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Evidence for antagonist activity of the dopamine D3 receptor partial agonist, BP 897, at human dopamine D3 receptor

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Abstract

The dopaminergic system has long been implicated in the mechanisms of reward and addiction. 1-(4-(2-Naphthoylamino)butyl)-4-(2-methoxyphenyl)-1A-piperazine HCl (BP 897) has been claimed to be a selective dopamine D3 receptor partial agonist and has recently been shown to inhibit cocaine-seeking behaviour, suggesting a role for dopamine D3 receptor agonists in the treatment of addiction. We have previously characterised the pharmacological profile of the human dopamine D3 and D2(long) receptors using microphysiometry and radioligand binding and we have now studied the interaction of BP 897 with the dopamine D2 and D3 receptors using these methods. At both human dopamine D3 and D2 receptors, BP 897 lacked agonist activity but was a potent and selective antagonist with pK_b values of 8.05 ± 0.16 (4) and 9.43 ± 0.22 (4) at human dopamine D2 and D3 receptors, respectively. These results, therefore, suggest that it may be the dopamine D3 receptor antagonist properties of BP 897 which have potential in the treatment of addiction and withdrawal.

Keywords: Dopamine; D3 receptor; BP 897; Microphysiometry; Radioligand binding

1. Introduction

There is a wide body of evidence suggesting that changes in dopaminergic neurotransmission play an important role in mediating the locomotor, discriminative stimulus and reinforcing effects of cocaine (for review, see Amalric and Koob, 1993). It is hypothesised that cocaine produces its reinforcing effects by inhibiting dopamine reuptake and thereby potentiating dopaminergic neurotransmission, particularly within the mesocorticolimbic dopamine system. Although it is not known which dopamine receptors are involved in mediating the reinforcing effects of cocaine, there is much evidence implicating an involvement of the dopamine D3 receptor. Thus, although the dopamine D3 receptor has a discrete distribution in the brain, it is present within the projection areas of the mesolimbic dopamine system (Sokoloff et al., 1990), a brain area thought to be involved in the action of cocaine (Amalric and Koob, 1993). Further, pharmacological studies have suggested that the dopamine D3 receptor subtype mediates the reinforcing effects of cocaine in animal models (Caine and Koob, 1993; Caine et al., 1997). Thus, for a series of dopamine agonists including quinpirole, quinelorane and 7-hydroxy-dipropylaminotetralin (7-OH-DPAT), there was a good correlation between their dopamine D3 agonist potency and their potency to decrease cocaine self-administration in rats, suggesting that these agonists mimic or substitute the effects of cocaine. Recently, BP 897 has been claimed as the first dopamine D3-receptor selective agonist, but displays partial agonist activity in an in vitro mitogenesis model in cell lines (Pilla et al., 1999). Further, it was shown that this compound inhibits cocaine-seeking behaviour but lacks any intrinsic reinforcing properties (Pilla et al., 1999), suggesting a moderate degree of dopamine D3 receptor stimulation may be involved in this drug-seeking response and that BP 897 may display antagonist properties.

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We have previously characterised a functional model of the human D3 receptor using the Cytosensor microphysiometer which measures changes in extracellular acidification rates as a determinant of changes in cellular metabolic activity (see Smart and Wood, 2000). As such it is independent of the signal transduction pathway employed by the receptor and this has enabled us to use the same system to profile human dopamine D2, D3 and D4 receptors, which couple to different signal transduction pathways, where we have characterised the interaction of a series of agonists, including dopamine and antagonists (Coldwell et al., 1999a,b). These studies confirmed that quinpirole, quinelorane and 7-OH-DPAT are full agonists and that they show some selectivity for the dopamine D3 receptor in functional studies. We also showed with 1*S*,2*R*,3,4-tetrahydro-5-methoxy-1-methyl-*N*,*N*-di-*n*-propyl-2-naphthalenamine hydrochloride (UH232) that the microphysiometer could identify and characterise partial agonists (Coldwell et al., 1999a). In view of the claimed partial agonist activity of BP 897, we have examined the selectivity and efficacy profile of this compound at the human dopamine D2 and D3 receptors using microphysiometry and radioligand binding. In order to further this study of partial dopamine D3 receptor agonists on the microphysiometer, we included 6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepin-2-amine, dihydrochloride (BHT 920) as it was also claimed to be a partial agonist in the mitogenesis model with similar efficacy (68%; Pilon et al., 1994) to BP 897 (53%; Pilla et al., 1999).

2. Materials and methods

2.1. Cells and materials

Cloned human dopamine D2(long) (Garvan Institute of Medical Research, Sydney) and D3 (INSERM, Paris) receptors were expressed in Chinese hamster ovary-K1 cells. Dopamine D2 Chinese hamster ovary cells were grown in 50:50 Dulbecco's modified Eagle's medium (DMEM; without sodium pyruvate, with glucose):Ham's F-12 containing 10% fetal bovine serum. The dopamine D3 cells were grown in DMEM as above containing 10% fetal bovine serum, 100 nM methotrexate, 2 mM glutamine, 500 nM (−)-sulpiride and 1% (v:v) essential amino acids. Cells were grown to confluence, harvested by scraping in fresh medium and grown at 37°C in 95%O_2/5% CO_2. All cell culture materials were from Gibco, Life Science UK. 6-Allyl-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepin-2-amine, dihydrochloride (BHT 920) was purchased from RBI (Sigma) and 1-(4-(2-naphthoylamino)butyl)-4-(2-methoxyphenyl)piperazine, monohydrochloride (BP 897) was synthesised in the Department of Discovery Chemistry, SmithKline Beecham (Harlow, UK). [^{125}I]Iodosulpride (2000 Ci/mmol) was from Amersham UK; quinpirole was obtained from RBI; YM-O9151 was obtained from Yamanouchi (Oxford, UK) all other chemicals were from Sigma (Poole, UK).

2.2. Radioligand binding studies

Radioligand binding assays were carried out on well washed membranes from Chinese hamster ovary cells stably expressing either human dopamine D2 or D3 receptors. Membranes (5–15 μg protein) were incubated with [^{125}I]iodosulpride (0.1 nM) in the presence and absence of competing ligands for 40 min at 37°C in a buffer (pH 7.4) containing (in mM): Tris, 50; NaCl, 120; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1. Non-specific binding was defined using 10 μM YM-09151.

2.3. Microphysiometry

Changes in extracellular acidification rates were determined using the Cytosensor microphysiometer (Molecular Devices, Sunnyvale, CA, USA) as described in detail previously (Coldwell et al., 1999a). Cells were seeded into transwell cups at a density of 300,000 cells per well and incubated for 6 h in fetal bovine serum-containing media. The cells were then incubated overnight in fetal bovine serum- and sulpiride-free medium and loaded into Cytosensor chambers. Cells were perfused with bicarbonate-free DMEM containing 2 mM glutamine and 44 mM NaCl at a flow rate of 100 μl min^{-1} at 37°C with a cycle of 60 s pump on followed by 30 s pump off. The acidification rate was determined between 68 and 88 s during the pump-off phase. Compounds were dissolved in dimethyl sulphoxide and diluted in low-buffered DMEM. Concentration–effect curves were obtained by exposing the cells sequentially to increasing concentrations of agonist for a 6-min period with 15 washout before a further challenge. The response was taken as the peak increase in acidification rate upon addition of agonist over basal taken immediately prior to agonist challenge. Data was normalised as a mean response to a maximal concentration (100%) of quinpirole (10 μM) carried out at the start and end of the agonist concentration–effect curve. For antagonist studies, a control concentration–response curve to quinpirole was conducted and the cells were then exposed to the antagonist for at least 42 min prior to construction of a further quinpirole concentration–effect curve. Each chamber therefore acted as its own control. Drug additions were performed using the Cytosampler autosampler (Molecular Devices) from deep well blocks.

2.4. Data analysis

In radioligand binding studies, IC_{50} values were converted to pK_i using according to Cheng and Prusoff (1973). Experiments were repeated and data expressed as the mean ± standard error of the mean (S.E.M.).

In microphysiometry studies, concentration–effect curves were and analysed using a four-parameter logistic equation to give EC_{50} (IC_{50} in inhibition studies), slope, minimum and maximum (Bowen and Jerman, 1995). The EC_{50} values were then expressed as pEC_{50} ($-\log_{10}(EC_{50})$). Antagonist data were analysed as the ability of the antagonist to shift the agonist concentration–effect curve and defined as K_b[antagonist] · M/concentration ratio − 1, where concentration ratio is the EC_{50} obtained in the presence of the antagonist divided by that obtained in the absence of the antagonist (Arunlakshana and Schild, 1959). Data were expressed as pK_b ($-\log_{10}(K_b)$).

3. Results

3.1. Radioligand binding studies

BP 897 displayed pK_i of 7.22 ± 0.05 (6) and 8.80 ± 0.07 (3) at human dopamine D2 and D3 receptors, respectively. BHT 920 was somewhat weaker with pK_i of 5.97 ± 0.03 (3) and 6.63 ± 0.07 (3) at human dopamine D2 and D3 receptors, respectively.

3.2. Agonist studies

BP 897 was devoid of any significant agonist activity at either human dopamine D2 or D3 receptors (Fig. 1). BP 897 therefore had an intrinsic activity value, compared to the presumed full agonist, quinpirole, of less than 0.1 at both receptors. In contrast, BHT 920 was a full agonist (Fig. 2) at both receptors with pEC_{50} 7.93 ± 0.25 (5) and 8.15 ± 0.05 (4) at dopamine D2 and D3, respectively. The maximal response (expressed as a percentage of the maximal fitted quinpirole response taken as 100%) for BHT

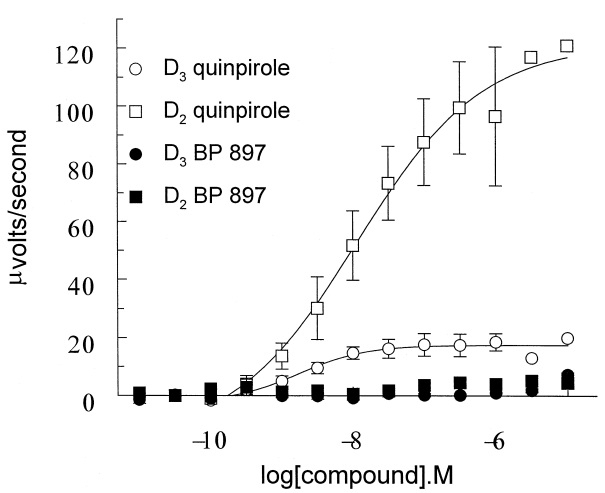

Fig. 1. Concentration–effect curves of quinpirole and BP 897 at human dopamine D2 and D3 receptors in Chinese hamster ovary cells using microphysiometry. Cells were stimulated by increasing the concentrations of either quinpirole or BP 897. Results are the acidification responses shown as µV/s from three to four separate experiments with data as the mean ± S.E.M.

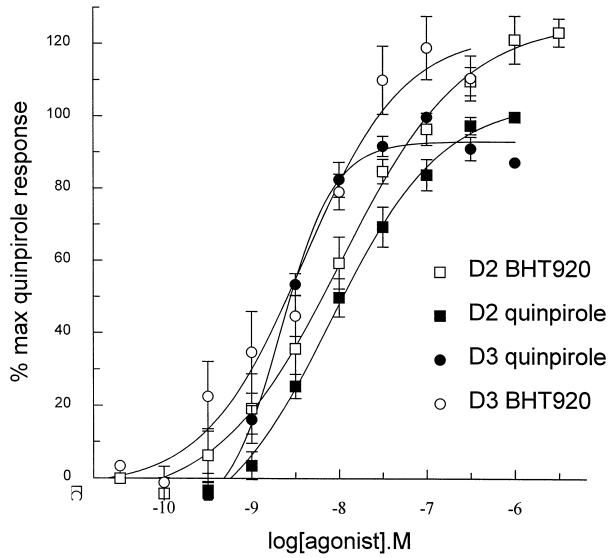

Fig. 2. Concentration–effect curves of quinpirole and BHT 920 at human dopamine D2 and D3 receptors in Chinese hamster ovary cells using microphysiometry. Cells were stimulated by increasing concentrations of either quinpirole or BHT 920. Results are the acidification responses shown as µV/s from three to four separate experiments with data as the mean ± S.E.M.

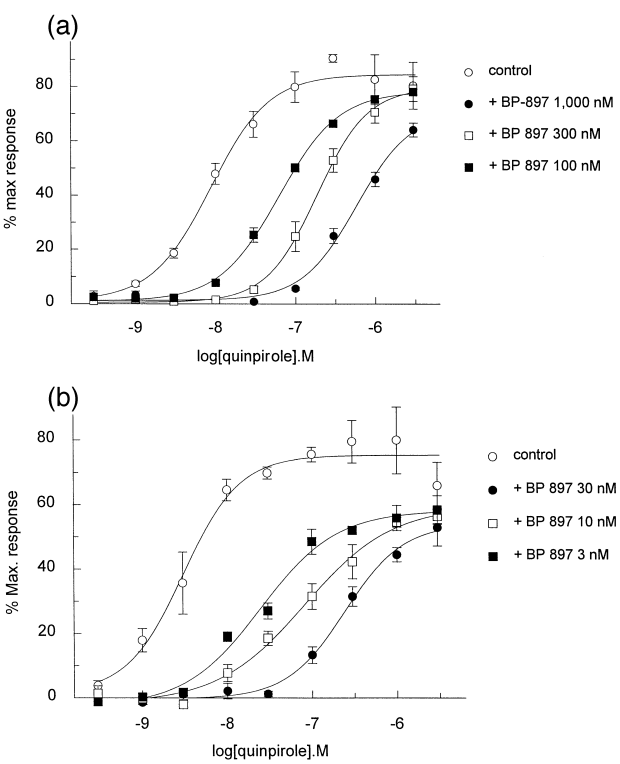

Fig. 3. Antagonism of functional responses at the human dopamine D2 receptor (a) and at the human dopamine D3 receptor (b) in Chinese hamster ovary cells by BP 897. Cells were stimulated with increasing concentrations of quinpirole in the presence and absence of varying concentrations of BP 897 and their acidification rate determined. The results are expressed as percent of the maximal response to quinpirole and are the mean ± S.E.M. from four separate experiments.

920 at the dopamine D2 and D3 receptors was 118% ± 8.7 and 117% ± 8.5 (not significantly different from quinpirole maximum, *F*-test), respectively and the slope factors were 0.94 ± 0.13 and 0.89 ± 0.11, respectively.

3.3. Antagonist studies

BP 897 was evaluated for antagonist activity at human dopamine D2 and D3 receptors. At both receptors, BP 897 shifted the quinpirole concentration–effect curve to the right in a parallel manner (Fig. 3). The calculated pK_b values were 8.05 ± 0.16 (4) and 9.43 ± 0.22 (4) at dopamine D2 and D3 receptors, respectively. BP 897 did reduce the maximal response to quinpirole at the dopamine D3 receptor (Fig. 3b) but this effect was not related to the concentration of BP 897 and Schild analysis indicated a slope not significantly different from unity.

4. Discussion

BHT 920 and BP 897 are both claimed to be partial agonists with similar efficacy at the dopamine D3 receptor (Pilla et al., 1999). In this study, BHT 920 was found to be a full agonist using real time microphysiometry. The large difference between its binding affinity and its functional potency suggests the presence of a large receptor reserve in the clonal cell line. It was therefore surprising to find that, using microphysiometry, BP 897 lacked agonist activity but instead appeared to be a selective dopamine D3 receptor antagonist. Although BP 897 did appear to depress the maximal response to quinpirole, this effect was not concentration related and Schild analysis gave a slope not significantly different from unity, suggesting that BP 897 is a competitive antagonist at the human dopamine D3 receptor. Further, a time-related decrease in the maximal response to quinpirole is often seen in this assay as the cells are maintained for a long time (6–8 h) to obtain before and after treatment curves.

Different results in the intrinsic activity of partial agonists can be obtained between different assay systems. Thus, there may be differences in the level of receptor reserve and in the efficiency of functional coupling which may change the observed efficacy of partial agonists between tissues. BHT 920 was identified as a partial agonist in a mitogenesis model at the dopamine D3 receptor expressed in NG 108 cells with a similar intrinsic activity to that of BP 897: maximal response 68% for BHT 920 (Pilon et al., 1994), 55% for BP 897 (Pilla et al., 1999). It is therefore unlikely that any changes in receptor reserve or functional coupling could explain why BHT 920 appears to be a full agonist and BP 897 appears as a full antagonist in microphysiometry. Indeed, the large increase in functional potency compared to binding affinity for BHT 920 suggests the presence of a large receptor reserve for both the dopamine D2 and D3 expression systems, suggesting that most partial agonists should display marked agonist activity.

Differences in agonist efficacy and potency between assay systems may also reflect different coupling mechanisms or species differences in the receptor. Greater agonist-induced responses for the dopamine D3 receptor, when expressed in human neuroblastoma cells (SH-SY5Y) as compared to human embryonic kidney cells (HEK 293) have been seen (Zaworski et al., 1999). Also, the D3 receptor has been shown to couple to Gi, Gs and Gz (Obadiah et al., 1999), suggesting different receptor-response coupling mechanism can occur. Differences in the interaction of partial agonists with receptors from different species may occur, but it should be noted that although the behavioural effects of BP 897 were studied in rodents, the functional effects of BP 897 at the dopamine D3 receptor were studied on the human orthologue (Pilla et al., 1999).

There are distinct differences between the microphysiometry and mitogenesis systems. The mitogenesis assay involves a 16- to 18-h incubation in the presence of compound, followed by 2-h incubation with [^{3}H]thymidine. The microphysiometer assay involves the perfusion of cells with the agonist for 6 min and the peak response is seen within this period, so that measurements are carried out in real time. The mitogenesis assay requires a sustained activation which may be complicated by desensitisation processes and stability of the agonist. Further, the repertoire of effectors and G proteins in the NG 108 cells may be more extensive than in Chinese hamster ovary cells (see Pilon et al., 1994). Although this may aid functional coupling of expressed receptors, it may complicate interpretation using non-selective pharmacological agents. To this point, BHT 920 is a known agonist at α_2-adrenoceptors (Gessi et al., 1999) and the presence of these receptors in NG 108 cells has been described (Holmberg et al., 1998). It is therefore possible that the response observed in the microphysiometer is a direct consequence of activation of the dopamine D3 receptor, whereas the response seen in the mitogenesis assay is an adaptation of the cell to continued receptor activation and may be complicated by the presence of endogenous receptors and regulators. It would be useful to study the interaction of BHT 920 and BP 893 in the mitogenesis model using shorter incubation times and to investigate the effect of an alkylating agent to reduce receptor reserve.

The present results therefore suggest that the primary effect of BP 897 at human dopamine D3 receptors is as an antagonist. These results raise the possibility that it may be the antagonist properties of BP 897 which mediate its inhibition of cocaine-seeking behaviour and further suggest that dopamine D3 receptor antagonists may have therapeutic utility in cocaine withdrawal. The determination of the profile of BP 897 at the human dopamine D3 receptor awaits its examination in functional studies in native tissue from man.

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References

Amalric, M., Koob, G.F., 1993. Functionally selective neurochemical afferents and efferents of the mesocorticolimbic and nigrostriatal dopamine system. In: Arbuthnott, G.W., Emson, P.C. (Eds.), Progress in Brain Research 99 Elsevier, New York, pp. 209–226.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. Chemother. 14, 48–58.

Bowen, W.P., Jerman, J.C., 1995. Nonlinear regression using spreadsheets. Trends Pharmacol. Sci. 16, 413–417.

Caine, S.B., Koob, G.F., 1993. Modulation of cocaine self-administration in the rat through D3 dopamine receptors. Science 260, 1814–1816.

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.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (Ki) and the concentration of an inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Coldwell, M.C., Boyfield, I., Brown, A.M., Stemp, G., Middlemiss, D.N., 1999a. Pharmacological characterisation of extracellular acidification rate responses in human D2(long), D3 and D4.4 receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 127, 1135–1144.

Coldwell, M.C., Boyfield, I., Brown, A.M., Hagan, J.J., Middlemiss, D.N., 1999b. Comparison of the functional potencies of ropinirole and other dopamine receptor agonists at human D2(long), D3 and D4.4 receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 127, 1696–1702.

Gessi, S., Campi, F., Varani, K., Borea, P.A., 1999. Alpha2-adrenergic agonist modulation of [^{35}S]-GTP gamma S binding to guanine-nucleotide-binding proteins in human platelet membranes. Life Sci. 64, 1403–1413.

Holmberg, C.I., Kukkonen, J.P., Bischoff, A., Nasman, J., Courtney, M.J., Miche, M.C., Akerman, K.E.O., 1998. Alpha(2B)-adrenoceptors couple to Ca^{2+} increase in both endogenous and recombinant expression systems. Eur. J. Pharmacol. 363, 65–74.

Obadiah, J., Avidor-Reiss, T., Fishburn, C.S., Carmon, S., Bayewitch, M., Vogel, Z., Fuchs, S., Levavi-Sivan, B., 1999. Adenylyl-cyclase interaction with the D2 dopamine receptor family: differential coupling to Gi, Gz and Gs. Cell. Mol. Neurobiol. 19, 653–664.

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

Pilon, C., Levesque, D., Dimitradou, 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 neuroblastome-glioma hybrid cell line. Eur. J. Pharmacol. 268, 129–139.

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

Smart, D., Wood, M.D., 2000. Cytosensor techniques for examining signal transduction of neurohormones. Biochem. Cell Biol. 78, 281–288.

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