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Agonist action at $D_{2(long)}$ dopamine receptors: ligand binding and functional assays

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1 The activities of a range of agonists at $D_{2(long)}$ dopamine receptors expressed in CHO cells have been determined in ligand binding and in a functional assay, the stimulation of [^{35}S]-GTPγS binding.

2 For several agonists (apomorphine, dopamine, pergolide, quinpirole, NPA, ropinirole, talipexole) binding in the absence of added guanine nucleotides was best described in terms of interaction at higher and lower affinity states, whereas for other agonists (bromocriptine, DHEC, lisuride, 3-PPP) a one binding site model was a good description of the data. In the presence of GTP (100 μM) all agonist binding data were best described by a one site model.

3 All of the agonists tested increased [^{35}S]-GTPγS binding above the basal level and the maximal effects and potencies of the agonists in this test were different. There was no clear relation betwen the ability of an agonist to stabilize the formation of the ternary complex of agonist/receptor/G-protein and the maximal activity of the agonist or the amplification factor (ratio of dissociation constant for binding to receptor to EC_{50} in functional assay).

4 A comparison was made between the profiles of the $D_{2(short)}$ and $D_{2(long)}$ receptor isoforms in these assays.

Keywords: Dopamine; receptors; ligand binding; [^{35}S]-GTPγS binding; agonist action

Introduction

Five subtypes of dopamine receptor have been identified (D_1–D_5) and these may be divided into two subfamilies (the $D_{1\text{-like}}$ receptors (D_1, D_5) and the $D_{2\text{-like}}$ receptors (D_2, D_3, D_4)) on the basis of DNA sequence and functional properties (Sibley & Monsma, 1992; Civelli *et al*., 1993; Neve & Neve, 1997). The dopamine receptors all signal via coupling to G-proteins and the members of the $D_{2\text{-like}}$ subfamily have all been shown to inhibit adenylyl cyclase when expressed in Chinese hamster ovary (CHO) cells (Chio *et al*., 1994; Gardner *et al*., 1996) although linkage to other second messenger responses has also been observed in other systems (Vallar & Meldolesi, 1989). The $D_{2\text{-like}}$ receptors also exist in variant forms e.g. the $D_{2(short)}$ and $D_{2(long)}$ receptors generated by alternative splicing of a common gene. These isoforms may have small differences in their abilities to bind drugs (Castro & Strange, 1993; Malmberg *et al*., 1993) and they may also signal via different G-proteins (Montmayeur *et al*., 1993; Senogles, 1994), although the precise specificity of these receptor/G-protein interactions has not been defined.

For G-protein linked receptors such as the dopamine receptors it is thought that an agonist binds to the receptor (R) to stabilize a form of the receptor (R*) that is able to couple to the G-protein (Lefkowitz *et al*., 1993; Samama *et al*., 1993). The agonist also stabilizes the receptor/G-protein complex (R*G) in the form of the ternary complex (AR*G). In the ternary complex guanosine 5′-diphosphate (GDP) attached to the G-protein is exchanged for guanosine 5′-triphosphate (GTP) and the ternary complex bearing GTP disintegrates to yield α and $\beta\gamma$ subunits of the G-protein that can influence the activity of effector proteins. There is also some suggestion, based on the results of mutations, that the breakdown of the ternary complex may be regulated by agonists (Hausdorff *et al*., 1990; Van Koppen *et al*., 1994). Therefore the agonist may have three or more effects on the pathway from agonist binding to the agonist-mediated signal.

For receptors such as the D_2 dopamine receptor a spectrum of chemically different agonists exists and these can exhibit different potencies and extents of agonism, i.e. some can be full, some can be partial agonists, some can have a high potency, some low potency. In terms of understanding drug action it is important to understand the molecular basis of these differences. Therefore, we have examined a range of agonists for their abilities to bind to the receptor and to stimulate [^{35}S]-guanosine-5′-*O*-(γ-thiotriphosphate) binding, a functional test for receptor/G-protein coupling (Gardner *et al*., 1996). We have used the human $D_{2(long)}$ receptor expressed in CHO cells as a model system for these studies. We have previously obtained data on agonist effects at the $D_{2(short)}$ dopamine receptor (Gardner *et al*., 1997) and a comparison between the two receptor isoforms can now be made using these assay systems.

Methods

Cell culture

CHO-D2L cells expressing the recombinant human $D_{2(long)}$ dopamine receptor gene (Hayes *et al*., 1992) were grown in RPMI 1640 medium supplemented with 2 mM glutamine, 5% foetal bovine serum and 200 μg ml^{-1} active geneticin in an atmosphere of 5% CO_2 at 37°C. Cells were passaged every 4–5 days.

Preparation of cell membranes

The cells were grown to confluency in 175 cm^2 flasks, the medium was removed, and the cells were washed with 10 ml of

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Buffer A (20 mM HEPES, 6 mM $MgCl_2$, 1 mM EDTA and 1 mM EGTA, pH 7.4) at 4°C. The wash buffer was removed and the cells were then scraped from the flasks into 5 ml of buffer A and homogenized with 30 strokes of a Dounce homogenizer. The homogenate was centrifuged at 1,700 *g* for 10 min at 4°C and the resulting supernatant was centrifuged at 48,000 *g* for 1 h at 4°C. The pellet from this centrifugation was resuspended in Buffer A and again centrifuged at 48,000 *g* for 1 h at 4°C. The resulting pellet was resuspended in Buffer A at a concentration of 2–3 mg protein ml^{-1} and stored at −80°C before use.

Radioligand binding assays

Cell membranes (25–75 μg) were incubated with [^{3}H]-spiperone (0.3 nM for competition experiments, 40 pM to 2 nM in saturation experiments) and competing drugs in Buffer B (20 mM HEPES, 10 mM $MgCl_2$ and 100 mM NaCl, pH 7.4 supplemented with 0.1 mM dithiothreitol) in a final volume of 1 ml for 45 min at 25°C. The assay was terminated by rapid filtration using a Brandel cell harvester and unbound radioligand removed with four washes of 4 ml of ice-cold phosphate buffered saline (0.14 M NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 5 mM Na_2HPO_4, pH 7.4). Filters were soaked for at least 6 h in 2 ml LKB optiphase 'Hisafe' 3 scintillation fluid after which bound radioactivity was determined by liquid scintillation counting. Non-specific binding was defined in saturation and competition experiments in the presence of 3 μM (+)-butaclamol. In saturation experiments the total binding was determined in the presence of 3 μM (−)-butaclamol.

[^{35}S]-GTPγS binding assays

The [^{35}S]-GTPγS binding assay was carried out essentially as described by Gardner *et al.* (1996). Cell membranes (25–75 μg) were incubated in Buffer B containing 0.1 mM dithiothreitol (DTT) and 1 μM GDP and drugs in a volume of 0.9 ml for 30 min at 30°C. This preincubation ensured that the agonists tested were at equilibrium when the [^{35}S]-GTPγS (50–150 pM, final concentration) was added (in 100 μl of Buffer B) to initiate the reaction. The assay mixture was incubated for a further 20 min unless otherwise stated. The assays were terminated by rapid filtration and bound radioactivity determined as described for the radio-ligand binding assays above. The total binding of [^{35}S]-GTPγS was less than 20% of that added.

Data analysis

Data from the [^{35}S]-GTPγS binding assays were analysed by non-linear regression analysis using the 'Inplot' curve fitting programme (GraphPad). The percentage stimulation of [^{35}S]-GTPγS binding by agonist was calculated by dividing the total binding of [^{35}S]-GTPγS observed in the presence of agonist by total binding observed in the absence of agonist. Radioligand binding experiments were analysed with 'LIGAND' (Elsevier-BIOSOFT). Radioligand binding data were assumed to conform to a one site model unless a statistically significant improvement was obtained with a two site fit.

Materials

[^{35}S]-GTPγS was purchased from Du Pont, [^{3}H]-spiperone was purchased from Amersham. Dopamine receptor agonists and (+)- and (−)-butaclamol were purchased from RBI. All other materials were of the highest commercial purity available.

Results

Ligand binding

Saturation analysis of [^{3}H]-spiperone binding to $D_{2(long)}$ dopamine receptors expressed in CHO cells (CHO-D2L cells) indicated a single class of high affinity binding sites (K_d 70 pM, pK_d 10.15±0.08, B_{max} 1.3±0.1 pmol mg^{-1} protein, mean± s.e.mean, $n=7$). The binding of agonists to membranes of the CHO-D2L cells was assessed by competition *versus* [^{3}H]-spiperone binding (Figure 1, Table 1). Competition of apomorphine, dopamine, quinpirole, R(−)-10,11-dihydroxy-N-n-propylnoraporphine hydrochloride (NPA), talipexole, pergolide and ropinirole *versus* [^{3}H]-spiperone gave data that were best described by a two site model in the absence of added guanine nucleotides and the affinities of the higher and lower affinity sites (K_h, K_l) were derived. However, in the presence of 100 μM GTP, the data were best described by a one site model. The affinity of the lower affinity site observed in the absence of GTP (K_l) and that of the single site observed in the presence of GTP ($K_{i,GTP}$) were similar. The % of the two classes of sites for these agonists was between about 25 and 50%. For dopamine and apomorphine, 100 μM GDP had a similar effect on the binding of these agonists to that seen with GTP.

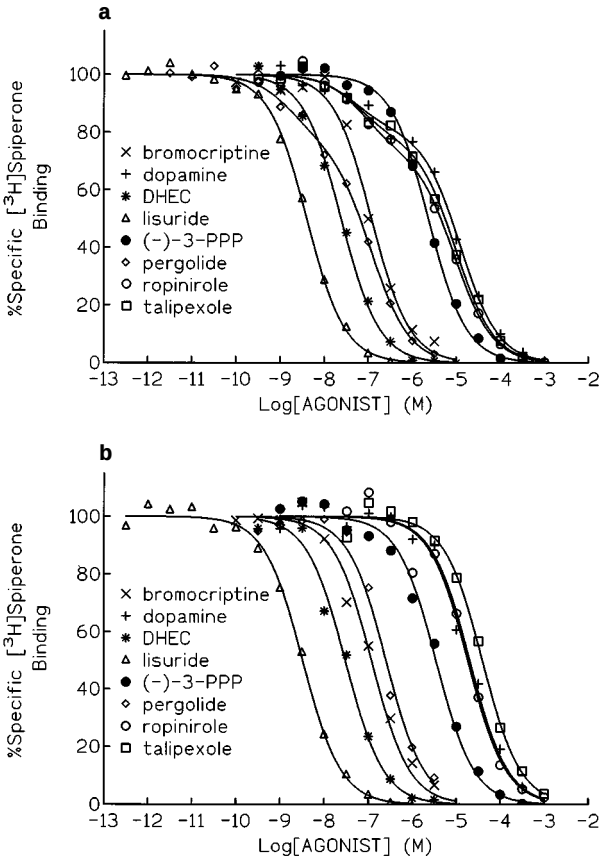

Figure 1 Agonist binding to $D_{2(long)}$ dopamine receptors expressed in CHO cells. The binding of the agonists bromocriptine, dopamine, DHEC, lisuride, (−)-3-PPP, pergolide, ropinirole and talipexole was determined as described in Methods in competition assays *versus* [^{3}H]-spiperone in the absence (a) and in the presence (b) of 100 μM GTP. The data shown are from single experiments replicated as in Table 1.

Table 1 The binding of agonists to $D_{2(long)}$ dopamine receptors

Agonist	pK_h (K_h, nM)	$\%R_h$	pK_l (K_i, nM)	n	pK_{iGTP} (K_{iGTP}, nM)	n	pK_{iGDP} (K_{iGDP}, nM)	n
Apomorphine	8.89±0.16 (1.3)	26±2	7.07±0.08 (85)	3	6.78±0.02 (166)	3	6.58±0.08 (266)	2
Bromocriptine	–		8.05±0.20 (8.8)	2	8.01±0.07 (9.7)	2	–	
DHEC	–		8.26±0.07 (5.5)	2	8.20±0.02 (6.2)	2	–	
Dopamine	7.82±0.18 (15)	27±4	5.48±0.11 (3300)	4	5.21±0.12 (6200)	4	5.05±0.15 (9000)	2
Lisuride	–		9.15±0.06 (0.7)	4	9.07±0.13 (0.9)	3	–	
NPA	9.42±0.13 (0.38)	45±5	7.79±0.04 (16)	3	7.73±0.04 (18)	3	–	
(−)-3-PPP	–		6.33±0.05 (464)	3	6.07±0.12 (848)	3	–	
Pergolide	9.60±0.23 (0.5)	24±4	7.83±0.09 (15)	3	7.66±0.09 (22)	3	–	
Quinpirole	6.92±0.15 (120)	36±6	5.28±0.06 (5300)	4	5.20±0.08 (6400)	4	–	
Ropinirole	7.41±0.21 (39)	35±3	5.70±0.07 (2000)	4	5.74±0.10 (1800)	3	–	
Talipexole	7.11±0.39 (78)	45±12	5.65±0.07 (2200)	3	5.49±0.13 (3200)	3	–	

Agonist binding was determined in competition *versus* [^{3}H]-spiperone in the absence and presence of GTP or GDP (100 μM) as described in the Methods section and the competition curves were analysed by non linear regression analysis to derive dissociation constants for the higher (K_h) and lower (K_l) affinity states and the percentage of higher affinity states ($\%R_h$) where a two site model provided a better description of the data; single values of the dissociation constant (K_l or $K_{i,GTP}$) are given where a one binding site model provides the better description. Data are mean±s.e.mean ($n \geqslant 3$) or mean±range ($n=2$).

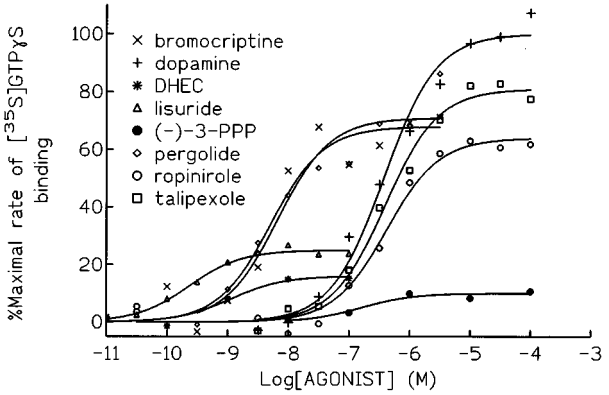

Figure 2 Agonist stimulation of [^{35}S]-GTPγS binding through $D_{2(long)}$ dopamine receptors expressed in CHO cells. The stimulation of [^{35}S]-GTPγS binding by the agonists bromocriptine, dopamine, DHEC, lisuride, (−)-3-PPP, pergolide, ropinirole and talipexole was determined as described in Methods. The data shown are from single experiments replicated as in Table 2.

Table 2 Stimulation of [^{35}S]-GTPγS binding by agonists at $D_{2(long)}$ dopamine receptors expressed in CHO cells

Agonist	pEC_{50} (EC_{50}, nM)	*Maximal effect* (% of dopamine effect)	n
Apomorphine	6.76±0.12 (174)	90±18	3
Bromocriptine	8.15±0.05 (7.1)	58±6*	7
DHEC	8.86±0.09 (1.4)	16±8*	2
Dopamine	6.25±0.11 (562)	100	9
Lisuride	9.29±0.06 (0.5)	36±6*	4
NPA	7.76±0.15 (18)	114±7	4
Quinpirole	5.85±0.10 (1400)	96±5	6
(−)-3-PPP	6.43±0.19 (241)	10±2*	2
Pergolide	8.13±0.15 (7.4)	73±1*	4
Ropinirole	6.46±0.08 (347)	64±4*	3
Talipexole	6.55±0.10 (281)	78±1*	3

Stimulation of [^{35}S]-GTPγS binding was determined for a range of agonist concentrations after a fixed time of incubation (20 min) as described in the Methods section. EC_{50} values and maximal effects were determined and data are expressed as mean±s.e.mean for n observations ($n \geqslant 3$) or mean±range ($n=2$). *Significantly different from the dopamine response ($P<0.05$).

For S(−)-3-(3-hydroxyphenyl)-N-propylpiperidine hydrochloride ((−)-3-PPP) the competition curves in the absence and presence of GTP were best described by a one site model although there was a significant decrease in the affinity observed in the presence of 100 μM GTP (paired t test, $P<0.05$). Competition curves for bromocriptine, dihydroergocristine (DHEC) and lisuride were best described by a one site model and there was no significant effect of the addition of 100 μM GTP on the competition curves ($P>0.05$).

Agonist stimulation of [^{35}S]-GTPγS binding

The stimulation of the rate of [^{35}S]-GTPγS binding by agonists was investigated over a range of appropriate concentrations of test compound. From the concentration-effect curves (Figure 2) the pEC_{50} and maximal agonist responses were determined for the agonists tested (Table 2). The Hill coefficients for all the agonists (except (−)-3-PPP and DHEC did not significantly differ from 1 (paired t test, $P>0.05$). For DHEC and (−)-3-

PPP the response was small and variable and so it was difficult to measure Hill coefficients accurately. Consequently all data were analysed with Hill coefficients constrained to 1. The agonists dopamine, quinpirole, apomorphine and NPA gave similar maximal responses whereas for all other agonists significantly smaller maximal responses were observed ($P<0.05$) (Table 2).

Discussion

In this study we have examined the effects of a series of dopamine agonists at the $D_{2(long)}$ dopamine receptor using ligand binding and a functional assay, the stimulation of [^{35}S]-GTPγS binding. The aim of the experiments was to try to understand the mechanistic basis of agonist action in more detail. The results show that using these *in vitro* assays it is possible to make measurements of agonist affinity, potency and maximal functional effect. However, no simple relationship was seen between the ability of an agonist to promote formation of the ternary complex (AR*G) and the maximal functional effect.

Currently the accepted view of agonist action at G-protein coupled receptors is summarized in the extended ternary complex model (Lefkowitz *et al*., 1993; Samama *et al*., 1993). In this model, in the absence of agonist, the receptor can exist in two forms R and R* and only the latter can couple to the G-protein. Agonists bind preferentially to R* and thus stabilize this form of the receptor. Formation of the R*G complex is also stabilized by the agonist so that the net result of agonist action is to promote formation of the ternary complex AR*G. The agonist, therefore, has two effects to promote the formation of the ternary complex, on the stabilization of R* and the stabilization of R*G. Once the ternary complex has formed, exchange of GDP attached to the G-protein for GTP is facilitated and when GTP is bound the complex dissociates in to the α- and $\beta\gamma$-subunits of the G-protein which can then alter the activities of effector molecules. There is some evidence that as well as promoting the formation of the ternary complex the agonist promotes its breakdown (Hausdorff *et al*., 1990; Birnbaumer *et al*., 1990; Van Koppen *et al*., 1994), so that there may be three places in this series of events where the agonist can act.

Different aspects of this series of events may be assayed in order to understand how agonists act. In ligand binding assays agonists are thought to bind with higher affinity to the receptor coupled to G-protein than to the uncoupled receptor (Wregget & De Lean, 1984; Lefkowitz *et al*., 1993). This can lead in agonist/^{3}H-antagonist competition experiments to complexity in agonist binding which may be interpreted in terms of higher and lower affinity binding states. These higher and lower affinity states cannot be directly equated with the coupled and uncoupled receptors (Wregget & De Lean, 1984; Lee *et al*., 1986) although the ratio of the two affinities is a measure of the stabilization of the ternary complex by the agonist. The guanine nucleotide exchange event may be assayed using the binding of [^{35}S]-GTPγS (Gardner *et al*., 1996). This assay provides a measure of the rate of GDP/GTP exchange which should be related to the overall functional response in the system.

In the present experiments we have used ligand binding assays *versus* [^{3}H]-spiperone to determine the properties of a series of agonists at human $D_{2(long)}$ dopamine receptors expressed in CHO cells. For many of the agonists (apomorphine, dopamine, NPA, pergolide, quinpirole, ropinirole, talipexole) competition curves could be resolved in to contributions from higher (K_h) and lower (K_l) affinity sites and in the presence of GTP a single class of sites was seen with an affinity ($K_{i,GTP}$) similar to that seen for the lower affinity population in the absence of GTP (K_l). As Lee *et al*. (1986) have suggested this pattern is consistent with a situation where there is an excess of receptor over G-protein, in which case the value for K_l($K_{i,GTP}$) corresponds to the affinity of agonist for the receptor uncoupled from G-protein. However, for other agonists (bromocriptine, DHEC, lisuride, 3-PPP), a single binding site model provided a good description of data in the absence or presence of GTP and in some cases the affinity was reduced significantly by GTP (3-PPP) and in other cases it was not. For two agonists, apomorphine and dopamine, GDP was found to have similar effects to GTP. Whereas GTP is thought to lead to breakdown of the ternary complex, GDP probably sequesters G-protein α-subunits preventing ternary complex formation, the net result in either case being low affinity agonist binding to the free receptor.

A functional measure of agonist action was obtained using the stimulation of [^{35}S]-GTPγS binding and this provided data on the maximal effect of the agonist as well as the potency. A spectrum of maximal activities was seen from partial to full agonists and the rank order of the values obtained was generally in agreement with data obtained in other functional assays (see for example McDonald *et al*., 1984; Meller *et al*., 1991). However, there was no relationship between maximal effect and potency, with some agonists being very potent but only producing a partial effect, e.g. lisuride, but others producing a high maximal effect but with rather lower potency, e.g. quinpirole.

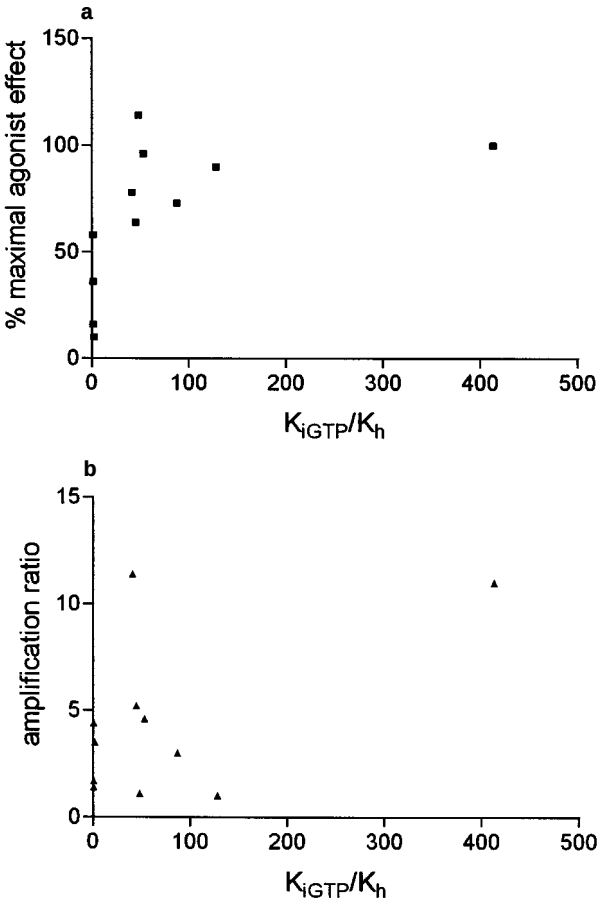

Figure 3 Relations between efficacy parameters for agonists at $D_{2(long)}$ dopamine receptors. The graphs show the relation between the K_{iGTP}/K_h ratio for the agonists tested and their maximal effect (a) and amplification ratio (b) as given in Table 3.

The ternary complex model discussed above predicts that the complexity seen in agonist binding experiments should relate to agonist efficacy in some way. In the present experiments the complexity in agonist binding is manifest in the higher and lower affinities seen for some agonists when they are used to inhibit [^{3}H]-spiperone binding and data are analysed in terms of two independent sites. It has been shown that the ratio of the affinities ($K_{i,GTP}/K_h$, see above) is a measure of the stabilization of the ternary complex by the agonist and this should correlate to agonist efficacy (De Lean *et al*., 1980; Wregget & De Lean, 1984; Samama *et al*., 1993; Kenakin, 1993). For D_2 dopamine receptors, Lahti *et al*. (1992) and Harley *et al*. (1995) tested this idea and determined a $K_{i,GTP}/K_h$ ratio and in both studies a correlation was claimed between this ratio and agonist efficacy (maximal agonist effect and relative agonist efficacy respectively). The method for determining the $K_{i,GTP}/K_h$ ratio was slightly different from that used here and involved agonist competition experiments *versus* a tritiated agonist in the absence of added guanine nucleotides (K_h), and *versus* a tritiated antagonist in the presence of GTP ($K_{i,GTP}$). There were also ionic differences in the buffers used for the two determinations. There is no theoretical basis for making these determinations under different conditions and the method resulted in values less than one for some agonists. Also Lahti *et al*. (1992) normalized their data for full agonists to take account of the fact that these gave different $K_{i,GTP}/K_h$ ratios. O'Boyle & Lawlor (1996) used a similar method for determining $K_{i,GTP}/K_h$ to that used in the present study together with the normalized efficacy data of Lahti *et al*. (1992) and although a correlation was obtained this included four agonists with $K_{i,GTP}/K_h$ ratios of unity.

In the present experiments we have sought correlations between the $K_{i,GTP}/K_h$ ratio and measures of agonist efficacy such as the maximal agonist effect in the functional assay, stimulation of [^{35}S]-GTPγS binding. No clear correlation emerges from a comparison of these data (Figure 3) and the agonists may be divided in to two sub-groups as follows. In the first sub-group the agonists show a high $K_{i,GTP}/K_h$ ratio ($> \sim 40$) and there is a high maximal agonist effect (64% or more of the dopamine effect). It seems that if the ternary complex is well stabilized then a high maximal agonist effect is observed and although there is a tendency for higher values of $K_{i,GTP}/K_h$ to be associated with higher maximal agonist activity and lower values with lower activity, no clear correlation is seen between the two quantities. This lack of correlation implies that there is further complexity in the system. We have described a similar lack of correlation but with less extensive data for the $D_{2(short)}$ dopamine receptor (Gardner *et al*., 1997). In the second sub-group the agonists show $K_{i,GTP}/K_h$ ratios close to one and these compounds are partial agonists. In this sub-group, therefore, agonism may be achieved in the absence of apparent stabilization of the ternary complex.

The lack of correlation between the ability of the agonist to stabilize the ternary complex and the maximal agonist effect implies additional complexity and this may reside in the models of agonism outlined earlier. The extended ternary complex model proposes that agonists may influence efficacy at two stages, stabilization of R* over R, and stabilization of R*G over R*. The binding of an agonist to the receptor in the absence of G-protein coupling ($K_{i,GTP}$) will lead to stabilization of R* to an extent that depends on the different affinities of the agonist for R and R*, but the apparent affinity of the agonist will be between the affinity for R and R*. Thus the $K_{i,GTP}$ values determined in the present study will not be an accurate estimate of the affinity for R unless there is little stabilization of R*, and this is unlikely except for the very poor agonists. Therefore estimates of stabilization of ternary complex formation based on the simple $K_{i,GTP}/K_h$ ratio may underestimate stabilization at the R*/R step. It is presently impossible to quantify this stabilization.

The ergolines and ergopeptines (bromocriptine, DHEC and lisuride) may be extreme examples of this effect. These compounds are agonists but exhibit no apparent stabilization of the ternary complex based on $K_{i,GTP}/K_h$ ratios. Similar observations have been made before for the D_2 dopamine receptor and 5-HT_{1A} (5-hydroxytryptamine) receptor (Sibley & Creese, 1983; Sundaram *et al*., 1995; Gardner *et al*., 1997). For these compounds binding to the receptor in the absence of G-protein coupling may strongly stabilize the activated form of the receptor (AR*) and there may be little energy gained in ternary complex (AR*G) formation, ie AR* is close in structure to AR*G. This would give agonism but there would be no difference between binding affinities in the absence and presence of GTP as described here. For these compounds the binding energy between receptor and ligand is fully expressed upon binding to the free receptor.

It would be useful to estimate the degree of amplification (the extent to which potency for an agonist in a functional assay is greater than its affinity for binding to the receptor) for different agonists as this is another important part of agonist efficacy. Indeed for full agonists this may be more informative than the maximal effect in probing efficacy, as depending on

Table 3 Comparison of agonist binding and functional parameters on $D_{2(short)}$ and $D_{2(long)}$ dopamine receptors

	$K_{i,GTP}$ (nM)		EC_{50} (nM)		*Maximal agonist effect*		$K_{i,GTP}/K_h$		*Amplification ratio*	
Agonist	$D_{2(short)}$	$D_{2(long)}$	$D_{2(short)}$	$D_{2(long)}$	$D_{2(short)}$	$D_{2(long)}$	$D_{2(short)}$	$D_{2(long)}$	$D_{2(short)}$	$D_{2(long)}$
Apomorphine	208	166	120	174	88	90	128	128	1.7	1
Bromocriptine	17	9.7	5.2	7.1	58	58	1	1	3.3	1.4
Dopamine	10700	6200	1300	562	100	100	25	413	8.2	11
DHEC	6.0	6.2	5.5	1.4	23	16	1	1	1.1	4.4
Lisuride	–	0.9	–	0.5	–	36	–	1	–	1.7
NPA	23	18	3.3	17.5	105	114	88	48	7.0	1.1
(−)-3-PPP	1300	848	288	241	9	10	2	2	4.5	3.5
Pergolide	–	22	–	7.4	–	73	–	87	–	3
Quinpirole	10000	6400	390	1400	94	96	385	53	25.6	4.6
Ropinirole	–	1800	–	347	–	64	–	45	–	5.2
Talipexole	–	3200	–	281	–	78	–	41	–	11.4

The parameters shown are $K_{i,GTP}$, (the dissociation constant in ligand binding assays in the presence of 100 μM GTP), the EC_{50} (the concentration of agonist giving half-maximal stimulation of [^{35}S]-GTPγS binding), the maximal agonist effect in the [^{35}S]-GTPγS binding assays, $K_{i,GTP}/K_h$ (the ratio of apparent dissociation constants in agonist binding assays in the absence of added guanine nucleotides) and the amplification ratio, ($K_{i,GTP}/EC_{50}$). These parameters are discussed in more detail in Gardner *et al*. (1997) and the data for $D_{2(short)}$ are taken from Gardner *et al*. (1997).

the system there may be a ceiling on the maximal agonist effect possible but agonists may still differ in their degree of amplification. In experiments on the $D_{2(short)}$ dopamine receptor we showed that the amplification ratio could be estimated from the $K_{i,GTP}/EC_{50}$ ratio (Gardner *et al*., 1997) and these data are given together with data for the $D_{2(long)}$ receptor in Table 3. It might be expected that the amplification ratio would be related at least in part to the ability of the agonist to stabilize the ternary complex ($K_{i,GTP}/K_h$). There is a slight tendency for a higher value of $K_{i,GTP}/K_h$ to be associated with a high amplification ratio (Figure 3) and a lower value with a low amplification ratio but there are many exceptions. Notable here are apomorphine and NPA which appear to stabilize the ternary complex well but provide a low amplification ratio. At the $D_{2(short)}$ dopamine receptor similar findings were obtained for apomorphine (Gardner *et al*., 1996). A possible explanation for these findings is that these compounds stabilize the ternary complex well, but that for these compounds breakdown of the ternary complex occurs poorly. This would be consistent with the observations of Birnbaumer *et al*. (1990), Hausdorff *et al*. (1990) and Van Koppen *et al*. (1994) indicating that ternary complex breakdown is an event regulated by agonists and can be rate determining. Alternatively, it is possible that for these compounds guanine nucleotide (GDP/GTP) exchange is slow.

The ternary complex models discussed here may also be incomplete (see for example Lee *et al*., 1986) and this may lead to some of the discrepancies highlighted in the present study. It has been proposed that there may not be free diffusion of receptors and G-proteins in the plane of the membrane (Wreggett, 1987; Neubig *et al*., 1988). Also it has been suggested that receptors and G-proteins may exist as heteroligomers (Jahangeer & Rodbell, 1993; Sinkins & Wells, 1993; Wreggett & Wells, 1995) and models to describe such behaviour have been outlined. For the β_2-adrenoceptor (Hebert *et al*., 1996) and the D_2 dopamine receptor (Ng *et al*., 1996) there is some evidence that the receptors may exist as dimers, although these observations require further validation.

It is also of some interest to compare agonist actions at the two isoforms of the D_2 receptor, $D_{2(short)}$ and $D_{2(long)}$, as these have been shown to couple to effectors via different G-proteins (Montmayeur *et al*., 1993; Senogles, 1994) and this may lead to different agonist efficacy patterns. Data for several agonists are given in Table 3 for the two isoforms. Whereas binding affinities at the two receptors are very similar for the low affinity state and maximal agonist effects are similar, there are significant differences where measures of agonist amplification are concerned. In particular there are differences in the rank orders for the amplification ratios for the set of agonists tested on the two receptors. This suggests that for the two isoforms of the D_2 receptor there is some difference in the activation process. This could reflect the coupling of the two isoforms to different G-proteins as has been suggested from some experimental work (Montmayeur *et al*., 1993; Senogles, 1994). This would then represent a form of agonist trafficking whereby the particular receptor/G-protein combination exhibits a particular amplification-ratio-pattern for a range of agonists. It may be possible, therefore, to use the amplification-ratio-pattern in functional *in vivo* tests to identify the contribution of receptor isoforms.

In conclusion, the data presented here show that it is possible to obtain measures of agonist binding and efficacy at D_2 dopamine receptors using simple *in vitro* tests. Differences in agonist efficacy patterns may be apparent for different receptor isoforms.

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