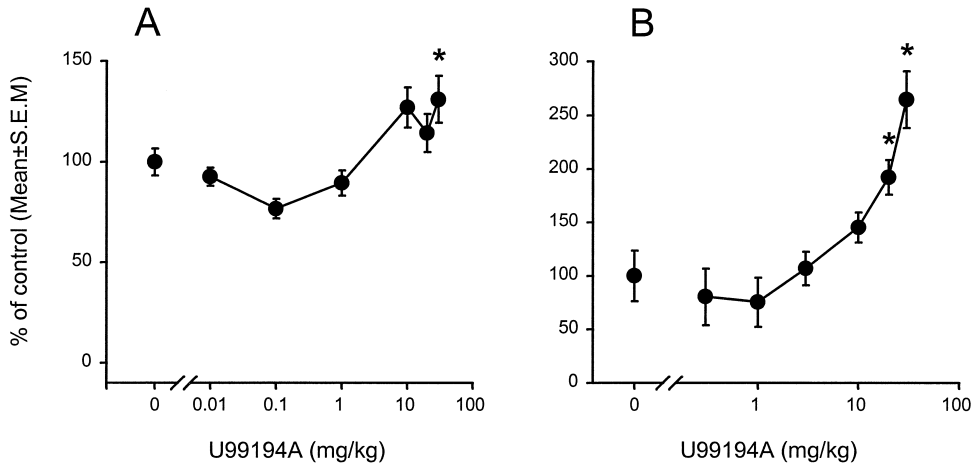

Fig. 2. Effects of U99194A on (A) spontaneous locomotor activity in normal mice and on (B) locomotor activity in habituated mice. Compounds were administered (s.c.) 20 min before the start of activity measurements. Results are shown as means ± S.E.M., expressed as a percentage of the control value ($n = 8$ mice per dose). * $P < 0.05$, compared to the control (one-way analysis of variance followed by Student–Newman–Keuls post-hoc test).

scope. Results obtained from animals in which the probe was positioned incorrectly were not used. Data are presented as the percentage change in baseline values in order to compare drug effects in different brain areas where the baseline value represents the mean of two basal values sampled immediately before injection.

2.8. Assay of dopamine and DOPAC in the perfusate

The concentrations of dopamine and DOPAC in the microdialysis samples were determined by using a high performance liquid chromatography (HPLC) system consisting of a high-pressure pump (Merch-Hitachi L-6200A) operating at a flow rate of 1.2 ml/min, and a Merck AS4000 autosampler with refrigerated sample tray (10°C). Twenty μl of a 24 μl sample (20 μl dialysate in 4 μl 2 mM perchloric acid), or of a dopamine/DOPAC standard solution, was injected on a 75 × 4.6 mm Supelcosil LC-18-DB column (3 mM particles). An electrochemical detector (BAS LC 4B) with glassy carbon working electrode set at +0.7 V was used to monitor sample elution. The column was eluted isocratically with a 10:1 (v/v) elution buffer/MeOH mixture purged with helium. The elution buffer consisted of 150 mM sodium dihydrogen-phosphate, 1 mM EDTA and 0.3 mM *n*-octane sulfonic acid adjusted to pH 3.6 with phosphoric acid. Dopamine and DOPAC peaks in the sample chromatogram were quantified by measuring peak heights relative to a standard solution consisting of 4 nM dopamine and 400 nM DOPAC dissolved in 0.2 mM perchloric acid.

2.9. Data analysis

Statistical analysis of the microdialysis data was performed in order to evaluate the effect of on drug effect on $[\text{dopamine}]_e$ and $[\text{DOPAC}]_e$. The accumulated drug responses over time (area under curve, AUC) were compared to the AUC of the control group by use of a one way analysis of variance followed by Student–Newman–Keuls post-hoc test. Log probit analysis was used to calculate ED_{50} values. Effect of drugs on locomotor activity, rotation behavior and conditioned avoidance responding were investigated by an analysis of variance followed by Student–Newman–Keuls post-hoc test.

3. Results

3.1. Locomotor activity in normal mice and rats

Cis-8-OH-PBZI monophasically inhibited spontaneous locomotor activity in normal mice ($P < 0.001$) in a dose-dependent manner as shown in Fig. 1A ($ED_{50} = 8.5$ mg/kg

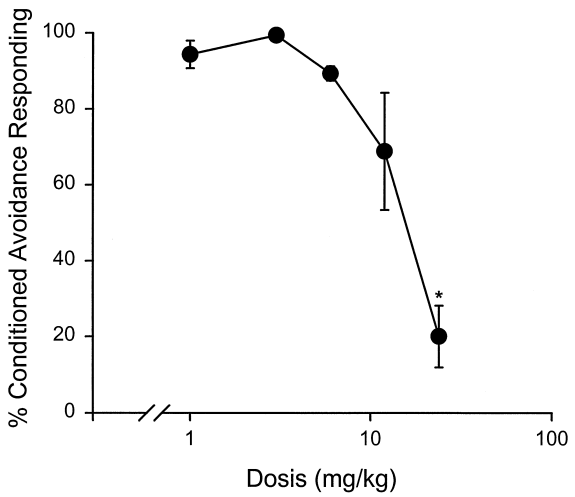

Fig. 3. Effect of *cis*-8-OH-PBZI on conditioned avoidance responding (effects on performance) in rats. The result is depicted as mean ± S.E.M., expressed as a percentage of the saline control value ($n = 4$ rats per dosage). The compound did not induce escape failures. *Cis*-8-OH-PBZI was administered s.c. 30 min before the start of each experiment. * $P < 0.05$, compared to the control (one-way analysis of variance followed by Student–Newman–Keuls post-hoc test).

Table 1
Inhibition of conditioned avoidance responding (CAR) and induction of catalepsy in rats, expressed as ED_{50} values (mg/kg)

		ED_{50} (mg/kg)	
		CAR	Catalepsy
Cis-8-OH-PBZI	(s.c.) −30 min	15.5	> 48
Haloperidol	(i.p.) −120 min	0.2	0.4
Clozapine	(i.p.) −120 min	10.0	> 100

s.c.). The ED_{50} value in rats was 4.2 mg/kg s.c. (data not shown). The effect of *cis*-8-OH-PBZI was effectively antagonized by the dopamine D_3 receptor antagonist U99194A (Fig. 1B) which at doses of 20 and 30 mg/kg (s.c.) of U99194A shifted the ED_{50} value from 10.7 mg/kg to 18.3 mg/kg and > 30 mg/kg, respectively. *Cis*-8-OH-PBZI also inhibited D-amphetamine-induced hyperlocomotion in mice ($P < 0.05$) with an ED_{50} value of 23.6 mg/kg, s.c. (Fig. 1C). The dopamine D_3 receptor preferring agonist 7-OH-DPAT induced a biphasic response ($P < 0.001$) with inhibition of spontaneous locomotor activity occurring at low doses (ED_{50} value of 0.07 mg/kg) and stimulation of locomotor activity occurring at higher doses (Fig. 1D). The same profile was observed with the dopamine D_3 receptor preferring agonist PD 128907 and the nonselective dopamine receptor agonist (−)-apomorphine with ED_{50} values of 0.57 and 0.36 mg/kg, respectively (data not shown). In contrast, the dopamine D_3 receptor antagonist U99194A did not inhibit spontaneous locomotor activity (Fig. 2A) at doses of up to 30 mg/kg (s.c.) in mice and actually increased locomotor activity at the highest dose ($P < 0.05$). When mice were habituated to the cages for a 2 h period, U99194A increased locomotor activity ($P < 0.001$) in a dose-dependent manner to a level which was approximately 250% of that observed in control animals (Fig. 2B).

3.2. Conditioned avoidance responding

Cis-8-OH-PBZI dose dependently inhibited conditioned avoidance responding in rats ($P < 0.001$) with an ED_{50} value of 15.5 mg/kg following subcutaneous administration (Fig. 3). The compound did not induce escape failures indicating that the observed inhibition of conditioned avoidance responding was not due to non-specific motor side effects. By comparison, the prototypical neuroleptic haloperidol and the atypical neuroleptic clozapine inhibited conditioned avoidance responding with ED_{50} values of 0.2 and 11 mg/kg, respectively, following i.p. administration (Table 1).

3.3. Catalepsy

At doses ranging from 3 to 48 mg/kg *cis*-8-OH-PBZI failed to induce catalepsy in rats (Table 1). Clozapine similarly showed no cataleptogenic effect at doses up to 100 mg/kg. By contrast, haloperidol effectively induced catalepsy at low doses (ED_{50} = 0.44 mg/kg).

3.4. Locomotor activity in reserpinized mice

Cis-8-OH-PBZI (0.001–30.0 mg/kg, s.c.) did not increase locomotor activity ($P > 0.05$) in reserpinized mice (the small effects observed at the lowest and highest doses were not statistically significant and were due to the behavior of a single mouse in each group) (Fig. 1A). In contrast, the dopamine D_3 receptor preferring agonist 7-OH-DPAT increased locomotor activity ($P < 0.001$) to more than 50% of the activity observed in saline treated, non-reserpinized mice (Fig. 1D). The same profile was observed with the dopamine D_3 receptor preferring agonist PD 128907 and the nonselective dopamine receptor agonist (−)-apomorphine (data not shown).

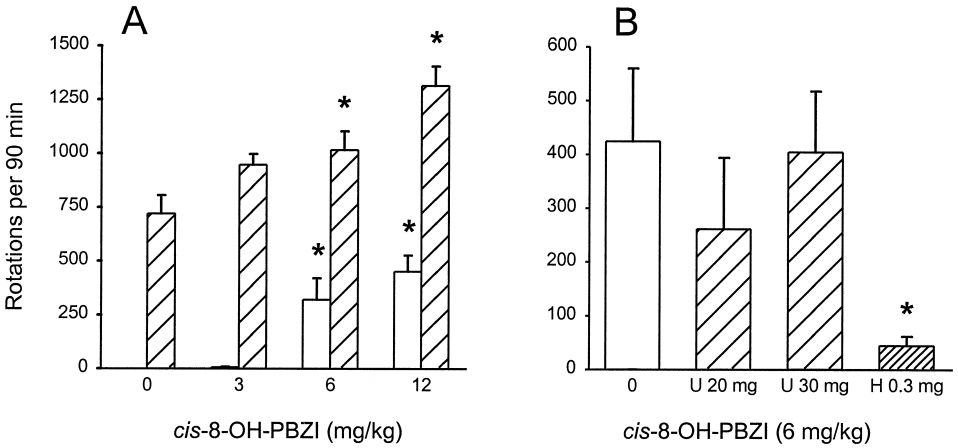

Fig. 4. (A) Contralateral rotation induced by *cis*-8-OH-PBZI administered to unilaterally 6-hydroxydopamine-lesioned rats alone (white bars) or together with a fixed dose of 7-OH-DPAT, 1.0 mg/kg (hatched bars). (B) Contralateral rotation induced by *cis*-8-OH-PBZI, 6 mg/kg alone (white bar) or together with the dopamine D_3 receptor antagonist U99194A, 20 and 30 mg/kg (hatched bars, U20 and U30) or the dopamine $D_{2,3,4}$ receptor antagonist haloperidol, 0.3 mg/kg (hatched bar, H 0.3). Results are expressed as the mean ± S.E.M. ($n = 8$ for each group). $^{*}P < 0.05$, compared to the control (one-way analysis of variance followed by Student–Newman–Keuls post-hoc test).

3.5. Rotation in unilaterally 6-hydroxydopamine-lesioned rats

Cis-8-OH-PBZI dose-dependently induced contralateral rotation ($P < 0.01$) in rats (Fig. 4A). Further, the compound increased ($P < 0.01$) the contralateral rotations induced by 7-OH-DPAT, 1 mg/kg (s.c.) in an additive manner (Fig. 4A). The rotation induced by 6 mg/kg of *cis*-8-OH-PBZI was not antagonized by the dopamine D_3 receptor antagonist U99194A but was completely blocked ($P < 0.01$) by pretreatment with 0.3 mg/kg (s.c.) of the dopamine receptor antagonist haloperidol (Fig. 4B). By contrast, 0.01 or 0.1 mg/kg doses (s.c.) of the dopamine-D_1 receptor antagonist SCH23390 had no inhibitory effect ($P > 0.05$) on the *cis*-8-OH-PBZI-induced rotation (data not shown).

3.6. Microdialysis, $[dopamine]_e$ and $[DOPAC]_e$

Interstitial levels of dopamine and DOPAC ($[dopamine]_e$ and $[DOPAC]_e$, respectively) were measured in the nucleus accumbens and dorsolateral striatum. Steady state levels of dopamine and DOPAC were reached within the first hour following the start of the perfusion (data not shown). *Cis*-8-OH-PBZI at doses of 6 and 12 mg/kg s.c. did not ($P > 0.05$) affect $[dopamine]_e$ and $[DOPAC]_e$ compared to the effect of saline (Fig. 5).

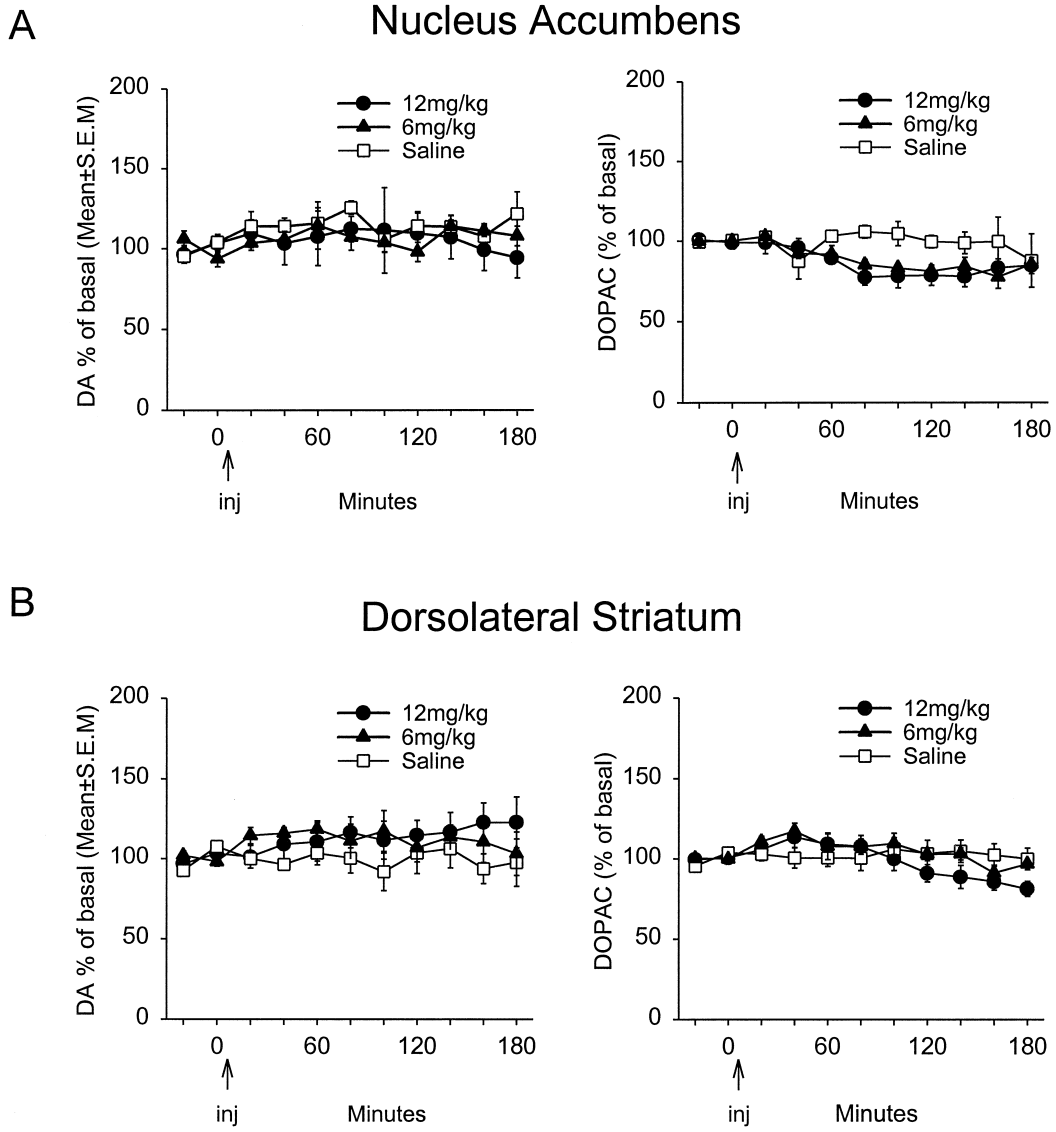

Fig. 5. Effect of *cis*-8-OH-PBZI (6 and 12 mg/kg s.c.) on $[dopamine]_e$ and $[DOPAC]_e$. Microdialysis measurements were made in the dorsolateral striatum (DlSt) and in the nucleus accumbens (NAc) of awake rats. Each curve shows the mean ± S.E.M. of $[dopamine]_e$ and $[DOPAC]_e$ measured in five animals. Individual time points were calculated as the percentage of the last two basal values measured prior to drug administration. Injection of the drug is indicated with an arrow.

4. Discussion

In the present report we investigated the in vivo pharmacological profile of the benz[e]indole *cis*-8-OH-PBZI, which was described in the accompanying article as a preferential dopamine D_3 receptor agonist (Scheideler et al., 1997).

Earlier reports have suggested that dopamine D_3 receptors are expressed both pre- and postsynaptically (Damsma et al., 1993; Waters et al., 1993; Svensson et al., 1994a,b; Kreiss et al., 1995). For this reason, we attempted to assess the pre- versus postsynaptic site of action of *cis*-8-OH-PBZI by studying its effect on locomotor activity in both normal and reserpinized mice, on rotation in unilaterally 6-hydroxydopamine-lesioned rats, and on interstitial levels of dopamine in rats. These results were further compared with those obtained for different dopamine receptor antagonists and agonists. Moreover, *cis*-8-OH-PBZI was assessed in both conditioned avoidance responding and catalepsy tests in order to evaluate its efficacy in preclinical tests in which efficacy is considered predictive of antipsychotic efficacy and motor side effect potential, respectively.

Cis-8-OH-PBZI monophasically inhibited spontaneous locomotor activity in mice. This profile has previously been observed for dopamine receptor antagonists (McLean et al., 1978), as well as for several partial dopamine receptor agonists active at dopamine D_2 receptors, including the substituted aryl-cyclohexenyl-alkyl-amine Cl-1007 (Meltzer et al., 1995) and the amino-ergolines SDZ 208–911 and SDZ 208–912 (Coward et al., 1990). This is in contrast to the effect of the preferential dopamine D_3 receptor antagonist U99194A, which in the present study increased spontaneous locomotor activity at the highest dose tested and dose-dependently increased the activity level in habituated mice, as it did in earlier studies (Waters et al., 1993). Importantly, reversal of the *cis*-8-OH-PBZI-induced decrease in spontaneous locomotor activity by U99194A shows that *cis*-8-OH-PBZI acts in vivo as a dopamine D_3 receptor selective ligand. The dopamine receptor agonists 7-OH-DPAT, (−)-apomorphine and PD 128907 also inhibited locomotor activity at low doses, probably mediated by stimulation of dopamine D_3 receptors, as suggested by Svensson et al. (1994a). However, each of these compounds further increased locomotor activity at higher doses, most likely mediated by postsynaptic dopamine D_2 receptor stimulation (Costall et al., 1981). In the present study a pretreatment interval of 20 min was used, and it may well be that several of the compounds would have had more potent effects if other dosing regimens were used.

Traditionally, it has been proposed that inhibition of locomotor activity by dopamine agonists is due to stimulation of presynaptic dopamine receptors whereas stimulation of locomotor activity by dopamine receptor agonists is due to interaction with postsynaptic dopamine receptors (Costall et al., 1981). Several neurochemical methods have been used to evaluate the degree to which a given compound has a pre- versus postsynaptic site of action: γ-butyrolactone blocks impulse flow in dopaminergic neurons, which results in increased dopamine synthesis (measured by accumulation of 3,4-dihydroxyphenylalanine). Stimulation of presynaptic dopamine receptors attenuates the accumulation of 3,4-dihydroxyphenylalanine. The dopamine D_3 preferring agonists (+)7-OH-DPAT and PD 128907 decrease γ-butyrolactone-induced 3,4-dihydroxyphenylalanine accumulation at doses which inhibit locomotor activity (Pugsley et al., 1995). Agonist action at presynaptic D_2-like receptors on somatodendrites or terminals is expected to decrease both dopamine release and interstitial levels of dopamine. A decrease in interstitial dopamine levels has been reported for agonists with a preference for dopamine D_3 receptors, including PD 128907 (Pugsley et al., 1995) and (+)7-OH-DPAT (Svensson et al., 1994a). However, *cis*-8-OH-PBZI did not alter dopamine release, measured by microdialysis, in the present study. Doses of *cis*-8-OH-PBZI which inhibited locomotor activity in rats (6 and 12 mg/kg) did not affect interstitial levels of dopamine or DOPAC in the rat dorsolateral striatum or in the nucleus accumbens. Interestingly, the theory that inhibition of locomotor activity is mediated by presynaptic dopamine receptor stimulation has been questioned, since the relation between the time-course/duration of the behavioral suppression elicited by dopamine receptor agonists and the reduction in dopamine release measured by in vivo microdialysis are often inconsistent (Ståhle, 1992). In addition, it has recently been reported that the lowest doses of (+)7-OH-DPAT which inhibit locomotor activity do not reduce DOPA accumulation or decrease interstitial levels of dopamine in the striatum or nucleus accumbens (Svensson et al., 1994a). Based on this observation, it has been suggested that the inhibition of locomotor activity is mediated by stimulation of a postsynaptic dopamine receptor, presumably the dopamine D_3 receptor. This information is in accordance with the observation that the dopamine D_3 receptor antagonist U99194 increases locomotor activity (Waters et al., 1993; present study). The behavioral effects of *cis*-8-OH-PBZI could then be explained by postsynaptic dopamine D_3 receptor stimulation having an inhibitory action on locomotor activity in normal mice and rats, without affecting interstitial levels of dopamine in the striatum or nucleus accumbens or locomotion in reserpinized mice. This is in accordance with the recent observation that homozygotic mice lacking the dopamine D_3 receptor exhibit increased locomotor activity (Accili et al., 1996).

In reserpinized animals, *cis*-8-OH-PBZI did not induce locomotor activity at any of the doses tested. By contrast, the dopamine receptor agonists 7-OH-DPAT, (−)-apomorphine and PD 128907 reversed the akinesia caused by reserpine, suggesting that these ligands act postsynaptically in a stimulatory fashion. However, if stimulating

postsynaptic dopamine D_3 receptors inhibits locomotor activity, as hypothesized by Waters et al. (1993), the effects of *cis*-8-OH-PBZI on locomotor activity in normal mice and its lack of effect in reserpinized mice would be consistent with a postsynaptic site of action.

Evaluation of conditioned avoidance responding in rats, a classical test for antipsychotic efficacy (Arnt, 1982), showed that *cis*-8-OH-PBZI inhibited the response with an ED_{50} of 15.5 mg/kg, an effect also observed with several neuroleptic drugs (Arnt, 1982; present study). In addition, *cis*-8-OH-PBZI did not induce catalepsy at test doses up to 48 mg/kg. The present data indicate that the observed efficacy of *cis*-8-OH-PBZI in conditioned avoidance responding, together with the lack of catalepsy, is associated with a dopamine D_3 receptor interaction. However, since high test doses were used for conditioned avoidance responding in order to elicit a pharmacological effect (ED_{50} = 15.5 mg/kg), we cannot exclude that *cis*-8-OH-PBZI may mediate its pharmacological effects through an action at both dopamine D_3 and D_2 receptors. In this case, the action on dopamine D_2 receptors is most likely a partial agonist mode of action, since *cis*-8-OH-PBZI at very high doses in vitro behaves like a partial agonist at dopamine D_2 receptors (Scheideler et al., 1997).

Cis-8-OH-PBZI was further tested in the very sensitive rat 6-hydroxydopamine model in which dopamine receptor agonist-induced contralateral rotation is mediated by postsynaptic dopamine receptors following lesioning of the presynaptic dopamine neurons. At doses of 3, 6 and 12 mg/kg, *cis*-8-OH-PBZI induced contralateral rotation, as has also been observed with the dopamine D_3 receptor agonists quinpirole and 7-OH-DPAT (Arnt and Hyttel, 1990; McElroy et al., 1993; Fink-Jensen, unpublished data). The effect of *cis*-8-OH-PBZI was additive to that of 1 mg/kg of 7-OH-DPAT and could be completely blocked by the dopamine D_2 subfamily receptor antagonist haloperidol but not by the dopamine D_1 subfamily receptor antagonist SCH23390 (data not shown), demonstrating that the effect is mediated by stimulation of a receptor belonging to the dopamine D_2 receptor subfamily. However, the response to *cis*-8-OH-PBZI could not be antagonized by the dopamine D_3 receptor antagonist U99194A. The 6-hydroxydopamine lesion has been shown to cause a selective decrease in dopamine D_3 receptor expression in the nucleus accumbens (Lévesque et al., 1995). However, the actual 6-hydroxydopamine lesion affects both the mesolimbic area and the motor nigrostriatal system. Thus, postsynaptic dopamine D_2 subtype receptors may be involved in the contralateral rotation response that we observed.

In conclusion, *cis*-8-OH-PBZI, a preferential dopamine D_3 receptor agonist in vitro, exhibits an in vivo profile suggestive of antipsychotic efficacy with low or no catalepsy potential. The effect of *cis*-8-OH-PBZI on spontaneous locomotor activity could be reversed by the dopamine D_3 selective receptor antagonist U99194A, thereby demonstrating that cis-8-OH-PBZI has a dopamine D_3 receptor agonist action also in vivo. Moreover, our data suggest that *cis*-8-OH-PBZI selectively stimulates postsynaptically located receptors mediating an inhibitory behavioral response.

Acknowledgements

Karina Lynge Laursen, Frank Strauss, Birthe Lehman are thanked for excellent technical assistance.

References

Accili, D., Fishburn, C.S., Drago, J., Steiner, H., Lachowicz, J.E., Park, B.H., Gauda, E.B., Lee, E.J., Cool, M.H., Sibley, D.R., Gerfen, C.R., Westphal, H., Fuchs, S., 1996. A targeted mutation of the D_3 dopamine receptor gene is associated with hyperactivity in mice. Proc. Natl. Acad. Sci. USA 93, 1945–1949.

Arnt, J., 1982. Pharmacological specificity of conditioned avoidance response inhibition in rats: Inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol. Toxicol. 51, 321–329.

Arnt, J., Hyttel, J., 1990. Dopamine D-2 agonists with high and low efficacies: Differentiation by behavioural techniques. J. Neural Transm. 80, 33–50.

Baldessarini, R.J., Tarsy, D., 1980. DA and the pathophysiology of dyskinesias induced by antipsychotic drugs. Annu. Rev. Neurosci. 3, 23–46.

Balk, J.H., Picetti, R., Salardi, A., Thiriet, G., Dierich, A., Depaulis, A., Le Meur, M., Borrelli, E., 1995. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 377, 424–428.

Costall, B., Lim, S.K., Naylor, R.J., 1981. Characterisation of the mechanisms by which purported dopamine agonists reduce spontaneous locomotor activity of mice. Eur. J. Pharmacol. 73, 175–188.

Coward, D.M., Dixon, A.K., Urwyler, S., White, T.G., Enz, A., Karobath, M., Shearman, G., 1990. Partial dopamine-agonistic and atypical neuroleptic properties of the amino-ergolines SDZ 208–911 and SDZ 208–912. J. Pharmacol. Exp. Ther. 252, 279–285.

Cruse, S.F., Lear, J., Klein, C.L., Andersen, P.H., Dick, R.M., Crider, A.M., 1993. *cis*-1,2,3a,4,5,9b-hexahydro-3H-benz[e]indoles: Synthesis and in vitro binding affinity at dopamine D_1 and D_2 receptors. J. Pharmacol. Sci. 82, 334–339.

Damsma, G., Bottema, T., Westerink, B.H.C., Tepper, P.G., Dijkstra, D., Pugsley, T.A., MacKenzie, R.G., Heffner, T.G., Wikström, H., 1993. Pharmacological aspects of *R*-(+)-7-OH-DPAT, a putative dopamine D_3 receptor ligand. Eur. J. Pharmacol. 249, R9–R10.

Fink-Jensen, A., Hansen, L., Hansen, J.B., Nielsen, E.B., 1996. Regional differences in the effect of haloperidol and atypical neuroleptics on interstitial levels DOPAC in the rat forebrain: An in vivo microdialysis study. J. Psychopharm. 10, 119–125.

Klemm, W.R., 1985. Neuroleptic-induced catalepsy: A D2 blockade phenomenon. Pharmacol. Biochem. Behav. 23, 911–915.

Kreiss, D.S., Bergstrom, D.A., Gonzalez, A.M., Huang, K.X., Sibley, D.R., Walters, J.R., 1995. Dopamine receptor agonist potencies for inhibition of cell firing correlate with dopamine D_3 receptor binding affinities. Eur. J. Pharmacol. 277, 209–214.

Landwehrmeyer, B., Mengod, G., Palacios, J.M., 1993. Differential visualization of dopamine D2 and D3 receptor sites in rat brain. A comparative study using in situ hybridization histochemistry and ligand binding autoradiography. Eur. J. Neurosci. 5, 145–153.

Lévesque, D., Matres, M.P., Diaz, J., Griffon, N., Lammers, C.H., Sokoloff, P., Schwartz, J.C., 1995. A paradoxical regulation of the dopamineD3 receptor expression suggests the involvement of an

anterograde factor from dopamine neurons. Proc. Natl. Acad. Sci. USA 92, 1719–1723.

McElroy, J., Zeller, K., Amy, K., Ward, K., Cawley, J., Mazzola, A., Keim, W., Rohrbach, K., 1993. In vivo agonist properties of 7-hydroxy-*n*,*n*-di-*n*-propyl-2-aminotetralin, a dopamine D_3-selective receptor ligand. Drug Dev. Res. 30, 257–259.

McLean, J.R., Parker, R.B., Coughenour, L.L., 1978. Effect of antipsychotic and other classes of drugs on spontaneous locomotor activity and neurotoxicity in mice. Pharmacol. Biochem. Behav. 8, 97–99.

Meltzer, L.T., Christoffersen, C.L., Corbin, A.E., Ninteman, F.W., Serpa, K.A., Wiley, J.N., Wise, L.D., Heffner, T.G., 1995. Cl-1007, a dopamine partial agonist and potential antipsychotic agent. II. Neurophysiological and behavioral effects. J. Pharmacol. Exp. Ther. 274, 912–920.

Morelli, M., Di Chiara, G., 1985. Catalepsy induced by SCH 23390 in rats. Eur. J. Pharmacol. 117, 179–185.

Pugsley, T.A., David, M.D., Akunne, H.C., MacKenzie, R.G., Shih, Y.H., Damsma, G., Wikstrom, H., Whetzel, S.Z., Georgic, L.M., Cooke, L.W., Demattos, S., Corbin, A.E., Glase, S.A., Wise, L.D., Dijkstra, D., Heffner, T.G., 1995. Neurochemical and functional characterization of the preferentially selective dopamine D_3 agonist PD 128907. J. Pharmacol. Exp. Ther. 275, 1355–1366.

Scheideler, M.A., Martin, J., Hohlweg, R., Rasmussen, J.S., Nærum, L., Ludvigsen, T.S., Larsen, P.J., Korsgaard, N., Crider, A.M., Ghosh, D., Cruse, S.F., Fink-Jensen, A., 1997. The preferential dopamine-D_3 receptor agonist, *cis*-8-OH-PBZI induces limbic *Fos* expression in rat brain. Eur. J. Pharmacol. 339, 259–268.

Sokoloff, P., Giros, B., Martres, M.-P., Bouthenet, M.-L., Schwartz, J.C., 1990. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347, 146–151.

Ståhle, L., 1992. Do autoreceptors mediate dopamine agonist-induced yawning and suppression of exploration? A critical review. Psychopharmacology 106, 1–13.

Svensson, K., Carlsson, A., Waters, N., 1994a. Locomotor inhibition by the D_3 ligand *R*-(+)-7-OH-DPAT is independent of changes in dopamine release. J. Neural Transm. 95, 71–74.

Svensson, K., Carlsson, A., Huff, R.M., Kling-Petersen, T., Waters, N., 1994b. Behavioral and neurochemical data suggest functional differences between dopamine D_2 and D_3 receptors. Eur. J. Pharmacol. 263, 235–243.

Waters, N., Svensson, K., Haadsma-Svensson, S.R., Smidt, M.W., Carlsson, A., 1993. The dopamine D_3-receptor: A postsynaptic receptor inhibitory on rat locomotor activity. J. Neural Transm. 94, 11–19.

Wikström, H., Andersson, B., Sanchez, D., Lindberg, P., Arvidsson, L.E., 1985. Resolved monophenolic 2-aminotetralins and 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines: Structural and stereochemical considerations for centrally acting pre- and postsynaptic dopamine-receptor agonists. J. Med. Chem. 28, 215–225.