The neurotransmitter dopamine has been implicated in the aetiology of brain disorders such as Parkinson's disease and schizophrenia, and dopamine receptors provide important targets for anti-parkinsonian and antipsychotic drugs. In particular, the beneficial use of dopamine D_2 or D_1/D_2 agonists in the drug treatment of Parkinson's disease is now well established [1], although the ideal agonist has not yet been identified. Alternative strategies for the treatment of this condition which are currently being evaluated include the use of D_2 partial agonists alone or in combination with a full-efficacy D_1 agonist [2]. Another proposed therapeutic application for D_2 receptor partial agonists is in the treatment of psychostimulant addiction [3]. Hence, elucidation of the molecular mechanisms underlying drug–receptor interaction and response and a simple procedure for predicting agonist intrinsic activity in vitro would facilitate the design and development of new agents with therapeutic potential.

**Background**

Agonist ligands (hormones, neurotransmitters or drugs) are defined by their ability to elicit effects in biological systems through interaction with receptors (i.e. agonists have intrinsic activity). Antagonist ligands, on the other hand, lack intrinsic activity and are defined by their lack of demonstrable biological activity when used in isolation; they are thought to act by passive occupancy of the agonist binding site, resulting in blockade of agonist functions. Partial agonists are defined as ligands with lower efficacy than a 'full' agonist and their efficacies are determined from the plateau of their concentration–response curves [4]. An understanding of the molecular mechanisms through which agonists activate specific receptors has been a major goal of modern pharmacology. This process begins with the agonist binding to the membrane-bound receptor and ends with a series of cellular responses; in between, there are a series of complex transduction steps that translate the action of receptor binding into biological responses. The receptors for many central nervous system transmitters, including those for dopamine, belong to the superfamily of seven transmembrane receptors that mediate their effects via coupling to guanine nucleotide-binding proteins (G-proteins) [5]. The dynamics of G-protein signal transduction have been the subject of much investigation [6,7]. Key steps involved in this process include receptor-induced, GTP-dependent G-protein activation, dissociation of G-proteins into free α-GTP complex and free βγ dimers, effector activation by α-subunits but also by βγ, and finally hydrolysis of GTP by α-subunits.

**Dopamine receptors: structural determinants of ligand binding**

Dopaminergic receptors fall into two groups on the basis of biochemical, pharmacological, physiological and, more recently, molecular biological criteria [8,9]. In common with other G-protein-coupled receptors, both D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) receptors are composed of a single polypeptide chain with seven relatively hydrophobic domains, which are thought to form membrane-spanning α-helices, interspersed between three sets of intra- and extracellular loops. The ligand binding site is thought to be formed by the bunching of these α-helices, and much effort has been directed towards identifying the sites on the receptor that are critical for ligand binding. Several amino acids that are conserved within the dopaminergic family and across the seven transmembrane superfamily have been identified [10] and are presumed to be involved in the ligand binding process. However, it is clear from studies using site-directed mutagenesis that particular amino acid residues may participate in ligand binding in different ways at different receptor classes. It is also apparent that the determinants of the binding of dopaminergic agonists and antagonists are not identical, although they may overlap [11]. Indeed, recent studies at the D_2 dopamine receptor suggest that agonist drugs interact with distinct subsets of binding sites and that the efficacy of agonists is determined by distinct, partially overlapping subsets of amino acids [12]. When this technology was initially developed it was hoped that it would prove to be a great asset to drug design programmes. However, to date it appears that no single hypothesis can be developed to describe
either the structural determinants of ligand binding to D₂ dopamine receptors or the structure–function relationships at these receptors. Furthermore, it has been argued that it is unlikely that a single pharmacophore for efficacy exists [12]. This point is particularly interesting in light of the proposal that the particular chemical structure of an agonist may determine which form of activated receptor results (if multiple forms are possible), and hence which second-messenger system is activated [13].

**Binding properties of agonists**
Against this background of largely unsuccessful efforts to generate a single model to describe how and where different agonists bind to the D₂ receptor, it is perhaps worthwhile revisiting an approach used in the past to explore the relationship between ligand binding data and agonist efficacy. Early studies on the mechanisms of interaction between ligands and the β-adrenoceptor identified several important properties that were unique to agonists and not shared by antagonists. For example, antagonist/³H-labelled antagonist competition curves are steep, indicative of homogeneous binding to a single site, whereas agonist/³H-labelled antagonist competition curves are typically shallow, reflecting heterogeneous binding to high and low-affinity sites [14]. In the presence of guanine nucleotides, agonist/³H-labelled antagonist competition curves are shifted to the right and become steeper owing to the interconversion of agonist high- and low-affinity states [15]. Similar observations have since been made for many G-protein-coupled receptors [16,17], including those for dopamine [18,19]. More recently, it has been reported that agonists interact with their receptors in a mixed competitive/non-competitive fashion, whereas antagonists act in a purely competitive manner [20,21]. Furthermore, many G-protein coupled receptors, such as the α₂-adrenoceptor and D₁ and D₂ dopaminergic receptors, exhibit decreased affinity for agonists in the presence of Na⁺ [22–24]. When the kinetics of agonist and antagonist binding to [³H]-agonist labelled β-adrenoceptors was examined it was found that, while the rate constants of dissociation were similar for agonists and antagonists, the rate constants of association were consistently slower for agonist binding than for antagonist binding [25]. When changes in thermodynamic parameters associated with binding of agonists and antagonists were compared, the affinity of agonist binding was seen to increase as the incubation temperature was decreased and there was a net decrease in entropy when agonists bound; antagonists, on the other hand, were relatively insensitive to temperature and binding was associated with an increase in entropy [26]. Many of these differences between agonist and antagonist binding relate to the ability of agonists to recognize two interconvertible affinity states of the receptor, while antagonists only recognize a single state. Transition of receptors from low- to high- and back to low-affinity states for agonists is an integral part of signal transduction by G-protein coupled receptors [6]. The prevailing view is that spontaneous equilibrium exists between free receptor (R) and free G-protein (G) on the one hand and a heterodimeric complex (RG) on the other [27]; agonists are thought to bind with higher affinity to RG than to R and thereby stabilize the RG complex [28]. In contrast, antagonists either show no preference for RG over R, or exhibit the opposite preference to agonists. This ternary complex model [29] has been reported to describe the binding of agonists to a number of G-protein-coupled receptors [30–32] including D₁ dopamine [33]. It must be noted, however, that a model based on co-operative interactions rather than ternary complex formation has been proposed to explain the binding of histamine agonists to [³H]-histamine-labelled sites [34]. Thus, across a number of different classes of G-protein-coupled receptors, there is broad agreement that the formation of high-affinity agonist–receptor–G-protein complexes are necessary for activation of the G-proteins.

**Relationship between agonist binding properties and intrinsic activity**
One of the first studies to address the issue of whether values derived from ligand binding data could be correlated with the intrinsic activities of partial agonists examined a series of β-adrenoceptor agonists [15]. In this study, the ability of agonists to stimulate adenylate cyclase was found to be closely correlated with the amount of high-affinity state formed in the presence of the agonists (%RH) and the ratio of dissociation constants of the agonist for the high- and low-affinity states of the receptor (Kᵣ/Kᵢ). All the partial agonists, even those with intrinsic activities less than 0.1, bound to a high-affinity site which amounted to at least 50% of total binding sites; full agonists bound to ≥ 80% of sites with
high affinity. When the relative efficacies of six muscarinic agonists for stimulation of phosphoinositide breakdown and Ca\(^{2+}\) mobilization was investigated, a similar correlation between intrinsic activity and KJKH ratio for each agonist was found [17]. However, a significant correlation between intrinsic activity and %RH was obtained only for Ca\(^{2+}\) mobilization and not for phosphoinositide breakdown. This was despite the fact that the rank order of agonist efficacy was the same for the two biochemical responses. The percentage of high-affinity sites recognized by agonists ranged from 0 to 58% of total sites. Using a more complex approach, Lahti et al. [35] made essentially the same observation for D\(_2\) receptor agonists, namely that intrinsic activity was correlated with KJKH ratios. They used \(^3\)H-labelled agonist to label agonist high-affinity state and \(^3\)H-labelled antagonist + GTP to label agonist low-affinity states, thereby ensuring that a K\(_H\) could be determined for all agonists. Analysis of the thermodynamic properties of agonist interaction with \(\beta\)-adrenoceptors showed a good correlation between agonist efficacy and both entropy and enthalpy changes [36]. Using an alternative approach to measure agonist interaction with high-affinity states of the D\(_2\) dopamine receptor, a strong correlation between agonist-induced masking of \(^3\)H-labelled antagonist and agonist intrinsic activity was noted [21]. There is not complete agreement between groups that agonist activity is related to a high-affinity site since at the 5-HT\(_{1A}\) receptor agonists potency was described as a function of affinity for the low-affinity state of \(^3\)H-labelled agonist binding [37]. This apparently anomalous finding may arise at least in part because the receptors were labelled with an agonist.

An interesting extension of the above investigations has been to determine the effect of receptor desensitization on agonist binding measures. A surprising result was obtained when \(\beta\)-adrenoceptors were desensitized by chronic exposure to desipramine. Despite a substantial loss of agonist efficacy, the K\(_l\)/K\(_H\) ratio was increased and there was no change in %R\(_H\) [38]. However, down-regulation of rat cortical 5-HT\(_2\) receptors resulted in a decrease in %R\(_H\) and a decrease in K\(_l\)/K\(_H\) ratio [39], which is more consistent with the above data.

**Binding properties of D\(_2\) agonists and partial agonists**

In the present study, the radioligand properties of a series of structurally diverse dopamine D\(_2\) agonists and partial agonists were investigated in displacement and saturation binding experiments with a view to identifying which binding measure(s), if any, best predicted agonist intrinsic activity. D\(_2\) receptors in bovine caudate membranes were labelled with \(^3\)H]spiperone; the intrinsic activity measures of the agonists used in this study were taken from a study by Lahti et al. [35], who used electrophysiological data to derive values for agonist intrinsic activity and normalized these values relative to that for dopamine.

The agonists used and their intrinsic activities were: quinpirole, 1.04; dopamine, 1; \(R(-)\)-apomorphine, 0.73; \(S(-)\)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (\(S(-)\)-3-PPP), 0.34; terguride, 0.16; N-[(8-\(\alpha\)-2,6-dimethylergoline-8-yl)-2,2-dimethylpropanamide (SDZ 208-911), 0.11; N-[(8-\(\alpha\)-2-chloro-6-methylergoline-8-yl]-2,2-dimethylpropanamide (SDZ 208-912), -0.12.

Results from displacement experiments are summarized in Table 1. For each experiment, the data were fitted to one- and two-site models using INPLOT 4; a two-site model was adopted only when it resulted in a statistically significant better fit. Estimates were obtained for agonist affinity for a single site (K\(_S\)), Hill slope (n\(_H\)), agonist affinity for the high- (K\(_H\)) and low- (K\(_l\)) affinity states and percentage of receptors in a high affinity state (%R\(_H\)). As can be seen from the table, displacement of \(^3\)H]spiperone binding by the high-efficacy agonists quinpirole, dopamine or apomorphine was best described by a two-site model; competition curves for the other four weak or moderate partial agonists were all best fitted to a single site. This contrasts with data for agonist binding to the \(\beta\)-adrenoceptor, for which even very weak partial agonists recognized at least 50% of the receptors with high affinity [15], but agrees with data for the muscarinic receptor, for which weak partial agonists did not bind to a high-affinity site [17].

The effects of each agonist on the characteristics of \(^3\)H]spiperone binding were also determined in saturation experiments. A family of saturation curves was constructed in the absence and presence of 0.5 x 2 x 10 x K\(_S\) concentration of agonist and the apparent dissociation constant for \(^3\)H]spiperone (K\(_d\)) and the maximum number of receptors (B\(_{max}\)) were calculated. All the agonists caused a dose-dependent loss of ligand binding affinity (data not shown), indicating a competitive component to their interaction with the \(^3\)H]spiperone-labelled site. The three
Effects of dopamine D2 agonists on [3H]spiperone binding to bovine caudate dopamine D2 receptors

Displacement of [3H]spiperone was carried out in the presence of 25 nM ketanserin to occlude 5-HT2 receptors. The displacement curves for quinpirole, dopamine and R(−)apomorphine (R(−)-apo) were best described by a two-site model. The curves for all other compounds best fitted a single-site model. Each binding measure was tested for correlations with agonist intrinsic activity [35] using Pearson's correlation coefficient (r). Data shown are the means ± SEM of n = 4–6 independent experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kᵣ (nM)</th>
<th>Hill slope (nH)</th>
<th>Kᵣ (nM)</th>
<th>%Rᵢ (%)</th>
<th>Kᵣ/Kᵣ</th>
<th>%Rᵢ at 10 × Kᵣ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinpirole</td>
<td>71.0 ± 88</td>
<td>−0.50 ± 0.05</td>
<td>34.5 ± 20.7</td>
<td>38.0 ± 3.7</td>
<td>4.55 ± 0.75</td>
<td>250 ± 68</td>
</tr>
<tr>
<td>Dopamine</td>
<td>617 ± 196</td>
<td>5.56 ± 0.01</td>
<td>51.4 ± 18.6</td>
<td>44.2 ± 7.6</td>
<td>3.40 ± 0.55</td>
<td>91 ± 29</td>
</tr>
<tr>
<td>R(−)-apo</td>
<td>109 ± 16</td>
<td>−0.69 ± 0.04</td>
<td>4.12 ± 2.37</td>
<td>22.2 ± 3.9</td>
<td>0.23 ± 0.03</td>
<td>122 ± 33</td>
</tr>
<tr>
<td>S(−)-3-PPP</td>
<td>421 ± 146</td>
<td>−0.82 ± 0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Terguride</td>
<td>0.89 ± 0.06</td>
<td>−1.05 ± 0.09</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>SDZ208-911</td>
<td>1.49 ± 0.16</td>
<td>−1.01 ± 0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>SDZ208-912</td>
<td>1.21 ± 0.17</td>
<td>−0.94 ± 0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>r</td>
<td>0.832*</td>
<td>0.941**</td>
<td>—</td>
<td>—</td>
<td>0.850*</td>
<td>0.952**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01.

High-efficacy agonists that had been shown in displacement experiments to have a high-affinity binding site caused a dose-dependent loss of ligand binding sites. The other agonists had no effect on Bₘₐₓ. Data for the effects of 10 × Kᵣ agonist are shown in Table 1. Thus, the agonists with high intrinsic activities also had a non-competitive component to their interaction with D2 dopamine receptors. An agonist-induced decrease in Bₘₐₓ has been observed by others and is generally interpreted as agonist masking of ligand binding to the agonist high-affinity site [40,41]. Correlations were sought between agonist intrinsic activity and the various binding values measured. Significant correlations were found between agonist intrinsic activity and Kᵣ value (r = 0.832, P < 0.05), Hill slope (r = 0.941, P < 0.01) and Kᵣ/Kᵣ (r = 0.850, P < 0.05) and percent decrease in Bₘₐₓ induced by 2 × Kᵣ or 10 × Kᵣ agonist (r = 0.90, P < 0.01, and r = 0.95, P < 0.01 respectively). Of these, the correlation between nH and intrinsic activity (Figure 1) is probably the most useful since it is a value which can be determined for any agonist without the requirement that the agonist binds to a high-affinity site. A similar difficulty in using binding data to separate weak partial agonists from antagonists has been previously noted [35]. A relationship between agonist-induced masking of 3H-labelled antagonist binding has previously been reported for the D₂ dopamine receptor [21]. Interestingly, there was no relationship between %Rᵢ and intrinsic activity, as seen in some [15] but not all [17] previous studies.

Further insights on the relationship between agonist binding properties and agonist efficacy are now emerging as newer technology permitting receptor mutation is being combined with the more traditional approach of computer-assisted analysis of binding data. It has been reported that the β₂-adrenoceptor can be
mutated in such a way that agonist high-affinity binding is retained but that agonist activation of adenylate cyclase is impaired [42]. These data, therefore, suggest that the two properties of high-affinity agonist binding and agonist efficacy are not indistinguishable. More recently, the effect of replacing the C-terminal portion of the third intracellular loop of the \(\beta_2\)-adrenoceptor with the homologous region of the \(\alpha_2B\)-adrenoceptor was examined. It was found that the mutant receptor exhibited an increased affinity for agonists that correlated with the intrinsic activity of the agonist and that the efficacy of partial agonists was increased [43].

Taking the binding data presented here in context with the discussion above, a reasonable consensus emerges across a number of G-protein-coupled receptors that agonist intrinsic activity is related to the magnitude of difference between agonist affinity for low- versus high-affinity states of the receptor. This can be measured in ligand binding experiments as \(K_{ih}/K_{hl}\) ratios, if an agonist high-affinity site can be detected, or, better, as \(n_{H}\) even when competition curves do not reveal two sites.

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The binding of agonists and antagonists to dopamine receptors

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The application of molecular biological techniques to the dopamine receptors has revolutionized the study of these important sites of drug action. It has been shown that at least five subtypes of dopamine receptor exist (D₁, D₂, D₃, D₄, D₅) with different pharmacological and functional properties and different localizations [1-3]. The dopamine receptor subtypes defined on the basis of cloned genes may be subdivided into two subfamilies on the basis of the structure of the gene product and its properties once expressed. The two subfamilies D₁, D₃ and D₂, D₃, D₄ correspond to the two classes of dopamine receptors (D₁, D₂) defined using pharmacological and biochemical techniques before the advent of gene cloning, and consequently the subfamilies are termed D₁-like and D₂-like. The dopamine receptors bind the native catecholamine dopamine and related compounds, but in addition they bind a very wide range of antagonists of several different structural classes. It is important now to understand the mechanism of the binding of these ligands to the dopamine receptors and, in the case of agonists, how this binding process leads to activation of the receptor signalling cascade.

The binding of ligands to receptors: theoretical considerations

The binding of agonists and antagonists to the ligand binding site of receptors is a fundamental process, leading in the case of agonists to activation of the receptor and in the case of antagonists to blockade of agonist action. Understanding this process in detail is central to the understanding of the receptor and to the design of drugs directed at receptors. See [4] for a more detailed discussion of this topic.

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Abbreviation used: n,n-ADTN, 2-amino-n,n-dihydroxy-1,2,3,4-tetrahydronaphthalene where n,n is 5,6 or 6,7.