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The dopamine D3 receptor partial agonist, BP 897, is an antagonist at human dopamine D3 receptors and at rat somatodendritic dopamine D3 receptors

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Abstract

Recent studies have fueled the interest in dopamine D3 receptor antagonists and partial agonist for the treatment of psychosis and drug abuse, respectively. *N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxamide (BP 897) is a dopamine D3 receptor selective ligand recently described as partial agonist with potential effects on drug-dependence. The aim of the present study was to determine both the functional activity of BP 897 at human dopamine D3 receptors expressed in Chinese hamster ovary (CHO) cells and in an electrophysiological in vivo model of dopaminergic activity. BP 897 failed to stimulate the human dopamine D3 receptor and showed antagonistic effects ($cpIC_{50} = 9.51$) in a [^{35}S]GTPγS binding assay in cells expressing the human dopamine D3 receptor. In vivo, BP 897 up to 8.2 mg/kg, i.v., had no agonistic effects on firing rate of substantia nigra dopaminergic neurons and antagonized the quinpirole-induced inhibition of firing ($DID_{50} = 1.1$ mg/kg). Our data demonstrate that BP 897 acts, in vivo and in vitro, as a dopamine D3 receptor antagonist.

Keywords: BP 897; Dopamine; Dopamine D3 receptor; Functional assay; Electrophysiology; Drug dependence

1. Introduction

The human dopamine D3 receptor belongs to the dopamine D2 receptor family. It shares high overall amino acid identity with other members of this family namely the dopamine D2 (52%) and the dopamine D4 (39%) receptors (Sokoloff et al., 1990; Van Tol et al., 1991). The human dopamine D3 receptor triggers second messenger cascades through the activation of G-proteins (Shafer and Levant, 1998; Vanhauwe et al., 1999). It has been reported that human dopamine D3 receptor is negatively coupled to adenylate cyclase and may induce different cellular responses such as cell proliferation and extracellular pH acidification (Pilon et al., 1994; Seabrook et al., 1994; Shafer and Levant, 1998; Zaworski et al., 1999). The anatomical distribution of dopamine D3 receptors in the brain, its binding affinity for neuroleptics, and genetic studies suggest a putative role in mechanisms of psychosis (Crocq et al., 1992; Sokoloff et al., 1992b; Shafer and Levant, 1998). The expression of dopamine D3 receptors in the brain is restricted to discrete areas of the limbic system, which are known to play a role in the pathophysiology of psychotic disorders. The highest levels of dopamine D3 receptor mRNA transcripts have been found in the olfactory bulb, nucleus accumbens and the Islands of Calleja (Sokoloff et al., 1990, 1992a,b; Murray et al., 1994). These data strongly suggest that compounds with high affinity and selectivity to human dopamine D3 receptors may have antipsychotic activity with an atypical neuroleptic profile, thus devoid of extrapyramidal side effects (Sokoloff et al., 1992a,b). It has also been suggested that dopamine D3 receptors may play an important role in the development of cocaine addiction (Caine et al., 1997). A recent study using *N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxamide (BP 897) further strengthens this hypothesis (Pilla et al., 1999).

BP 897 is a new potent and selective ligand at human dopamine D3 receptors (Pilla et al., 1999). It has been shown that BP 897 has anti-craving activity in an animal model of cocaine-seeking behavior (Pilla et al., 1999). The intrinsic activity of BP 897 remains controversial because

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contradictory data have been reported depending on the assay used. BP 897 was able to stimulate human dopamine D3 receptors as a partial agonist in heterologous cellular systems while it behaves either as an agonist or an antagonist in vivo (Pilla et al., 1999). The potent dopamine D3 receptor preferring compounds (+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3*b*]-1,4-oxasin-9-ol (PD 128907), and 7-hydroxy-dipropyl-aminotetralin-hydrobromide (7-OH-DPAT) have been used in different systems (Vanhauwe et al., 1999) and are important reference compounds to characterize agonism and partial agonism at human dopamine D3 receptors. To determine the putative therapeutic value of BP 897 as a dopamine D3 receptor selective ligand, it is important to clearly elucidate its intrinsic pharmacological activity. The present study aimed to characterize the intrinsic activity of BP 897 by measuring the human dopamine D3 receptor mediated G protein activation in transfected Chinese hamster ovary (CHO) cells, in vitro, and its electrophysiological activity in the dopaminergic system in rat brain, in vivo. The effects of BP 897 are discussed in comparison to the prototypic dopamine D3 receptor ligands PD 128907 and 7-OH-DPAT.

2. Materials and methods

2.1. Drugs

Dopamine hydrochloride, *R*(+)-7-OH-DPAT (7-hydroxy-dipropylaminotetralin-hydrobromide), (±)-7-OH-DPAT, PD 128907 ((+)-*trans*-3,41,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3*b*]-1,4-oxasin-9-ol), and quinpirole were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany). BP 897 (*N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxamide) was synthesized in the Department of Chemistry at KNOLL (Ludwigshafen, Germany). [^{35}S]GTPγS (1000 Ci/mmol) was from Du Pont de Nemours, NEN (Bad Homburg, Germany). GTPγS, GDP, EGTA, EDTA and dithiothreitol were from Sigma. All other reagents were of analytical grade from Merck (Darmstadt, Germany).

2.2. Cell culture and membrane preparation

CHO cells (clone hD3/CHO) stably transfected with the human dopamine D3 receptor gene were cultured in Dulbecco's modified Eagle's Medium F-12 (DMEM) medium containing 10% fetal calf serum, at 37°C in an atmosphere of 5% CO_2 and 95% O_2. Cells were grown to confluence, detached and pelleted ($100 \times g$). Cells were lysed and homogenized at 4°C in 5 mM Tris–HCl buffer (containing 5 mM EDTA, 5 mM EGTA, 0.1 mM phenylmethylsulphonyl fluoride and 3 mM benzamidine; pH 7.6). Cell homogenates were centrifuged ($1000 \times g$) at 4°C for 1 min. The pellet was resuspended in buffer and centrifuged at $40{,}000 \times g$, for 20 min at 4°C; this step was performed twice. Membranes were resuspended in 5 mM Tris-HCl buffer, frozen and stored at −80°C, until used.

2.3. [^{35}S]GTPγS binding experiments

The [^{35}S]GTPγS binding assay was performed using the method of Hilf and Jakobs (1992) with minor modifications. Briefly, cell membranes (60 μg protein) were incubated with 50 mM triethanolamine–HCl buffer (pH 7.5) containing 6.75 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 10 μM GDP and 5.4 nM [^{35}S]GTPγS. Following a 60-min incubation period at 30°C in the absence or in the presence of different concentrations of drug, the assay mixture (100 μl) was rapidly filtered through Whatman GF/B filters using a Skatron® filtration device. Filters were quickly washed with 9 ml of 50 mM Tris–HCl (4°C) containing 100 mM NaCl and 5 mM $MgCl_2$ at pH 7.5. Radioactivity retained on the filters was determined by liquid scintillation counting.

2.4. Electrophysiology

Male Sprague–Dawley rats weighing 200–310 g were anaesthetized with chloralhydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Body temperature was controlled at 37 ± 0.5°C. After an incision in the scalp, a hole was drilled in the skull above the midbrain and the dura was removed. Micropipette recording electrodes (impedance 3–7 MΩ) were filled with 0.5 M Na-acetate (1% Pontamine sky blue). With extracellular recording methods, spontaneously active dopaminergic neurons of substantia nigra were identified with extracellular recording methods and by following criteria (Bunney et al., 1973): spike length ≥ 2 ms, frequency 1–10 Hz and triphasic action potential (positive/negative/small positive). The stereotactic coordinates according to Paxinos and Watson (1986) were interaural 3.0 to 3.4 mm, lateral 1.8 to 2.2 mm and 7.0 to 9.0 mm ventral from brain surface. The baseline firing rate of each cell was recorded for 5 min. Thereafter, BP 897, (±)-7-OH-DPAT, PD 128907 or quinpirole were given i.v. in cumulative doses for testing their potential agonistic activity.

In a second set of experiments, the firing of dopaminergic neurons of the substantia nigra was inhibited by cumulative doses of quinpirole. Each animal was dosed individually with the highest dose of quinpirole leading to a complete inhibition of firing. After total inhibition of discharge, BP 897 was administered i.v. in cumulative doses to test its antagonistic potency. In all experiments, the time interval between two consecutive doses of the test compound was 60–90 s. Only one neuron per animal was recorded. Housekeeping, treatment and sacrificing of the animals used in electrophysiological experiments were done in compliance with the German Animal Protection Act.

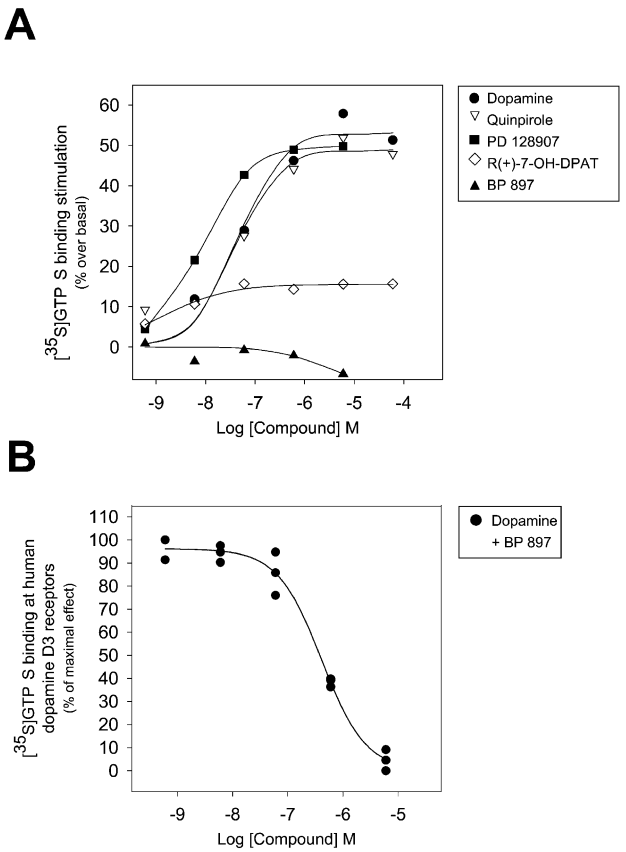

Fig. 1. Agonism and antagonism at human dopamine D3 receptor expressed in CHO cells. The effect of dopaminergic agonists and BP 897 on the stimulation of [^{35}S]GTPγS binding was determined as described in Materials and methods. (A) Agonist-stimulated basal level of [^{35}S]GTPγS binding. Note that BP 897 failed to increase it. (B) BP 897 dose-dependent inhibition of the dopamine stimulatory effect. Stimulation curves in (A) correspond to one typical experiment representative of at least three, each concentration done in triplicate. In (B), it is shown in three different experiments, each point performed in triplicate. The inhibition curve was obtained by simultaneous fitting.

2.5. Data analysis

Drug effects in in vitro experiments were expressed either as drug-induced increase in binding over basal binding (in the absence of drugs) or as percentage of increase in binding induced by 60 μM dopamine (100% effect). Curves were fitted by non-linear regression analysis (Sigma Plot V5.0, SSS, Chicago, USA) to the equation $E = (LE_{max})/(L + EC_{50})$, where E is the effect, L the drug concentration, E_{max} the maximal effect and EC_{50} the concentration inducing 50% of the maximal effect. In the case of inhibitory effects of BP 897, we used a similar equation to obtain the IC_{50} values. The cIC_{50} of BP 897 was defined as the inhibition constant on the effect of dopamine (DA) and was calculated using the transformation $cIC_{50} = IC_{50}/(1 + [DA]/DAEC_{50})$ (Vanhauwe et al., 1999). We refer to the negative logarithm of EC_{50}, IC_{50} or K_{ia} as pEC_{50}, pIC_{50} and $cpIC_{50}$, respectively.

For the determination of ED_{50}s and disinhibitory doses (DID_{50}) in the electrophysiological experiments, a sigmoid fitting routine ($f = 100/1 + \exp(-(x - x50)/b)$) of SigmaPlot V5.0 was used, x is the drug concentration, $x50$ the dose inducing 50% of the maximal effect and b is the slope.

3. Results

Dopamine, $R(+)$-7-OH-DPAT, PD 128907 and quinpirole dose-dependently induced an increase of [^{35}S]GTPγS binding to membranes of hD3/CHO cells (Fig. 1A). Dopamine and quinpirole stimulated human dopamine D3 receptors as full agonists with comparable potency (Table 1). PD 128907, a dopamine D3 receptor-selective compound stimulated the binding of [^{35}S]GTPγS also as a full agonist ($E_{max} = 86\%$) but with a higher potency ($pEC_{50} = 8.08$) than dopamine or quinpirole. $R(+)$-7-OH-DPAT was the most potent agonist tested in the present study ($pEC_{50} = 8.56$), however, its intrinsic activity was clearly lower ($E_{max} = 46\%$) than that of dopamine, quinpirole or PD 128907, respectively (Fig. 1A, Table 1). No significant differences were found between $R(+)$-7-OH-DPAT and $(\pm)$-7-OH-DPAT for neither the intrinsic activity nor the potency (data not shown). Therefore, the racemate form of the compound was used for in vivo experiments. BP 897 failed to induce any increase in [^{35}S]GTPγS binding to human hD3/CHO cell membranes, suggesting antagonist properties at human dopamine D3 receptors. Accordingly, BP 897 dose-dependently antagonized the effect of 60 μM dopamine in hD3/CHO membranes with a $pIC_{50} = 6.38$ (Fig. 1B, Table 1).

The effect of cumulative i.v. applications of BP 897 is shown in Fig. 2A. In doses of 64–8192 μg/kg, it did not induce significant changes in the firing rate of substantia

Table 1
Agonism and antagonism at human dopamine D3 receptors expressed in CHO cells
Stimulation of human dopamine D3 receptor by dopaminergic agonists and BP 897 in CHO cells transfected with human dopamine D3 receptors. Receptor activation was determined by measuring the increase in [^{35}S]GTPγS binding in cell membranes.

	IA (%)[a]	PEC_{50}[b]	pIC_{50}[c]	$cpIC_{50}$[d]
Dopamine	100	7.35 ± 0.07	–	–
Quinpirole	96 ± 6	7.29 ± 0.06	–	–
$R(+)$-7-OH-DPAT	43 ± 4	8.56 ± 0.12	–	–
PD 128907	86 ± 3	8.08 ± 0.05	–	–
BP 897	–	–	6.38 ± 0.04	9.51 ± 0.03

Values are means ± S.E.M. of three experiments.
[a] Intrinsic activity (IA) in % of the dopamine effect.
[b] pEC_{50} is −log of the concentration giving 50% of the maximal dopamine effect.
[c] pIC_{50} is −log of the concentration inhibiting maximal effect of dopamine by 50%.
[d] $cpIC_{50}$ was calculated as indicated in Materials and methods.

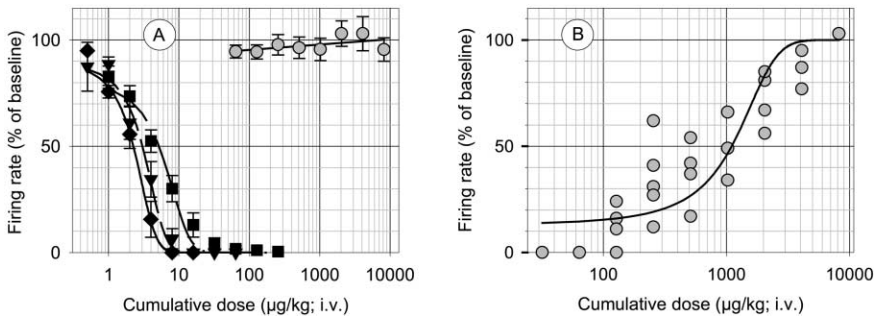

Fig. 2. The effects of dopamine D3 receptor-preferring agonists on the firing of substantia nigra dopaminergic neurons. (A) (±)-7-OH-DPAT (diamonds), PD 128907 (triangles), and quinpirole (square) inhibit the firing with ED_{50}s of 2.3, 3.0 and 5.2 μg/kg ($n = 3–13$ experiments/substance). In contrast, BP 897 in doses up to 8.2 mg/kg has no effect. However, in the same dose range, BP 897 ($n = 5$) antagonizes quinpirole-induced inhibition (inhibition not shown) of the firing of substantia nigra dopaminergic neurons with a DID_{50} of 1.1 mg/kg, as shown in (B).

nigra dopaminergic neurons ($n = 5$). If at all, there might be a slight trend to increase the firing rate at higher doses. The dopamine D3 receptor preferring partial agonist (±)-7-OH-DPAT as well as the full agonists PD 128907 and quinpirole were used as references. In contrast to BP 897, cumulative i.v. application of these compounds led to a significant decrease in firing of substantia nigra dopaminergic neurons (Fig. 2A). ED_{50} values for these three compounds were 2.3, 3.0 and 5.2 μg/kg body weight, respectively.

In the second set of experiments, BP 897 was used to antagonize the quinpirole-induced inhibition of firing of substantia nigra dopaminergic neurons (Fig. 2B). In five out of five experiments, the application of BP 897 lead to a disinhibition of firing. Disinhibition was always complete, indicated by the fact that the baseline-firing rate was reestablished in all experiments. The DID_{50} for this effect has been calculated with 1.10 mg/kg body weight.

4. Discussion

In the present study, we have characterized the pharmacological effects of BP 897 at human dopamine D3 receptors in stable transfected CHO cells and in an in vivo model of electrophysiological activity of the dopaminergic system. Our data clearly show that BP 897 acts as an antagonist at human dopamine D3 receptors expressed in CHO cells and also in vivo by inhibiting the firing of dopaminergic neurons of substantia nigra.

The potency and intrinsic activities of dopamine, quinpirole and PD 128907 at human dopamine D3 receptors ectopically expressed in CHO cells agree with previous studies (Seabrook et al., 1994; Malmberg et al., 1998; Newman-Tancredi et al., 1999; Vanhauwe et al., 1999). The potent dopamine D3 receptor-preferring ligand 7-OH-DPAT activated human dopamine D3 receptors as partial agonist, which supports the findings made in alternative heterologous systems (Malmberg et al., 1998; Newman-Tancredi et al., 1999; Vanhauwe et al., 1999). In our study, BP 897 was unable to increase [^{35}S]GTPγS binding in hD3/CHO cells, and potently inhibited the increase induced by dopamine. These findings are in contrast to previous data reporting BP 897 effects in a different cellular system. It has been shown that BP 897 acts as a partial agonist by inhibiting forskolin-induced cAMP accumulation and inducing mitogenesis in NG-108-15 cells transfected with human dopamine D3 receptors (Pilon et al., 1994; Pilla et al., 1999; Perachon et al., 2000). Discrepancy to the data reported here might be attributable to the different heterologous expression systems used in both studies. Activation of second messengers by receptors expressed in heterologous systems may vary depending on the particular cell line, receptor expression level or particular G-protein coupling (Newman-Tancredi et al., 1997). Actually, 7-OH-DPAT activated human dopamine D3 receptors in CHO cells as a potent partial agonist, which is in full agreement with previous studies (Malmberg et al., 1998; Newman-Tancredi et al., 1999; Vanhauwe et al., 1999). However, in hD3/NG-108-15 cells, 7-OH-DPAT induced mitogenesis almost as a full agonist (Pilon et al., 1994; Perachon et al., 2000). It has been demonstrated that intrinsic activity of 7-OH-DPAT at human dopamine D3 receptor was dependent on the expression levels (Vanhauwe et al., 1999). It is unlikely that differences between CHO and NG-108-15 is due to this factor because hD3/NG-108-15 cells used in previous studies display an even lower level of dopamine D3 receptor binding than CHO cell lines (Pilon et al., 1994). Data discrepancies between both cell types may be rather attributed to additional factors, such as effector mechanisms. Functional activity measured in the present study (GTPγS binding) targets a mechanism directly associated to ligand–receptor interaction; therefore, it is an appropriate in vitro assay to clearly differentiate dopamine D3 receptor-mediated activity as already suggested (Newman-Tancredi et al., 1999). In contrast, the mitogenesis assay used in NG-108-15 cells is an end-point event, downstream in different signaling pathways, and certainly encompasses other effects due to promiscuity of cellular signals and not only to dopamine

D3 receptor-mediated activity. BP 897 behaved as a clear and potent antagonist with a cIC_{50} value (0.39 nM) which is in full agreement with its reported K_i (0.92 nM) at human dopamine D3 receptor (Pilla et al., 1999). This conclusion is further strengthened by a very recent report showing antagonist effects of BP 897 in the acidification rate modulated by human dopamine D3 receptors (Wood et al., 2000). The antagonism of BP 897 observed in our in vitro assay is also confirmed by the in vivo pharmacological activity observed in our electrophysiological experiments in rats (see below).

In a second set of experiments, BP 897 was tested in anaesthetized rats for its effects on firing of dopaminergic neurons of the substantia nigra. No significant effect on the firing rate could be observed if BP 897 was given alone in cumulative doses up to 8.2 mg/kg body weight. We also observed this lack of any effect on firing rate with a number of mixed dopamine D3 receptor antagonists including haloperidol and clozapine (data not shown). In the same model, the application of the dopamine D3 receptor-preferring partial agonist (±)-7-OH-DPAT or the full agonists PD 128907 and quinpirole, clearly induced dose dependent inhibition of firing. The ED_{50}s for both compounds were similar to that reported for the dopaminergic neurons of the ventral tegmental area and the substantia nigra (Bowery et al., 1994, 1996; Lejeune and Millan, 1995). It has been shown that inhibition of substantia nigra dopaminergic cell firing correlates with the binding affinities of agonists at dopamine D3 receptors but not with their affinities to dopamine D2L receptor (Kreiss et al., 1995). Therefore, it is likely that the agonistic effects of (±)-7-OH-DPAT and PD 128907 observed in our model reflect dopamine D3 receptor activity. Studies using knockout mice have suggested that firing of dopaminergic neurons in this species is controlled by dopamine D2 but not by dopamine D3 receptors (Mercuri et al., 1997; Koeltzow et al., 1998). It remains open, whether this discrepancy is due to species-/strain-specific differences or to the appearance of compensatory mechanisms (Mercuri et al., 1997). On the other hand, BP 897 like numerous mixed dopamine D3 receptor antagonists including haloperidol (Kelland et al., 1989) and clozapine (Bowery et al., 1994) clearly antagonized the quinpirole-induced inhibition of firing rate of substantia nigra dopaminergic neurons in rats. Inhibition of nigral firing by the approximately 100-fold dopamine D3/D2 receptor selective compound S33084 further suggests an important function of dopamine D3 receptors in the control of firing of rat dopaminergic neurons (Millan et al., 2000). Nevertheless, by the doses used, a participation of dopamine D2 receptors in control of firing cannot be fully excluded. Highly dopamine D3 receptor selective compounds will help to clarify this controversial issue. The electrophysiological data obtained with potent dopamine D3 receptor agonists in vivo are in accordance with the effects observed in human dopamine D3 receptor transfected in CHO cells. The effects of BP 897 in models of drug dependence/craving are thought to be mediated by its partial agonism (Pilla et al., 1999). The results of the in vitro study of Wood et al. (2000) and our in vitro and in vivo findings make it improbable that partial agonistic activity at dopamine D3 receptors is the cause of the positive results of BP 897 in drug dependence/craving.

In conclusion, we demonstrated the antagonistic effects of BP 897 in an in vivo and in vitro system. This is in contrast to the previously reported partial agonistic activity at the human dopamine D3 receptor. The unique effects of BP 897 on drug dependence/craving may depend on other functions than the previously reported dopamine D3 receptor partial agonism.

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References

Bowery, B., Rothwell, L.A., Seabrook, G.R., 1994. Comparison between the pharmacology of dopamine receptors mediating the inhibition of cell firing in rat brain slices through the substantia nigra pars compacta and ventral tegmental area. Br. J. Pharmacol. 112, 873–880.

Bowery, B.J., Razzaque, Z., Emms, F., Patel, S., Freedman, S., Bristow, L., Kulagowski, J., Seabrook, G.R., 1996. Antagonism of the effects of (+)PD 128907 on midbrain dopamine neurons in rat brain slices by a selective D2 receptor antagonist L 741,626. Br. J. Pharmacol. 119, 1491–1497.

Bunney, B.S., Walters, J.R., Roth, R.H., Aghajanian, G.K., 1973. Dopaminergic neurons: effects of antipsychotic drugs and amphetamine on single cell activity. J. Pharmacol. Exp. Ther. 185, 560–571.

Caine, S.B., Koob, G.F., Parsons, L.H., Everitt, B.J., Schwartz, J.C., Sokoloff, P., 1997. D3 receptor test in vitro predicts decreased cocaine self-administration in rats. NeuroReport 8, 2373–2377.

Crocq, M.A., Mant, R., Asherson, P., Williams, J., Hode, Y., Mayerova, A., Collier, D., Lannfelt, L., Sokoloff, P., Schwartz, J.C., 1992. Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. J. Med. Genet. 29, 858–860.

Hilf, G., Jakobs, K.H., 1992. Agonist-independent inhibition of G protein activation by muscarinic acetylcholine receptor antagonists in cardiac membranes. Eur. J. Pharmacol. 225, 245–252.

Kelland, M.D., Freeman, A.S., Chiodo, L.A., 1989. (±)-3,4-Methylenedioxymethamphetamine-induced changes in the basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons. Eur. J. Pharmacol. 169, 11–21.

Koeltzow, T.E., Xu, M., Cooper, D.C., Hu, X.-T., Tonegawa, S., Wolf, M.E., White, F.J., 1998. Alterations in dopamine release but not

dopamine autoreceptor function in dopamine D3 receptor mutant mice. J. Neurosci. 18, 2231–2238.

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 D3 receptor binding affinities. Eur. J. Pharmacol. 277, 209–214.

Lejeune, F., Millan, M.J., 1995. Activation of dopamine D3 autoreceptors inhibits firing of ventral tegmental dopaminergic neurons in vivo. Eur. J. Pharmacol. 275, R7–R9.

Malmberg, A., Mikaels, A., Mohell, N., 1998. Agonist and inverse agonist activity at the dopamine D3 receptor measured by guanosine 5′ (gamma thio)triphosphate (S 35) binding. J. Pharmacol. Exp. Ther. 285, 119–126.

Mercuri, N.B., Saiardi, A., Bonci, A., Picetti, R., Calabresi, P., Bernardi, G., Borrelli, E., 1997. Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 79, 323–327.

Millan, M.J., Gobert, A., Newman-Tancredi, A., Lejeune, F., Cussac, D., Rivet, J.-M., Audinot, V., Dubuffet, T., Lavielle, G., 2000. S33084, a novel, potent, selective and competitive antagonist at dopamine D3 receptors: I. Receptorial, electrophysiological and neurochemical profile compared with GR218,231 and L741,626. J. Pharmacol. Exp. Ther. 293, 1048–1062.

Murray, A.M., Ryoo, H.L., Gurevich, E., Joyce, J.N., 1994. Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. Proc. Natl. Acad. Sci. U. S. A. 91, 11271–11275.

Newman-Tancredi, A., Conte, C., Chaput, L., Verriele, L., Millan, M.J., 1997. Agonist and inverse agonist efficacy at human recombinant serotonin $5HT_{1A}$ receptors as a function of receptor: G protein stochiometry. Neuropharmacology 36, 451–459.

Newman-Tancredi, A., Cussac, D., Audinot, V., Pasteau, V., Gavaudan, S., Millan, M.J., 1999. G protein activation by human dopamine D3 receptors in high-expressing Chinese hamster ovary cells: a guanosime-5′-*O*-(3-[^{35}S]thio)-triphosphate binding and antibody study. Mol. Pharmacol. 55, 564–574.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.

Perachon, S., Betancur, C., Pilon, C., Rostene, W., Schwartz, J.C., Sokoloff, P., 2000. Role of dopamine D3 receptors in thermoregulation: a reappraisal. NeuroReport 11, 221–225.

Pilla, M., Perachon, S., Sautel, F., Garrido, F., Mann, A., Wermuth, C.G., Schwartz, J.C., Everitt, B.J., Sokoloff, P., 1999. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 400, 371–375.

Pilon, C., Levesque, D., Dimitriadou, V., Griffon, N., Martres, M.P., Schwartz, J.C., Sokoloff, P., 1994. Functional coupling of the human dopamine D3 receptor in a transfected NG 108 15 neuroblastoma glioma hybrid cell line. Eur. J. Pharmacol., Mol. Pharmacol. Sect. 268, 129–139.

Seabrook, G.R., Kemp, J.A., Freedman, S.B., Patel, S., Sinclair, H.A., McAllister, G., 1994. Functional expression of human D3 dopamine receptors in differentiated neuroblastoma×glioma NG108-15 cells. Br. J. Pharmacol. 111, 391–393.

Shafer, R.A., Levant, B., 1998. The D3 dopamine receptor in cellular and organismal function. Psychopharmacology 135, 1–16.

Sokoloff, P., Giros, B., Matres, 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.

Sokoloff, P., Giros, B., Martres, M.P., Andrieux, M., Besancon, R., Pilon, C., Bouthenet, M.L., Souil, E., Schwartz, J.C., 1992a. Localization and function of the D3 dopamine receptor. Drug Res. 42, 224–230.

Sokoloff, P., Martres, M.-P., Giros, B., Bouthenet, M.-L., Schwartz, J.-C., 1992b. The third dopamine receptor (D3) as a novel target for antipsychotics. Biochem. Pharmacol. 43, 659–666.

Vanhauwe, J.F., Fraeyman, N., Francken, B.J., Luyten, W.H., Leysen, J.E., 1999. Comparison of the ligand binding and signaling properties of human dopamine D(2) and D(3) receptors in Chinese hamster ovary cells. J. Pharmacol. Exp. Ther. 290, 908–916.

Van Tol, H.H.M., Bunzow, J.R., Guan, H.-C., Sunahara, R.K., Seeman, P., Niznik, H.B., Civelli, O., 1991. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350, 610–614.

Wood, M.D., Boyfield, I., Nash, D.J., Jewitt, F.R., Avenell, K.Y., Riley, G.J., 2000. Evidence for antagonistic activity of the dopamine D3 receptor partial agonist, BP 897, at human dopamine D3 receptor. Eur. J. Pharmacol. 407, 47–51.

Zaworski, P.G., Alberts, G.L., Pregenzer, J.F., Im, W.B., Slightom, J.L., Gill, G.S., 1999. Efficient functional coupling of the human D3 dopamine receptor to G(o) subtype of G proteins in SH-SY5Y cells. Br. J. Pharmacol. 128, 1181–1188.