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The agonist activities of the putative antipsychotic agents, L-745,870 and U-101958 in HEK293 cells expressing the human dopamine $D_{4.4}$ receptor

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1 Dopamine D_4 receptor antagonists are being developed by several pharmaceutical companies as putative novel antipsychotics, possibly with low propensity to side-effects. Two such compounds, L-745,870 and U-101958 have been recently introduced.

2 The radioligand binding and functional activities of L-745,870 and U-101958 were investigated in human embryonic kidney (HEK)293 cells expressing the human recombinant dopamine $D_{4.4}$ receptor (HEK293/D_4 cells). [^{3}H]-spiperone binding experiments were performed and inhibition of forskolin-stimulated cyclic AMP accumulation was used as the functional response.

3 [^{3}H]-spiperone was found to label a homogeneous and saturable population of specific binding sites in HEK293/D_4 cell homogenates (B_{max} 505 ± 90 fmol mg^{-1} protein, pK_D 9.5 ± 0.1, $n=3$). Inhibition of specific [^{3}H]-spiperone binding was observed with spiperone (pK_i 9.6 ± 0.1, $n=3$), clozapine (pK_i 7.4 ± 0.1, $n=4$), L-745,870 (pK_i 8.5 ± 0.1, $n=3$) and U-101958 (pK_i 8.9 ± 0.1, $n=3$). By contrast, raclopride was very weak ($pK_i < 5$, $n=3$).

4 Dopamine inhibited forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells in a concentration-dependent fashion (E_{max} $71 \pm 2\%$ inhibition of forskolin-stimulated levels, pEC_{50} 8.7 ± 0.1, $n=10$). This effect was mimicked by the dopamine D_2-like receptor agonists, quinpirole and 7-hydroxy-2-dipropylaminotetralin (7-OH-DPAT).

5 L-745,870 and U-101958 also inhibited forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells in a concentration-dependent way. L-745,870 was less efficacious than dopamine (71% the efficacy of dopamine), whereas U-101958 behaved as a full agonist compared to dopamine. Potencies (pEC_{50}) values of L-745,870 and U-101958 were 9.0 ± 0.2 ($n=4$) and 8.7 ± 0.3 ($n=3$), consistent with pK_i values determined in radioligand binding studies.

6 Dopamine, L-745,870 and U-101958 (up to 1 μM) were devoid of effect on forskolin-stimulated cyclic AMP accumulation in control, non-transfected HEK293 cells.

7 The agonist effects of dopamine, L-745,870 and U-101958 in HEK293/D_4 cells could be antagonized by spiperone (pK_B 8.2–8.8) and clozapine (pK_B 7.1), but not by raclopride ($pK_B < 5$). None of these antagonists had any significant agonist activity at concentrations up to 10 μM.

8 These results show that the putative dopamine D_4 receptor antagonists, L-745,870 and U-101958 are not devoid of intrinsic activity at human recombinant dopamine $D_{4.4}$ receptors. Therefore, they may not represent the most appropriate drugs for testing the benefit of D_4 receptor antagonism in schizophrenic patients, if agonism should translate *in vivo*.

Keywords: L-745,870; U-101958; dopamine D_4 receptor; cyclic AMP inhibition; [^{3}H]-spiperone binding; schizophrenia

Introduction

The endogenous catecholamine, dopamine is involved in important brain functions, such as the control of locomotion, emotion and in the regulation of neuroendocrine and cognitive processes. Dopamine receptors have long been considered therapeutic targets in the treatment of neurological and psychoaffective disorders, like schizophrenia, Alzheimer's, Huntington's or Parkinson's diseases (Seeman, 1987; Seeman & Niznik, 1990). They have originally been divided into two subtypes, called D_1 and D_2 (Kebabian & Calne, 1979). More recent work using molecular biology techniques has led to the discovery of five distinct dopamine receptors, transcribed from five distinct genes. These receptors can be classified into two families, the D_1-like and the D_2-like families, on the basis of their primary structures, operational or pharmacological properties and second messenger coupling (for a review, see Hartmann & Civelli, 1997). Thus, the D_1-like family consists of the original D_1 and of the D_5 receptors that coupled to adenylate cyclase and formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in a positive way. On the other hand, D_2, D_3 and D_4 receptors constitute the D_2-like family and are negatively linked to cyclic AMP accumulation.

The D_2-like family is of particular interest in psychiatric diseases because neuroleptics are potent dopamine D_2 receptor antagonists. Furthermore, plasma concentrations of clinically used neuroleptics have been shown to correlate with the drug affinities for D_2 receptors (Seeman, 1992). However, a major drawback of most neuroleptics resides in the fact that they produce a number of side effects, presumably related to their D_2 receptor antagonist activity in the nigrostriatal pathway (extrapyramidal side effects, e.g., dyskinesia, tremor, etc...) or in the pituitary (hyperprolactinaemia). In this context, the

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cloning and expression of the dopamine D_4 receptor raised much interest because clozapine, an atypical neuroleptic that does not induce extrapyramidal side effects, has significant affinity for D_4 receptors and some selectivity over D_2 receptors (Van Tol *et al*., 1991). In addition, dopamine D_4 receptor mRNA is expressed in the mesolimbic system and related brain areas thought to be involved in the regulation of emotion and cognition (Matsumoto *et al*., 1996; Meador-Woodruff *et al*., 1996). Low levels of D_4 receptor mRNA have been detected in brain regions associated with the control of locomotor activity, such as striatum (Matsumoto *et al*., 1996; Meador-Woodruff *et al*., 1996). Recent autoradiographic studies, using the novel radioligand [^{3}H]-NGD 94-1, indicate that the *in situ* hybridization results compare well with the binding site distribution (Primus *et al*., 1997). Finally, an up-regulation of dopamine D_4 receptors has been reported in *postmortem* brain tissues from schizophrenics as compared to controls (Seeman *et al*., 1993; Murray *et al*., 1995; Sumiyoshi *et al*., 1995), although this has been a matter of controversy (see Reynolds & Mason, 1995; Reynolds, 1996).

All these findings strongly suggest that a selective dopamine D_4 receptor antagonist may represent a novel, attractive therapy for schizophrenia with a low propensity to trigger the side effects encountered with conventional drugs. The development of a selective dopamine D_4 receptor antagonist has therefore become the goal of many pharmaceutical companies. Several dopamine D_4 receptor selective ligands have recently been introduced (see review by Hartmann & Civelli, 1997). In the present work, we have characterized the functional activities of two such compounds, L-745,870 (3-[[4-(4-chlorophenyl)piperazin-1-yl]-methyl]-1*H*-pyrrolo[2,3-*b*]pyridine; Kulagowski *et al*., 1996) and U-101958 ((1-benzyl-piperidin-4-yl)-(3-isopropoxy-pyridin-2-yl)-methyl-amine; Schlachter *et al*., 1997) at human recombinant dopamine D_4 receptors stably expressed in human embryonic kidney (HEK)293 cells. L-745,870 is of particular relevance, since this compound has already been tested in the clinic (Bristow *et al*., 1997). By determination of the inhibition of forskolin-stimulated cyclic AMP accumulation as a functional response to dopamine D_4 receptor stimulation, it is shown in the following that both L-745,870 and U-101958 can exhibit agonist properties at human recombinant dopamine D_4 receptors, contrary to spiperone and clozapine. The results question the value of using these two compounds for testing the benefit of dopamine D_4 receptor antagonism in schizophrenia.

A preliminary account of this work has been presented to the British Pharmacological Society (Gazi *et al*., 1998).

Methods

Cloning of a human D_4 receptor cDNA

A human retinal cDNA library (10^6 plaques) constructed in the bacteriophage λgt10 was screened for human dopamine D_4 receptor-encoding cDNA using two 5′-end ^{32}P-labelled oligonucleotides (specific activity 10^6 c.p.m. pmol^{-1}). These oligonucleotides, D4.1, 5′-GCCATGGGGAACCGCCAG-CACC-3′ and D4.3, 5′-TAACGTACAAAAGCGCCCTCC-3′ correspond to sequences encoding the N-terminus of the D_4 receptor and to untranslated sequences of the D_4 receptor cDNA immediately 3′ of the stop codon, respectively. Phage plaques hybridizing with one of the two oligonucleotides were isolated and inserts were subcloned into Bluescript SK(−) (no phage hybridizing with both probes was detected). Following verification by Southern blotting, one clone (1300 base pairs) was sequenced and confirmed as encoding the human dopamine $D_{4.4}$ receptor (Van Tol *et al*., 1992), but lacked 102 base pairs of the 5′-coding sequence including the initiation codon. This sequence was obtained by polymerase chain reaction (Mullis & Faloona, 1987) from genomic DNA in the presence of 10% dimethyl sulphoxide. Oligonucleotides 5′-GCAAGCTTGTCCGCGGTGCTCAG-3′, representing the 5′ untranslated sequence and 5′-GGCGCACAGGTTGAA-GATGG-3′, complementary to the sequence encoding amino acid residues IFNLCA of the human D_4 receptor, served as primers. The conditions of the polymerase chain reaction were 50 cycles of 93°C for 30 s, 55°C for 30 s, 65°C for 3 min. The obtained 410 base pair fragment was digested with HindIII (5′ oligonucleotide borne restriction site) and NotI (endogenous restriction site) and ligated to the partial D_4 receptor cDNA at the common NotI site.

Production of cell line

The full length cDNA was subsequently subcloned into a mammalian expression vector with the CMV promoter (Gorman *et al*., 1990). This expression construct was cotransfected with the pRSVNeo plasmid (in a 9:1 ratio) into HEK293 cells by the calcium phosphate precipitation method of Chen and Okayama (1987). Selection for stable integration was performed by adding 0.8 mg ml^{-1} of geneticin to the culture medium (minimum essential medium (with Earle's salts) supplemented with 10% foetal calf serum, 100 iu ml^{-1} penicillin, 100 μg ml^{-1} streptomycin).

Cell culture

HEK293/D_4 receptors were propagated in minimum essential medium (with Earle's salts) supplemented with 10% foetal calf serum, 100 iu ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 0.8 mg ml^{-1} geneticin. Cells were grown in a humidified atmosphere at 37°C in the presence of 5% CO_2. They were split twice a week using a trypsin/EDTA solution. For radioligand binding experiments, cells were grown in 24.5 cm squared ('bio-assay') dishes. For cyclic AMP measurements, cells were seeded at a density of $2-3\times10^5$ cells/well in collagen-coated 24-well plates.

Radioligand binding assay

HEK293/D_4 cells grown to confluence in bio-assay dishes were collected by scraping in 50 mM HEPES buffer, pH 7.4, containing 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1% (w/v) bovine serum albumin, 0.025% (w/v) bacitracin and 0.025% (w/v) sodium azide. They were then centrifuged at 1200 r.p.m. for 10 min at 4°C. After the removal of the supernatant, the cells were frozen at −70°C until the day of the experiment. For each binding assay experiment, the cells were resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM EDTA, 1.5 mM $MgCl_2$ and 5 mM KCl, using a Polytron tissue homogenizer at setting 3–4 for 15 s. One hundred and fifty μl of the cell suspension (corresponding to ~250,000 cells/assay) were added to 96-well microtitre plates containing 50 μl drug and 50 μl (~40,000 c.p.m./assay) of [^{3}H]-spiperone (110 Ci mmol^{-1}, Amersham, Rahn AG, Zürich, Switzerland). The plates were incubated at room temperature for 120 min, rapidly filtered through Packard Unifilter-96, GF/C plates and washed 3 times with 300 μl ice-cold 10 mM Tris-HCl buffer containing 154 mM NaCl, pH 7.5. Filter-bound radioactivity was counted in 40 μl Micro-

scint 40 in a Packard TopCount scintillation counter. Non-specific binding was defined in the presence of 10 μM spiperone. Saturation experiments were performed using 8 concentrations of the radioligand, ranging from approximately 0.4 to 25 nM. Assays were performed in triplicate and the determination was replicated 3 times.

Measurement of cyclic AMP accumulation

Cells grown to confluence in 24-well plates were washed with 0.5 ml of HEPES-buffered salt solution (in mM: NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 0.8, NaH_2PO_4 0.9, glucose 25, HEPES 20, pH 7.4, containing phenol red 5 mg l^{-1}) and incubated with 6 μCi ml^{-1} of [8-^{3}H]-adenine (23 Ci $mmol^{-1}$, Anawa Trading SA, Wangen, Switzerland) at 37°C for two hours in 0.5 ml of the same buffer. They were then washed twice with 0.5 ml of the buffer solution supplemented with 1 mM isobutylmethylxanthine. The cells were incubated in 1 ml of the same solution at 37°C, in the presence and absence of forskolin (10 μM) and of test compounds at the indicated concentrations. Experiments were conducted in duplicate. After 15 min, the medium was removed and replaced by 1 ml of 5% trichloroacetic acid solution containing cyclic AMP and ATP (both 0.1 mM). After 30 min at 4°C, the trichloroacetic acid extracts were directly subjected to sequential chromatography on Dowex AG 50W-X4 and alumina columns (Salomon, 1991). Cyclic AMP accumulation was calculated as the ratio [^{3}H]-cyclic AMP/([^{3}H]-cyclic AMP + [^{3}H]-ATP). The recovery of cyclic AMP, as measured in separate experiments using a [^{3}H]-cyclic AMP standard, was 76 ± 1% (n = 5).

Analysis of data

Cyclic AMP data were expressed as percentage of forskolin-stimulated cyclic AMP accumulation. Concentration-dependent inhibition of binding and concentration-response curves for agonists in the cyclic AMP studies were analysed using SCTFIT, a non linear regression computerised program (DeLean *et al*., 1980). Values of K_i, E_{max} (maximal effect) and EC_{50} (concentration producing half the maximal effect) were derived from this analysis. The apparent pK_B values of antagonists were calculated according to the formula: $pK_B = \log[B] - \log(CR-1)$ where [B] is the concentration of the antagonist used and CR (concentration-ratio) is the ratio of agonist EC_{50} measured in the presence of antagonist over that measured in the absence of antagonist. Results are given as mean ± s.e.mean of the indicated n values.

Drugs and biochemicals

The substances were obtained from the following sources: forskolin, isobutylmethylxanthine and dopamine (Sigma, Fluka, Buchs, Switzerland); quinpirole hydrochloride (Research Biochemicals International, Rahn, Zürich, Switzerland); 7-hydroxy-2-dipropylaminotetralin hydrobromide (Tocris, Bristol, U.K.); raclopride tartrate (a gift from Astra, Sweden). L-745,870 (3-[[4-(4-chlorophenyl)piperazin-1-yl]-methyl]-1*H*-pyrrolo[2,3-*b*]pyridine; Dr R. Swoboda), U-101958 ((1-benzyl-piperidin-4-yl)-(3-isopropoxy-pyridin-2-yl)-methyl-amine; Dr R. Swoboda), spiperone and clozapine were from Novartis Pharma (Basel, Switzerland). Except for forskolin (10 mM stock solution in ethanol), the substances were prepared daily at 40 mM, either in distilled water or in a mixture of 1-methyl-2-pyrrolidone:ethanol (1:1) containing 20 mg ml^{-1} ascorbic acid, and further diluted with water.

Results

[^{3}H]-spiperone binding

In saturation binding experiments, [^{3}H]-spiperone was found to label a homogeneous and saturable population of specific binding sites in HEK293/D_4 cell homogenates. The B_{max} value amounted to 505 ± 90 fmol mg^{-1} protein and the pK_D value was 9.5 ± 0.1 (n = 3). The specific binding of [^{3}H]-spiperone was inhibited in a monophasic manner by spiperone (pK_i 9.6 ± 0.1, n = 3), clozapine (pK_i 7.4 ± 0.1, n = 4), L-745,870 (pK_i 8.5 ± 0.1, n = 3) and U-101958 (pK_i 8.9 ± 0.1, n = 3). By contrast, raclopride was very weak ($pK_i < 5$, n = 3).

Effects of dopamine and dopamine receptor agonists on forskolin-stimulated cyclic AMP accumulation

Forskolin (10 μM) induced a 20 to 30 fold stimulation of cyclic AMP accumulation in HEK293/D_4 cells. This effect was

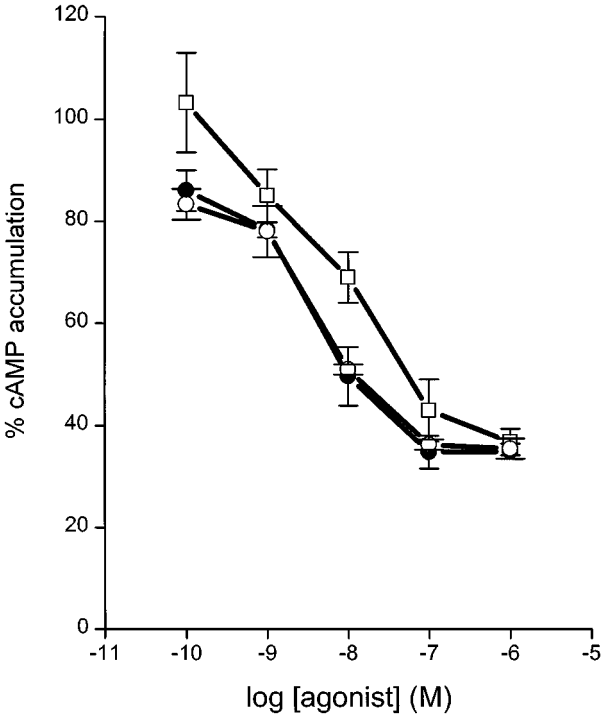

Figure 1 Concentration-response curves of dopamine (DA), quinpirole and 7-hydroxy-2-dipropylaminotetralin (7-OH-DPAT) for inhibition of forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells. Data are means and vertical lines show s.e.mean from three experiments.

Table 1 Agonist parameters in HEK293/D_4 cells

Agonists	*Efficacy (% relative to dopamine)*	*pEC_{50}*	n
Dopamine	100	8.7 ± 0.1	10
Quinpirole	98 ± 4	8.8 ± 0.2	3
7-OH-DPAT	100 ± 3	8.3 ± 0.2	3
L-745,870	71 ± 3	9.0 ± 0.2	4
U-101958	93 ± 4	8.7 ± 0.3	3

Efficacy values are E_{max} values expressed as percentage of the E_{max} of dopamine (which amounted to 71 ± 2% inhibition of forskolin-stimulated cyclic AMP accumulation).

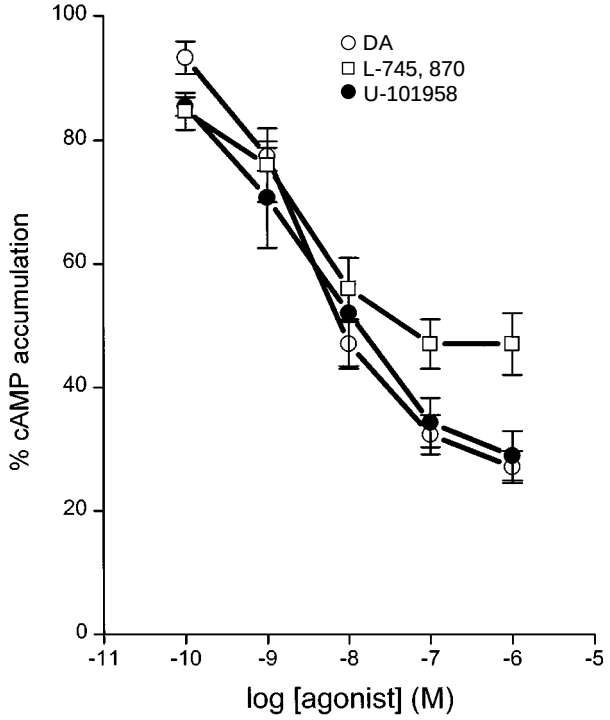

Figure 2 Concentration-response curves of dopamine (DA), L-745,870 and U-101958 for inhibition of forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells. Data are means and vertical lines show s.e.mean from seven, four and three experiments, respectively.

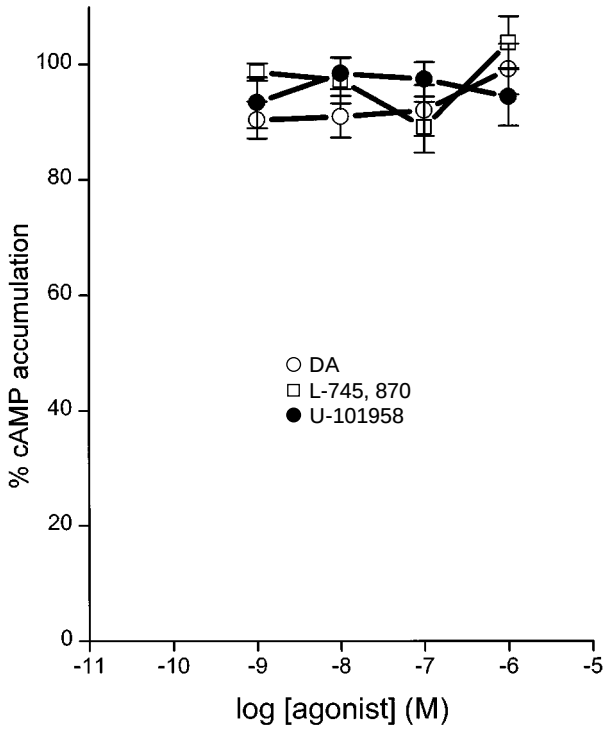

Figure 3 Lack of effect of dopamine (DA), L-745,870 and U-101958 on forskolin-stimulated cyclic AMP accumulation in non-transfected HEK293 cells. Data are means and vertical lines show s.e.mean from five experiments.

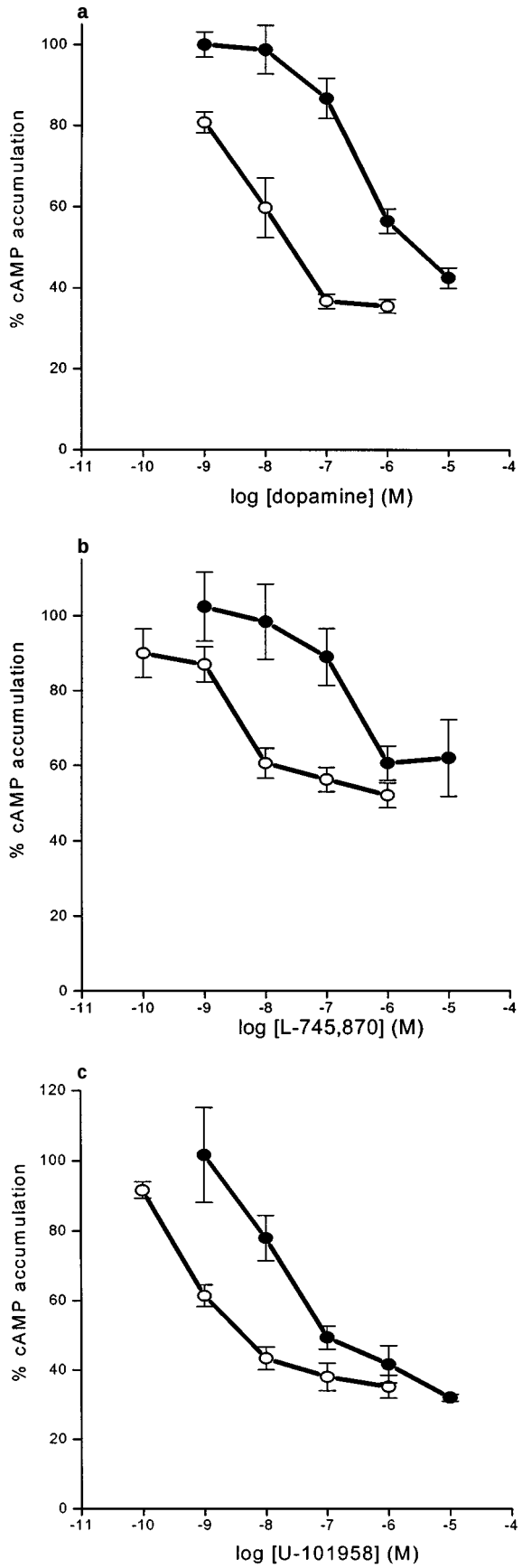

Figure 4 Concentration-response curves of (a) dopamine, (b) L-745,870 and (c) U-101958 for inhibition of forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells, in the absence (open symbols) and in the presence of spiperone (0.1 μM; solid symbols). Data are means and vertical lines show s.e.mean from four, five and three experiments, respectively.

inhibited in a concentration-dependent fashion by dopamine (E_{max} 71±2% inhibition of forskolin-stimulated levels, pEC_{50} 8.7±0.1, $n=10$) and mimicked by the dopamine D_2-like receptor agonists, quinpirole and 7-hydroxy-2-dipropylaminotetraline (7-OH-DPAT; Figure 1, Table 1).

Effects of L-745,870 and U-101958 on forskolin-stimulated cyclic AMP accumulation

Both L-745,870 and U-101958 inhibited forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells in a concentration-dependent way (Figure 2). L-745,870 was less efficacious than dopamine (71% efficacy, compared to dopamine) whereas U-101958 behaved as a full agonist compared to dopamine (Table 1). Dopamine, L-745,870 and U-101958 (up to 1 μM) were devoid of effect on forskolin-stimulated cyclic AMP accumulation in control, non-transfected HEK293 cells (Figure 3).

Effects of spiperone, raclopride and clozapine

The dopamine D_2-like receptor antagonists, spiperone, raclopride and clozapine did not show significant agonist activity up to 10 μM in HEK293/D_4 cells ($n=3$ each, not shown). Spiperone (0.1 μM) shifted the concentration-response curves of dopamine, L-745,870 and U-101958 to the right in a parallel manner (Figure 4). Derived pK_B values were comprised between 8.2 and 8.8 (Table 2). In the presence of raclopride (10 μM), concentration-response curves for dopamine, L-745,870 and U-101958 were not significantly altered (Figure 5). The concentration-response curves for dopamine, L-745,870 and U-101958 were shifted to the right in the presence of clozapine (1 or 10 μM; Figure 6), with a mean pK_B value of 7.1 in each case (Table 2).

Discussion

The present data constitute compelling evidence that L-745,870 and U-101958 act as agonists in HEK293 cells expressing the human recombinant dopamine $D_{4.4}$ receptor. Both compounds mimicked the effects of dopamine and of the dopamine D_2-like receptor agonists, quinpirole and 7-OH-DPAT, at inhibiting forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells. In doing so, U-101958 was as efficacious as dopamine, whereas L-745,870 acted as a partial agonist, with substantial intrinsic activity (71%, when compared to dopamine). Like dopamine, L-745,870 and U-101958 were without effect in the parental, non-transfected HEK293 cells, implying that the effects observed in HEK293/D_4 cells are mediated by the receptor for which the dopamine D_4 receptor cDNA codes. The antagonist profile, almost

Table 2 Antagonist pK_B values of spiperone, raclopride and clozapine obtained against dopamine, L-745,870 and U-101958 in HEK293/D_4 cells

Agonist used	*Spiperone*	*Raclopride*	*Clozapine*
Dopamine	8.8±0.2 (4)	<5 (3)	7.1±0.3 (9)
L-745,870	8.4±0.4 (5)	<5 (3)	7.1±0.2 (4)
U-101958	8.2±0.1 (3)	<5 (3)	7.1±0.2 (6)

Values derived from the experiments illustrated in Figures 4 to 6. Numbers of determinations are indicated in parentheses. Raclopride was ineffective at 10 μM.

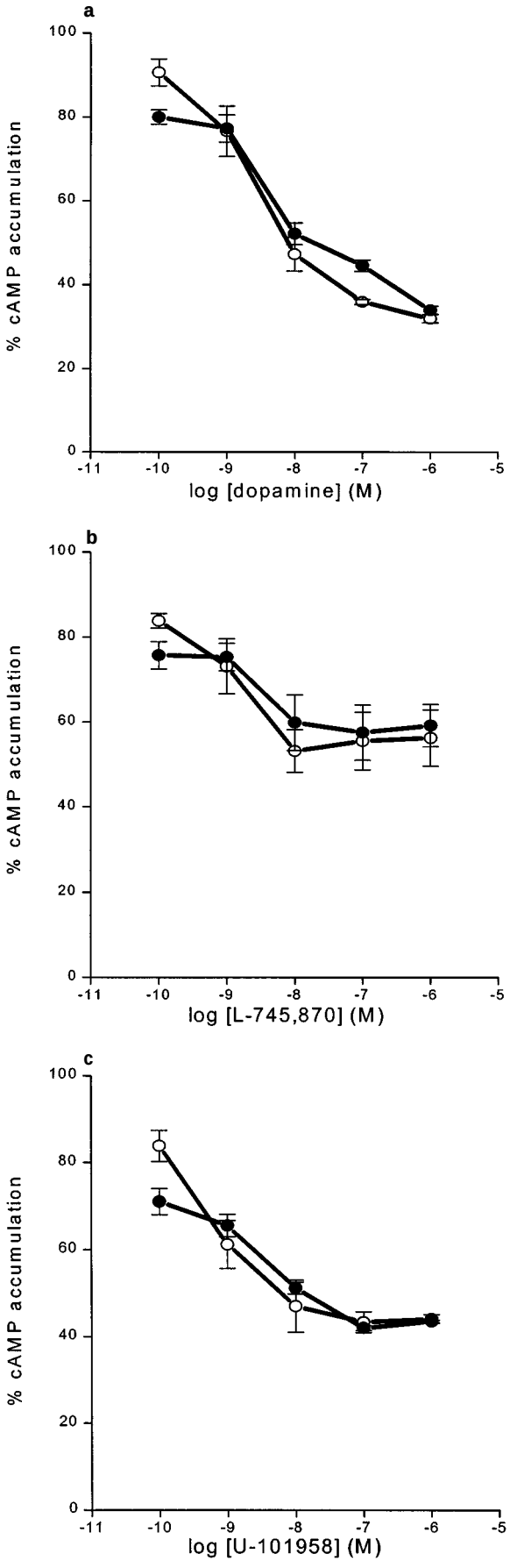

Figure 5 Concentration-response curves of (a) dopamine, (b) L-745,870 and (c) U-101958 for inhibition of forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells, in the absence (open symbols) and in the presence of raclopride (10 μM; solid symbols). Data are means and vertical lines show s.e.mean from three experiments.

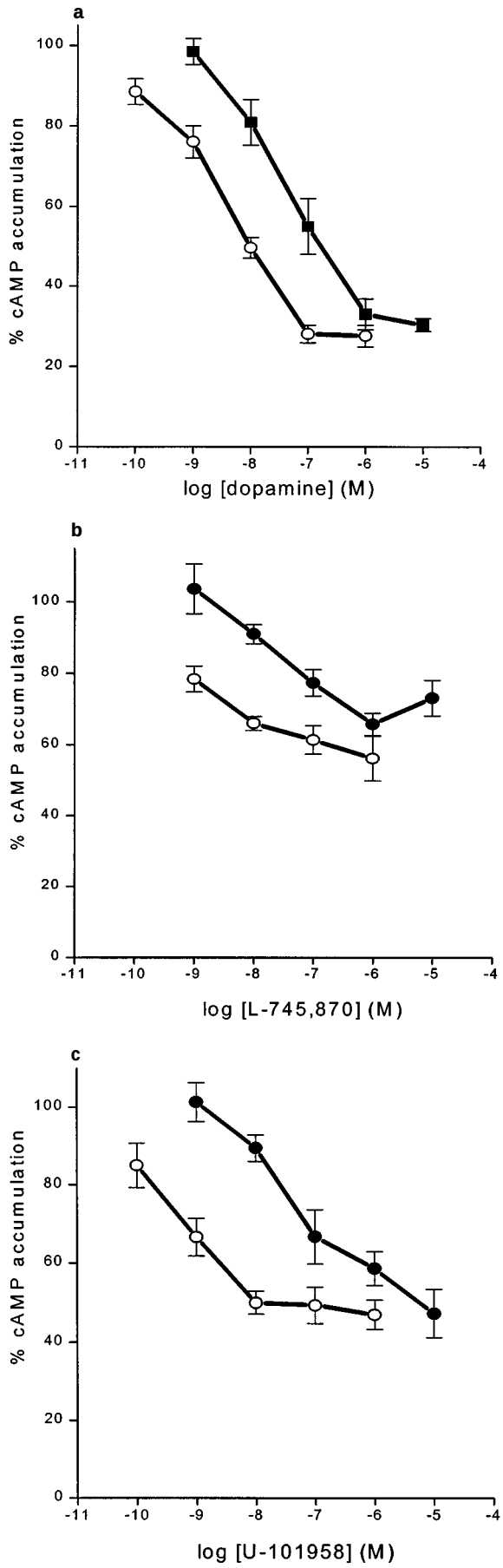

Figure 6 Concentration-response curves of (a) dopamine, (b) L-745,870 and (c) U-101958 for inhibition of forskolin-stimulated cyclic AMP accumulation in HEK293/D_4 cells, in the absence (open symbols) and in the presence of clozapine (1 μM; solid squares, or 10 μM; solid circles). Data are means and vertical lines s.e.mean from nine, four and six experiments, respectively.

identical when using either dopamine, L-745,870 or U-101958 as an agonist, fully confirms that view. Spiperone and clozapine antagonized the agonist responses with potencies (pK_Bs) consistent with their affinities for D_4 receptors, as estimated in the radioligand binding assay and known from the literature (Van Tol *et al*., 1991; Durcan *et al*., 1995). Also, raclopride was a very weak antagonist, in agreement with its low affinity for D_4 receptors (Van Tol *et al*., 1991; Lahti *et al*., 1993).

Both L-745,870 and U-101958 have been described as dopamine D_4 receptor antagonists (Schlachter *et al*., 1997; Kulagowki *et al*., 1996; Patel *et al*., 1997). In a recent study focusing on the binding of [^{35}S]-guanosine-5′-O-(3-thio)triphosphate ([^{35}S]-GTPγS) in human recombinant dopamine $D_{4.4}$ receptors, L-745,870 was found to be a silent antagonist of dopamine (Newman-Tancredi *et al*., 1997). Since the latter study was performed in Chinese hamster ovary (CHO) cells as the host cells, it might be that agonism of L-745,870 is dependent on the functional model and/or the host cell used. Examples of such variations have been reported (see Schoeffter *et al*., 1997). In line with this, it is noteworthy that dopamine appears to be markedly less potent in stimulating [^{35}S]-GTPγS binding (EC_{50} 100 nM; Newman-Tancredi *et al*., 1997) than in inhibiting cyclic AMP accumulation in the present study (EC_{50} ca 2 nM). Yet the number of receptors expressed in the former case is reportedly much higher (1400 versus 505 fmol mg^{-1} protein). Thus, our functional model (inhibition of cyclic AMP accumulation in HEK293/D_4 cells) seems to be more suitable than others for detecting dopamine D_4 receptor agonist activities. However, it should be pointed out that pure (or 'silent') antagonism can be shown in this model, as examplified by spiperone or clozapine. The reason why others have reportedly failed to see any detectable intrinsic activity of L-745,870 using the same functional model remains unclear (Kulagowski *et al*., 1996; Bristow *et al*., 1997). One possible explanation is that the latter studies may have been performed on a variant of the human D_4 receptor different from the $D_{4.4}$ receptor, which was used in the present work. Human dopamine D_4 receptors have been shown to exist as multiple allele forms, which differ by the number of a 16 amino acid repeat sequence in the putative third intracellular loop (Van Tol *et al*., 1992). The $D_{4.4}$ form (including 4 of these sequences) is the most commonly found one (Lichter *et al*., 1993; Shaikh *et al*., 1993). However, all D_4 receptor alleles investigated appear to couple to cyclic AMP in a similar fashion and no major differences, apart from their sensitivities to monovalent cations, have been found (Ashghari *et al*., 1994; 1995).

It has been hypothesized that compounds such as L-745,870 and U-101958, designed as selective dopamine D_4 receptor antagonists, would have antipsychotic activity, without eliciting the side-effects encountered with most neuroleptics (see Introduction). In doing so, they would resemble the atypical neuroleptic, clozapine. Since there is no animal model fully predictive of antipsychotic activity, direct testing of this hypothesis has to await the development of selective antagonists. L-745,870 is the first selective dopamine D_4 receptor antagonist to have entered clinical trials. Recently published data point to its being ineffective as an antipsychotic in man (Bristow *et al*., 1997). In the absence of other information, this seems to indicate that dopamine D_4 receptor antagonism is of no benefit for the treatment of schizophrenia.

However, our results showing that L-745,870 may under some circumstances behave as a dopamine D_4 receptor agonist, should temper this conclusion. Since clozapine and spiperone were pure antagonists in the same model, it is entirely possible that L-745,870 will not mimic the action of clozapine in schizophrenic patients. The same applies to U-101958, for which no clinical data are available. Therefore, pure, selective dopamine D_4 receptor antagonists are still needed to probe the D_4 receptor antagonism hypothesis in the treatment of schizophrenia.

In conclusion, it is shown in the present study that L-745,870 and U-101958, in contrast to spiperone and clozapine, are agonists at human recombinant dopamine $D_{4.4}$ receptors expressed in HEK293 cells. Both compounds have been introduced as dopamine D_4 receptor antagonists with potential therapeutic application as atypical antipsychotics, like clozapine. Our results therefore question the relevance of their use to test the benefit of dopamine D_4 receptor antagonism in schizophrenia.

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