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Relationship between inhibition of cyclic AMP production in Chinese hamster ovary cells expressing the rat $D_{2(444)}$ receptor and antagonist/agonist binding ratios

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1 Radioligand binding assays using [^{3}H]-(−)-sulpiride, in the presence of 1 mM ethylenediaminetetraacetic acid (EDTA) and 100 μM guanylylimidodiphosphate (GppNHp) and [^{3}H]-N0437 were developed to label the low and high agonist affinity states of the $rD_{2(444)}$ receptor (long form of the rat D_2 receptor) respectively. The ratios of the affinities of compounds in these two assays (K_{app} [^{3}H]-(−)-supiride/K_{app} [^{3}H]-N-0437) were then calculated.

2 The prediction that the binding ratio reflected the functional efficacy of a compound was supported by measurement of the ability of a number of compounds acting at dopamine receptors to inhibit $rD_{2(444)}$-mediated inhibition of cyclic AMP production. When the rank order of the ratios of a number of these compounds was compared to their ability to inhibit the production of cyclic AMP, a significant correlation was seen (Spearman rank correlation coefficient = 0.943, $P = 0.01$).

3 In conclusion, the sulpiride/N-0437 binding ratio reliably predicted the efficacy of compounds acting at dopamine receptors to inhibit cyclic AMP production mediated by the $rD_{2(444)}$ receptor.

Keywords: Rat $D_{2(444)}$ receptor; [^{3}H]-(−)-sulpiride; [^{3}H]-(−)-N-0437; cyclic AMP production; CHO cell

Introduction

It is now well known that dopamine receptors exist as two major classes, D_1-like and D_2-like, according to their amino acid sequence homology, functional and pharmacological properties. The D_1-like group consists of the D_1 (Dearry *et al.*, 1990; Monsma Jr. *et al.*, 1990) and D_5 (Sunahara *et al.*, 1991) receptors whereas the D_2-like group consists of the D_2, D_3 (Sokoloff *et al.*, 1990) and D_4 receptors (Van Tol *et al.*, 1991). The D_2 receptor can also exist as either a short ($D_{2(415)}$) or long form ($D_{2(444)}$), due to alternative splicing where an extra 29 amino acids are present in the third intracellular loop of the long form (humans: Dal Toso *et al.*, 1989; Grandy *et al.*, 1989; rats: Bunzow *et al.*, 1988; Giros *et al.*, 1989; Monsma Jr. *et al.*, 1989). The significance of this latter observation is unknown, although preliminary studies suggest that these two forms may have different binding properties (Castro & Strange, 1993). Additionally, alternative splicing has been reported for the D_3 receptor (Giros *et al.*, 1991; Fishburn *et al.*, 1993), and polymorphism of the D_4 receptor, where between 2 and 10 repeats (excluding 9) of a 48 base pair sequence in the third intracellular loop have been reported (Van Tol *et al.*, 1992; Lichter *et al.*, 1993). The significance of these latter findings has yet to be determined.

The distribution, pharmacology and function of these receptors are being investigated at present both *in vivo* and in transfected cell systems. For example, by use of Northern blotting and *in situ* hydbridization techniques, the mRNA for the D_2 receptor has been shown to be present in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra and ventral tegmental areas (Civelli *et al.*, 1991; Sibley & Monsma Jr., 1992; Gingrich & Caron, 1993; Civelli *et al.*, 1993). Indeed, the long form of the D_2 receptor has been shown to be generally more abundant than the short in rodent striatal tissue (Giros *et al.*, 1989). Other studies have shown that the D_2 receptors in transfected cell systems are linked to inhibition of adenylyl cyclase activity (Dal Toso *et al.*, 1989; Bates *et al.*, 1991; Meller *et al.*, 1991), inhibition of prolactin release (Meller *et al.*, 1991) and the stimulation of arachidonic acid release (Kanterman *et al.*, 1991; Lahti *et al.*, 1992).

We have previously shown that the rat $D_{2(444)}$ receptor ($rD_{2(444)}$) expressed in Chinese hamster ovary (CHO) cells is a good pharmacological model of rat striatal D_2 receptors (Harley *et al.*, 1994). In the present study we have investigated the use of simple radioligand binding studies to predict functional efficacy at these receptors. Some of the radioligand binding data presented here have been published previously (Harley *et al.*, 1994).

Methods

Lysed cell membrane preparation

Cells transfected with the long form of the rat D_2 receptor ($rD_{2(444)}$) were grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) containing 10% foetal calf serum (FCS), 1% penicillin/streptomycin, 1% glutamine and 100 μM non-essential amino acids, at 37°C in a humidified incubator with 95%O_2/5%CO_2. These were harvested in 20 mM HEPES Krebs buffer pH 7.4 (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 5, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11, HEPES 20), followed by lysis in 5 mM HEPES buffer pH 7.4 and resuspension in 20 mM HEPES Krebs buffer as described by Harley *et al.* (1994).

[^{3}H]-(−)-sulpiride binding to lysed cell membranes

[^{3}H]-(−)-sulpiride binding to the low agonist affinity state of the $rD_{2(444)}$ receptor was performed in 20 mM HEPES Krebs buffer containing 10 mM EDTA and 10 μM pargyline, pH 7.4, in the presence of 100 μM GppNHp (guanylylimidodiphosphate), as detailed by Harley *et al.* (1994).

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[^{3}H]-N-0437 binding to lysed cell membranes

Assays were performed in 20 mM HEPES Krebs buffer containing 10 μM pargyline, pH 7.4. Membranes were prepared as above and diluted to give 0.01–0.02 mg protein per assay tube (Bradford, 1976). Saturation experiments used a range of radioligand concentrations (0.01–10 nM). For displacement studies a single low concentration of 1 nM was used to label the high agonist affinity state of the receptor. Non-specific binding was defined by use of 1 μM (−)-sulpiride, with a final assay volume of 0.5 ml. Incubations were for 60 min at 30°C, terminated by 2 min at 4°C, followed by filtration through 0.05% PEI soaked GF/C filters on a Brandel cell harvester. These were then washed through with 15 ml of ice cold buffer, the individual filter discs removed and 10 ml of Hydrofluor scintillation fluid added. They were then counted for tritium on a β scintillation counter.

cyclic AMP production in whole CHO cells transfected with the $rD_{2(444)}$ receptor

Cells transfected with the $rD_{2(444)}$ receptor were grown to confluence on 24 well plates, in DMEM containing 10% FCS, 1% penicillin/streptomycin, 1% glutamine and 100 μM non-essential amino acids. Prior to assay, the cells were washed twice with 1 ml of DMEM (with no additives). Assays were performed in DMEM (no additives), in the presence of 1 mM IBMX, 0.1% ascorbic acid, 10 μM pargyline and 10 μM forskolin (to stimulate cyclic AMP production), with a final assay volume of 1 ml. Incubations were for 30 min in a humidifed incubator (95%O_2/5%CO_2), at 37°C, and were terminated by aspiration of the medium, followed by three washes with 1 ml of ice cold 20 mM HEPES Krebs buffer, pH 7.4. The cyclic AMP was extracted with 200 μl of 5% TCA (trichloroacetic acid) at 4°C for 15 min. These extractions were washed three times with 1 ml of water saturated ether, then a 150 μl aliquot of the aqueous layer was removed and dried under vacuum. The samples were then assayed for cyclic AMP using the Amersham (Slough, England) [^{3}H]-cyclic AMP kit.

Materials

[^{3}H]-(−)-sulpiride (60–87 Ci mmol^{-1}) was obtained from New England Nuclear (Stevenage, England), [^{3}H]-N-0437 (2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin) (50–80 Ci mmol^{-1}) from Amersham (Slough, England) and the cyclic AMP detection kit (sensitivity ~16 pg/tube) from Amersham (Slough, England). All tissue culture reagents were obtained from Gibco (Paisley, Scotland), other than non-essential amino acids which were from Flow (Rickmansworth, England). Single well tissue culture plates (245 × 245 × 20 mm) were from Nunc (Roskilde, Denmark) and twenty four well tissue culture plates from Costar (Cambridge, U.S.A.). Ascorbic acid, α-ergocryptine, dopamine (3-OH-tyramine), ethylenediaminetetra-acetic acid, ethyl ether, foetal calf serum (FCS), forskolin, guanylylimidodiphosphate (GppNHp), isobutylmethyl xanthine (IBMX), pargyline hydrochloride, polyethyleneimine (PEI) and trichloroacetic acid were obtained from Sigma (Dorset, England). All other chemicals were obtained from Research Biochemicals International (SEMAT, St Albans, England). The rat $D_{2(444)}$ receptor transfected into chinese hamster ovary cells (CHO) was a kind gift of Drs Sokoloff and Schwartz (Paris, France). N-0437 was a kind gift from Nelson Research (Irvine, U.S.A.), and hydrofluor scintillation fluid was obtained from National Diagnostics (Atlanta, Georgia, U.S.A.). SCH23390(7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1-H-3-benzazepine hydrochloride), SKF38393((±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride) and (−)-3PPP(preclamol; (−)-3-(3-hydroxyphenyl)-N-propylpiperidine hydrochloride were obtained from Research Biochemicals International.

Calculations

Binding curves were analysed by a non-linear least squares regression analysis (RS1, BBN Research Systems Cambridge, Mass, U.S.A.) and a computerised iterative procedure. IC_{50} determinations were corrected for ligand occupancy (Cheng & Prusoff, 1973) to give K_{app} values, expressed as the geometric mean. Levels of cyclic AMP were normalized by expressing the results as the percentage inhibition compared to that by dopamine, i.e. 10 μM forskolin-stimulated samples were defined as 0% inhibition and samples also containing 10 μM dopamine were defined as 100% inhibition. Functional dose-response curves were analysed with Allfit, a non-linear regression with a four parameter logistic curve fitting programme (DeLean *et al.*, 1978). The rank orders of potency for compounds were compared by the Spearman rank correlation coefficeint (one-tailed test, significance was defined by a P value of less than 0.05). Groups of data were compared by Student's single non-paired t tests, significance was defined by $P<0.05$. Receptor occupancy was calculated according to the equation from Clark (1933):-

$$[RA]/[R_T]=[A]/(K_A+[A])$$

Where R_T = total receptor concentration; RA = concentration of receptor occupied by agonist; [A] = agonist concentration and K_A = dissociation constant obtained from [^{3}H]-(−)-sulpiride binding to the low agonist affinity state of the receptor.

The K_{app} for each compound from low agonist affinity binding studies was used as the best approximation of the K_A, where the binding conditions were similar to those used in the functional studies. The low agonist affinity state was used as it has been suggested that this state is linked to receptor-mediated functional responses (Birdsall *et al.*, 1980; Kenakin, 1993). Percentage functional response was plotted against percentage receptor occupancy, and the occupancy required for 50% of the functional response determined. The ratio of the occupancy required by a reference full receptor agonist (quinpirole) to that required by a partial agonist to produce this level of response was defined as the relative efficacy (Furchgott & Burtsztyn, 1967).

Results

Radioligand binding to lysed cell membranes

In CHO cells transfected with the $rD_{2(444)}$ receptor under the low agonist affinity state binding conditions described, a single saturable binding site was obtained with the receptor antagonist [^{3}H]-(−)-sulpiride, as reported previously (Harley *et al.*,

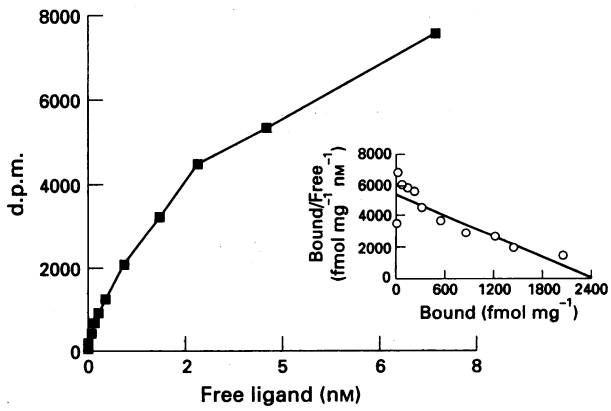

Figure 1 Saturation analysis of [^{3}H]-N-0437 binding at $rD_{2(444)}$ receptors in lysed CHO cells. A typical example of specific high agonist affinity state binding ([^{3}H]-N-0437) in lysed cell preparations of $rD_{2(444)}$ receptors in CHO cells. Methods were as described in the text, performed in duplicate on at least three separate occasions. Mean K_D(−s.e.mean; +s.e.mean) = 2.1(1.7;2.7) nM. Mean B_{max} = 3400 ± 930 fmol mg^{-1} protein.

1994). The K_D (−s.e.mean; +s.e.mean) was 3.0(2.8;3.1) nM with a maximal binding capacity of 3000 ± 1000 fmol mg^{-1} protein ($n=3$). In contrast, receptor agonist binding with [^{3}H]-N-0437 did not appear to saturate due to the presence of a low agonist affinity binding state (Figure 1). However, as this state could not clearly be separated from the higher agonist affinity state binding a single site was defined with a K_D of 2.1(1.7;2.7) nM and B_{max} of 3400 ± 930 fmol mg^{-1} protein ($n=3$). The affinities of a number of dopamine receptor compounds at both the low and high agonist affinity states of the $rD_{2(444)}$ receptor were determined by use of [^{3}H]-(−)-sulpiride (in the presence of EDTA and GppNHp, described by Harley *et al.*, 1994) and [^{3}H]-N-0437 binding respectively.

The receptor antagonists studied displayed a profile consistent with the D_2 receptor (Table 1), where spiperone and eticlopride showed very high affinity (low K_{app} ([^{3}H]-(−)sulpiride + EDTA + GppNHp binding): 0.14, 0.11 nM; high K_{app} ([^{3}H]-N-0437 binding): 0.10, 0.18 nM respectively) and a marked preference was shown by the receptor for the (−)-isomer of sulpiride (18 fold). The D_1-selective compounds, (+)-SCH23390 and SKF38393 showed much lower affinity for this receptor, as expected in a cell line expressing a D_2 type receptor. Neither the affinity nor Hill coefficient values of the antagonists changed greatly between the two assays, which resulted in values close to unity for the ratio of affinity for the [^{3}H]-(−)-sulpiride and [^{3}H]-N-0437 binding (sulpiride/N-0437 ratio).

In contrast, full receptor agonists such as dopamine showed a preference for the high agonist affinity state binding (low K_{app}: 1300 nM; high K_{app}: 180 nM) and produced a sulpiride/N-0437 ratio of 7.2 (Table 2). In the case of compounds known to be partial receptor agonists, such as lisuride, a higher affinity was seen in both assays, but with a smaller preference for the high agonist affinity binding state (low K_{app}: 0.78 nM; high K_{app}: 0.28 nM). This resulted in a sulpiride/N-0437 ratio of 2.8 (Table 2). All but one of these compounds exhibited a Hill coefficient close to unity, presumably due to mainly one agonist affinity state being labelled in both assays. The exception was quinpirole, which had a Hill coefficient of 0.73 for high agonist affinity state binding. This suggested the presence of both low and high agonist affinity state binding which was only discernible by very efficacious compounds.

Inhibition of cyclic AMP production in whole cells

A standard concentration of forskolin (10 μM) was used to stimulate cyclic AMP production, in order for a time course of this production and its inhibition by dopamine (10 μM) to be studied. As can be seen in Figure 2, cyclic AMP production was approximately linear up to 60 min, although dopamine reached a maximal inhibition of forskolin-stimulated cyclic AMP levels of 80–90% at 20–30 min. A standard assay time of 30 min was used for all further experiments. A forskolin dose-response curve for cyclic AMP production was determined (Figure 3), which gave an EC_{50} value of 150 μM. A concentration of 10 μM was seen to give a significant ($P<0.001$) stimulation (23 fold) above basal levels (0.932 ± 0.440 pmolwell cyclic AMP) and was used in all further experiments.

A number of compounds of varying efficacy predicted from the sulpiride/N-0437 ratio were examined for inhibition of cyclic AMP production via $rD_{2(444)}$ receptors transfected into

Table 1 Displacement of [^{3}H]-(−)-sulpiride and [^{3}H]-N-0437 binding in lysed rD_2CHO cell membranes

Compound	[^{3}H]-(−)-sulpiride + GppNHp + EDTA K_{app}(nM)	n_H	[^{3}H]-N0437 K_{app}(nM)	n_H	Ratio
D_2 antagonists					
Spiperone	0.14 (0.13–0.14)	1.55 ± 0.14	0.10 (0.063–0.13)	1.61 ± 0.17	1.4
Eticlopride	0.11 (0.077–0.15)	1.29 ± 0.14	0.18 (0.12–0.31)	1.79 ± 0.28	0.61
(+)-Sulpiride	72 (62–80)	0.91 ± 0.20	200 (170–290)	1.02 ± 0.16	0.36
(–)-Sulpiride	3.9 (2.8–7.2)	0.93 ± 0.09	11 (8.1–17)	0.88 ± 0.06	0.35
D_1 compounds					
SKF38393	8000 (6700–9900)	1.06 ± 0.14	3400 (2600–4000)	0.97 ± 0.09	2.4
(+)-SCH23390	360 (170–540)	0.92 ± 0.19	240 (140–390)	0.98 ± 0.16	1.5

Assays were carried out on lysed cell membranes in 20 mM HEPES Krebs buffer pH7.4 as detailed in the Methods. Curves consisted of a minimum of eight concentration points carried out in duplicate on at least 3 separate occasions.
K_{app}: IC_{50} values corrected for ligand occupancy (Cheng & Prusoff, 1973), expressed as the geometric mean, numbers in parentheses refer to the range of values obtained.
n_H: Hill coefficient ± s.e.mean.
Ratio: [^{3}H]-(−)-sulpiride K_{app} (+GppNHp + EDTA)/[^{3}H]-N-0437 K_{app}.

Table 2 Agonist displacement of [^{3}H]-(−)-sulpiride and [^{3}H]-N-0437 binding in lysed rD_2CHO cell membranes

Compound	[^{3}H]-(−)-sulpiride + GppNHp + EDTA K_{app}(nM)	n_H	[^{3}H]-N0437 K_{app}(nM)	n_H	Ratio
Quinpirole	4500 (3600–5400)	1.03 ± 0.11	340 (220–540)	0.73 ± 0.03	13
Dopamine	1300 (1100–1500)	0.88 ± 0.01	180 (140–270)	1.10 ± 0.11	7.2
N-0437	9.8 (8.5–11)	0.84 ± 0.05	2.1 (0.80–2.4)	1.10 ± 0.06	4.7
N-0434	8.7 (5.5–11)	0.90 ± 0.07	2.4 (2.0–3.2)	0.96 ± 0.04	3.6
α-Ergocryptine	0.60 (0.36–0.99)	1.27 ± 0.10	0.19 (0.15–0.25)	1.18 ± 0.07	3.2
Lisuride	0.78 (0.50–1.3)	1.41 ± 0.18	0.28 (0.22–0.33)	1.24 ± 0.09	2.8
(–)-Apomorphine	59 (53–62)	0.93 ± 0.08	30 (15–49)	1.15 ± 0.18	2.0
(–)-3PPP	90 (43–140)	0.83 ± 0.14	110 (96–120)	1.22 ± 0.02	0.82

Assays were carried out on lysed cell membranes in 20 mM HEPES Krebs buffer pH7.4 as detailed in the Methods. Curves consisted of a minimum of 8 concentration points carried out in duplicate on at least 3 separate occasions.
K_{app}: IC_{50}values corrected for ligand occupancy (Cheng & Prusoff, 1973), values are expressed as the geometric mean, numbers in parentheses refer to the range of values obtained.
n_H: Hill coefficient ± s.e.mean.
Ratio: [^{3}H]-(−)-sulpiride K_{app} (+GppNHp + EDTA)/[^{3}H]-N-0437 K_{app}.

CHO cells. The results were expressed as a percentage of the maximal dopamine inhibition (defined as 100% inhibition, example curves in Figure 4). From the results shown in Table 3 (also Figures 4 and 5) it can be seen that (−)-3PPP was the only compound to exhibit partial inhibition of forskolin-stimulated cyclic AMP production (85.5% of the 10 μM dopamine effect). In addition, all the compounds except for (−)-3PPP had EC_{50} values that were much lower than the low agonist affinity K_{app} values, which indicated that less than 50% of the receptors needed to be occupied to produce this response. The receptor occupancy required to elicit a 50% inhibition of cyclic AMP production was calculated using the Clark equation as detailed in the Methods. This showed that quinpirole needed to occupy 0.18%, dopamine 0.36%, N-0434 1.5%, (−)-apomorphine 2.3%, lisuride 7.3% and (−)-3PPP 49% of the receptors to produce a 50% response. This supported the hypothesis that (−)-3PPP was a partial agonist, and that a rank order of efficacy could be determined for the other compounds. Thus the relative efficacy was determined for each compound (Furchgott & Burtsztyn, 1967), using quinpirole as the reference agonist (Figure 5). This resulted in a relative efficacy value of 1.0 for quinpirole, 0.5 for dopamine, 0.12 for N-0434, 0.078 for (−)-apomorphine, 0.025 for lisuride and 0.0037 for (−)-3PPP (Table 4). Finally, the antagonist, (−)-sulpiride (1 μM), showed no activity on its own, but was able to block the activity of EC_{50} concentrations of the receptor agonist studied, which indicated that a specific $rD_{2(444)}$-mediated inhibition of cyclic AMP production was being measured.

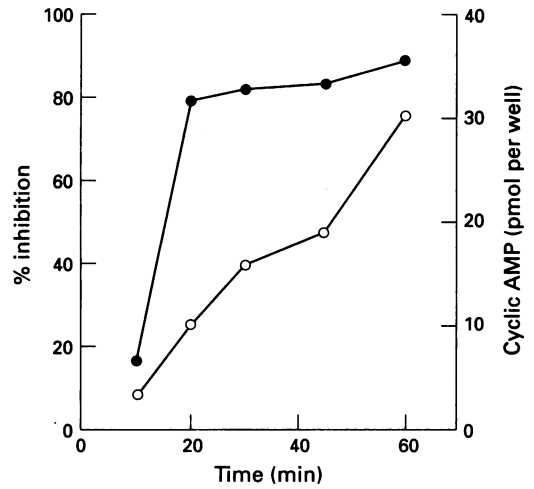

Figure 2 Time course of cyclic AMP production and its inhibition by dopamine in CHO cells transfected with the $rD_{2(444)}$ receptor. An example of a time course of 10 μM forskolin stimulation (○) of cyclic AMP production and 10 μM dopamine inhibition (●) of forskolin stimulated cyclic AMP production, in $rD_{2(444)}$ transfected CHO cells. Methods were as described in the text, samples were performed in duplicate on at least three separate occasions.

Table 3 Inhibition of forskolin stimulated cyclic AMP production in rD_2CHO cells

Compound	EC_{50}(nM)	*% max*
Quinpirole	5.2 ± 0.49	98.6 ± 1.6
Dopamine	5.7 ± 0.97	99.5 ± 2.7
N-0434	0.12 ± 0.038	104.6 ± 5.2
(−)-Apomorphine	1.0 ± 0.21	100 ± 2.6
Lisuride	0.084 ± 0.010	96.1 ± 1.6
(−)-3PPP	46 ± 24	85.5 ± 2.2

Inhibition of forskolin-stimulated cyclic AMP production was measured as described in the Methods. Curves consisted of a minimum of eight concentration points, performed in duplicate, on at least three separate occasions. Curves were analysed using Allfit, where the values are the mean ± s.e.mean derived from these analyses.
EC_{50}: concentration which elicited half the individual maximal inhibition.
% max: individual percentage maximal inhibition, compared to 10 μM dopamine.

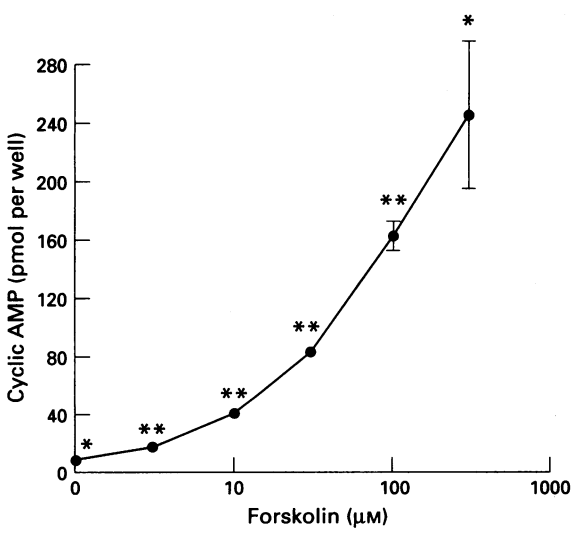

Figure 3 Forskolin stimulation of cyclic AMP production in CHO cells transfected with the $rD_{2(444)}$ receptor. Stimulation of cyclic AMP production above basal levels (0.932 ± 0.440 pmol per well) by forskolin was measured as described in the text. An EC_{50} of 150 μM was determined with a maximal stimulation of 376 ± 12 pmol per well of cyclic AMP. Values are the mean ± s.e.mean, performed in duplicate on at least three separate occasions. Significance of forskolin-stimulated cyclic AMP levels above basal were determined by Student's one tailed t test ($^*P<0.01$; $^{**}P<0.001$).

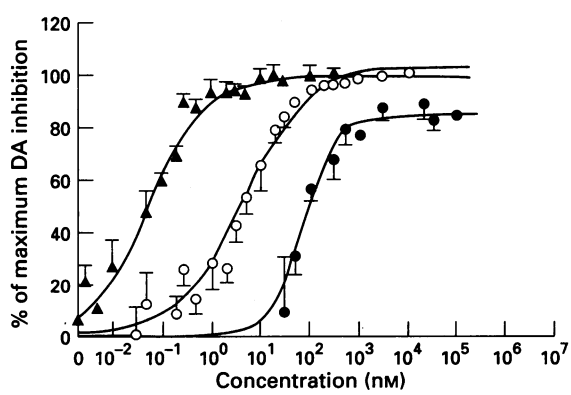

Figure 4 Inhibition of cyclic AMP production in CHO cells transfected with the $rD_{2(444)}$ receptor. Examples of agonist-induced inhibition of forskolin-stimulated cyclic AMP production via the $rD_{2(444)}$ receptor expressed in CHO cells, as described in the Methods. Values are the mean ± s.e.mean. (% inhibition compared to that produced by 10 μM dopamine, as detailed in Calculations), from curves performed in duplicate on at least three separate occasions. Curves are dopamine (○), lisuride (▲) and (−)-3PPP (●).

Table 4 Comparison of predicted and measured functional efficacy

Compound	*Ratio*	*Relative efficacy*
Quinpirole	13	1.0
Dopamine	7.2	0.5
N-0434	3.6	0.12
(-)-Apomorphine	2.0	0.078
Lisuride	2.8	0.025
(-)-3PPP	1.1	0.0037

The relative efficacy was calculated using data from the 50% level of inhibition of forskolin stimulated cyclic AMP production in $rD_{2(444)}$ transfected CHO cells (Figure 4), as detailed in the Calculations and Methods. The ratio was calculated from the K_{app} values for [^{3}H]-(-)-sulpiride (+EDTA, +GppNHp) and [^{3}H]-N-0437 binding as in Tables 1 and 2.

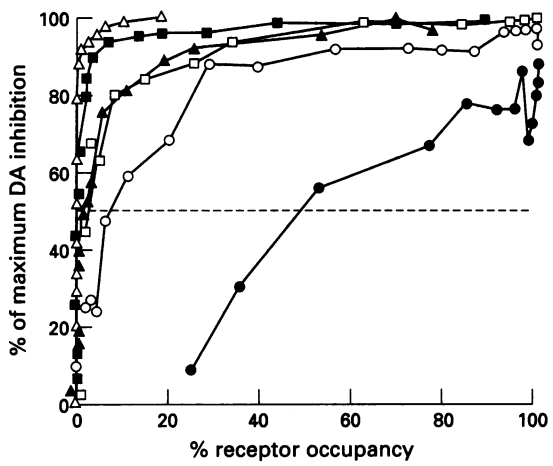

Figure 5 Transformation of the data from inhibition of cyclic AMP production in CHO cells transfected with the $rD_{2(444)}$ receptor. Plot of the percentage inhibition of forskolin-stimulated cyclic AMP production against the percentage receptor occupancy (calculated from the K_{app} for each compound at the low affinity state of the receptor, as detailed in Calculations), CHO cells transfected with the $rD_{2(444)}$ receptor. The dotted line represents 50% of the maximal inhibition of cyclic AMP production seen with 10 μM dopamine (DA). Curves are quinpirole (△), dopamine (■), N-0434 (▲), (-)-apomorphine (□), lisuride (○) and (-)-3PPP (●). Values used are the means from samples performed in duplicate on at least three separate occasions.

Comparison of the sulpiride/N-0437 ratio and inhibition of cyclic AMP production

A significant correlation was observed between the rank orders of the sulpiride/N-0437 ratio and relative efficacy measurements in the cyclic AMP assay (Table 4), with a Spearman rank correlation coefficient value of 0.943 ($P=0.01$). Indeed these data are linearly related with a correlation coefficient of 0.99 ($P<0.001$). This suggested that the sulpiride/N-0437 ratio was an accurate estimation of functional efficacy in CHO cells transfected with the $rD_{2(444)}$ receptor.

Discussion

The aim of this study was to develop simple radioligand binding assays for the prediction of functional efficacy in $rD_{2(444)}$ transfected CHO cells. This was achieved by utilizing the ternary complex model of drug/receptor interactions (Kenakin, 1993) where the K_{app} at the low agonist affinity state of the receptor approximated the true agonist dissociation constant. However, the K_{app} at the high agonist affinity state of the receptor also incorporated efficacy due to the promotion by agonists of receptor/G-protein coupling, leading to a functional response. A ratio of these two K_{app} values for various compounds would therefore give an indication of their efficacy, which could then be compared to actual efficacy by their ability to promote the production of cyclic AMP.

The low and high agonist affinity states of the $rD_{2(444)}$ receptor in CHO cells were radiolabelled with an antagonist ([^{3}H]-(-)-sulpiride) and agonist ([^{3}H]-N-0437) respectively. Displacement curves were then produced using compounds acting at dopamine receptors, and the ratio of their affinities at the low and high agonist affinity states of the receptor calculated and expressed as an antagonist/agonist ratio (sulpiride/N-0437 ratio). This ratio was then used to predict the efficacy of compounds acting via this receptor and compared to their measured functional efficacy.

Dopamine D_2 receptors have classically been thought to have either no effect or to inhibit the activity of adenylyl cyclase (Kebabian & Calne, 1979). Therefore, a number of compounds acting at dopamine receptors and thought to have varying degrees of efficacy from the antagonist/agonist ratio were examined for their ability to inhibit adenylyl cyclase activity. All, except for (-)-3PPP, showed a maximal inhibition of forskolin stimulated cyclic AMP production, which indicated that this compound was a partial agonist, in agreement with its low antagonist/agonist ratio. In order to identify a rank order of efficacy for all these compounds, the receptor occupancy required to produce a 50% inhibition of cyclic AMP production was determined (Furchgott & Burtszyn, 1967) and the relative efficacy calculated relative to quinpirole. This resulted in a rank order of quinpirole > dopamine > N-0434 > (-)-apomorphine > lisuride > (-)-3PPP, which was significantly correlated with the rank order of the antagonist/agonist ratios ($P=0.01$), and was also correlated linearly ($P<0.001$). Thus the antagonist/agonist ratio was shown to predict reliably the functional efficacy of these compounds for the $rD_{2(444)}$ receptor-mediated inhibition of forskolin stimulated cyclic AMP production.

In recent years a number of studies have examined D_2 receptor-mediated inhibition of cyclic AMP production in isolated cell systems. For example, Barton *et al.* (1991) reported on D_2 receptors expressed in Y-79 cells (human retinoblastoma cells). They showed that dopamine, N-0434 and (-)-apomorphine all exhibited the same maximal inhibition of adenylyl cyclase, as seen in the present study. However, the reported EC_{50} values were somewhat higher (dopamine: 1.95 μM; N-0434: 0.18 μM; (-)-apomorphine: 0.11 μM), possibly due to their use of cell lysates compared to whole cells in the present study and/or the available receptor reserve. A smaller receptor reserve would have the effect of increasing the EC_{50} and possibly reducing the maximal response of partial agonists, as can be seen after treatment of $rD_{2(444)}$ transfected CHO cells with 1 μM phenoxybenzamine for 30 min prior to assay (results not shown). In addition, the efficiency of coupling of the D_2 receptor to adenylyl cyclase may differ between the two cell systems, according to which G-proteins are available. The phenomenon of a receptor coupling to different effector systems has previously been described by Vallar *et al.* (1990) for D_2 receptors in pituitary GH_4C_1 and Ltk^- fibroblast cells. Results in good agreement with the present study have also been reported by Kanterman *et al.* (1991) and Albert *et al.* (1990) using $rD_{2(444)}$ receptors in CHO cells and rat D_2 receptors in GH_4C_1 cells respectively. They both demonstrated that dopamine could inhibit cyclic AMP production, and both reported EC_{50} values of 5.5 nM, which is in agreement with that obtained in the present study (5.7 nM). Thus the assay system used in the present study was a reliable and reproducible measure of functional efficacy.

Very few detailed studies have been carried out on transfected cells, with regard to agonist radioligand binding and functional studies. In addition, comparison of functional efficacy with the efficacy predicted from radioligand receptor

binding assays have not been extensively reported for the dopamine system, although this technique has already been used successfully at muscarinic M_1 receptors (Freedman *et al.*, 1988). However, a report by Lahti *et al.* (1992) did compare antagonist and agonist radioligand binding with functional efficacy as determined by the stimulation of arachidonic acid release, using CHO cells transfected with the $D_{2(444)}$ receptor. They defined low and high agonist affinity state binding with [^{3}H]-raclopride and [^{3}H]-U-86170 respectively. The rank order of antagonist/agonist ratios was similar to that obtained in the present study although the absolute values were somewhat higher. These ratios were then shown to reflect the ability of these compounds to stimulate arachidonic acid release, where quinpirole was again shown to be a full agonist and (−)-3PPP a partial agonist.

In conclusion, this study has validated the use of antagonist/agonist ratios obtained from simple radioligand binding studies to predict the functional activity of compounds at the $rD_{2(444)}$ receptor transfected in CHO cells.

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