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# COMMENTARY

# BINDING ENERGY AND THE ACTIVATION OF HORMONE RECEPTORS

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Many theories have been advanced to explain the nature of the interactions between hormonal and pharmacological receptors and their specific ligands. However, in addition to an absence of knowledge of the precise molecular details of the binding interactions and the activation of receptors, there is still a lack of understanding of the nature of agonist efficacy and its dependence upon the molecular structure of individual agonists. In this paper I wish to draw attention to the implications for receptor theory of a masterly review by Jencks [1] on the subject of binding energy and enzymic catalysis. In my view, the concepts provided by Jencks for the explanation of substrate specificity and enzymic efficiency have a direct relevance to some of the problems of receptor phenomenonology.

*Binding energy and efficacy of agonists*

Synthetic organic chemistry has provided many chemical derivatives of naturally occurring hormones with biological activities that range from an ability to initiate a biological response comparable with that of the natural hormone, i.e. full agonism, to powerful antagonists that prevent the action of hormones by competitively but inertly occupying their specific receptors. Between these extremes are partial agonists which, though active as stimulants, cannot elicit the full biological effect with which a tissue responds to the appropriate concentration of a natural or full agonist. The level of agonism possessed by a hormone analogue relative to that of the full agonist is generally referred to as the *efficacy* of the compound. It became clear many years ago that the efficacy of agonists is not related to their affinity for the receptors [2]. Partial agonists often exhibit greater affinity for their receptor than full agonists and at equimolar concentrations, therefore, occupy a greater fraction of the receptor population than full agonists but nevertheless fail to elicit the appropriate biological response. Furthermore, some agonists apparently elicit a maximal biological response while only occupying a fraction of the total receptors available. Several attempts have been made to account for the efficacy of agonists. Paton's 'rate' theory [3] conceived receptor activation as a quantal event associated with the moment of interaction between the receptor and the agonist. Occupation of the receptor after this results in blockade, while dissociation of the agonist from the receptor renders it again available for activation. A high dissociation rate constant, therefore, implies high efficacy, whereas a low dissociation rate results in prolonged occupancy and antagonism. Partial agonists, according to the 'rate' theory, have intermediate dissociation rate constants. Unfortunately, this theory simply transformed the efficacy parameter to a dissociation rate constant and no explanation was offered to account for the differences in dissociation rate constants.

Another theory [4] to account for agonist efficacy and partial agonism postulates that there are two states of the receptor, a ground state and an excited state, that are in equilibrium with each other. A biological response is initiated by the agonist binding to the excited state, thus displacing the equilibrium in favour of the excited state, whereas in the absence of an agonist the equilibrium between the two states of the receptor is heavily weighted in favour of the inert ground state. Antagonists are held to bind only to the ground state, thus preventing formation of the excited receptor state. On this model, partial agonists interact with both states of the receptor. Their efficacy is therefore determined by the ratio of their affinities for the ground and excited states of the receptor. The two state model depends on the ability of ligands to modify the equilibrium between the two states by a mass action mechanism.

The rapid initiation of a biological response by an agonist or its inhibition by an antagonist therefore requires high values for the forward and reverse specific rate constants that govern the conformational transition between ground and excited states of the receptor. Conformational changes in proteins indeed may be very fast when small intramolecular motions are involved and rate constants in excess of $10^3$ $sec^{-1}$ may be envisaged easily. It is therefore possible that the excitation of certain receptors could result from a rapid displacement of the equilibrium between the ground and excited states brought about by preferential ligand binding to one state or the other. However, it is also known that many conformational rearrangements in proteins occur over relatively long time periods. Thus the NADH- and GTP-induced dissociation of glutamate dehydrogenase has a forward rate constant of approximately 60 $sec^{-1}$ [5] and the substrate-induced activation of $\alpha$-chymotrypsin is even slower, with a forward rate constant of 1–3 $sec^{-1}$ [6]. In the case of such major conformational rearrangements, it seems quite possible that the ligands take an active role in inducing the transitions by enhancing the forward rate constants rather than by trapping the activated enzyme as a ligand-bound complex. If excitatory ligands do in fact accelerate the rate at which new equilibria are established between two conformational states

of proteins, then the reduction in the activation energy required for the transition must be derived from the free energy of binding of the ligand to the ground state of the protein.

Jencks [1] drew attention to the fact that the values of the Michaelis constant ($K_m$) for many enzymes with highly specific substrates are surprisingly high, i.e. in the range 0.1–0.01 mM. Assuming that the condition is satisfied that $K_m = K_s$, where $K_s$ is the dissociation constant for the initial non-covalent interaction between an enzyme and its substrate, a $K_m$ value between 0.1 and 0.01 mM apparently implies a relatively weak binding between an enzyme and its substrate, a conclusion that is difficult to reconcile with the exquisite specificity of many such interactions. This apparent discrepancy is resolved by Jencks [1] in the following way. Using many examples, he demonstrates that part of the intrinsic standard free energy change, $\Delta G_{int}$, that occurs when the substrate binds to the active site of an enzyme, must be used to lower the activation energy required to achieve the transition state intermediate and therefore contribute to the acceleration of the catalytic rate. The intrinsic standard free energy change is therefore not fully reflected in the observed dissociation constant, given by the $K_m$, and the value is higher than it would be if part of the binding energy did not contribute to the catalytic efficiency of the enzyme. The intrinsic binding energy may be used as follows:

(1) to diminish the requirement for the reduction in translational and rotational entropy involved in the formation of an enzyme–substrate complex;

(2) to induce conformational changes in the enzyme that may be involved in enzymic catalysis;

(3) to destabilize the substrate by geometric distortion, desolvation and electrostatic mechanisms.

Thus

$$\Delta G_{obs} = \Delta G_{int} + \Delta G_D - T\Delta S_{int}, \qquad (1)$$

where

$\Delta G_{obs}$ = observed free energy of binding,
$\Delta G_{int}$ = intrinsic free energy of binding,
$\Delta G_D$ = unfavourable free energy change from destabilization of the binding ligand and induced fit/conformation changes in the enzymes,
$T\Delta S_{int}$ = intrinsic entropy changes exclusive of solvation effects.

The terms $\Delta G_D$ and $T\Delta S_{int}$ may be large in relation to $\Delta G_{int}$ so that the observed binding parameter, $K_m$, is substantially poorer than would otherwise be the case. Jencks [1] discusses the concept of 'non-productive' binding which proposes that a proportion of the free energy of binding is not used to lower the activation energy and therefore enhance $V_{max}$ but is, instead, expressed as tighter binding. Non-productive binding is apparent in substrate analogues whose $K_m$ and also $V_{max}$ are both diminished compared with 'good' substrates. In the extreme case, the free energy change on binding is entirely non-productive, resulting in a very low $K_m$, and a $V_{max}$ that approaches zero. A compound with these properties would, of course, be an effective enzyme inhibitor.

An obvious parallel exists between enzyme substrates, partial substrates and inhibitors on one hand and agonists, partial agonists and antagonists on the other. I shall assume that in many instances agonists (L) may initiate biological responses by *inducing* a conformational change in the receptor proteins (R) to form the excited state R*,

i.e.

$$R + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R^*L, \qquad (2)$$

where $k_1$, $k_{-1}$, $k_2$ and $k_{-2}$ are specific rate constants. There is no clear evidence that in intact cells, RL → R*L is effectively reversible or whether $R^*L \rightleftharpoons R^* + L$ is involved. Recent discoveries concerning the desensitization and also the internalization of activated receptors in viable cells further complicate the picture [7]. It is therefore doubtful, perhaps, whether a true equilibrium can ever be established *in vivo* either with endogenous hormones or with administered drugs. An activation energy barrier separates the two conformational states of the receptor which, in the absence of an agonist, must be high enough effectively to minimize the steady state level of R*.

In the case of enzymic reactions, only the free energy released from 'productive' binding of a substrate to an enzyme may be used to lower the free energy of activation to reach the transition state. Other elements of the binding interaction yield a 'non-productive' free energy change that is expressed in the tightness of binding [1]. Applying this concept to the induction of a conformational change, the availability of productive free energy must therefore have a major influence on the rate, $k_2$, at which the new conformational state of the receptor is reached. Presumably, maximum values of $k_2$ occur when natural agonists interact with their receptors since the structural features of the binding interaction will have evolved to achieve this end. In the case of synthetic analogues of natural agonists, I suggest that their efficacy depends on the level of productive binding that occurs with the receptor. The interaction of partial agonists and antagonists with the receptor is associated with a greater proportion of the intrinsic free energy change being non-productive. The rate, $k_2$, at which conformational change in the receptor occurs is therefore less with these agents. At the same time, the affinities of such ligands for the receptor may increase if the non-productive free energy change is expressed as tightness of binding in much the same way that structural analogues of enzyme substrates that are resistant to catalytic transformation often bind more tightly to their enzymes than proper substrates. This is strikingly illustrated by certain ligands that interact with the $\beta$-adrenergic receptor. A number of antagonists such as l-propranolol, pindolol, IPS 339 and MK 950 have much lower dissociation constants, as measured by the radioligand displacement method, than agonists such as epinephrine, norepinephrine and isoproterenol [8]. Antagonists with significant intrinsic agonism, i.e. partial agonists, are seen as ligands whose binding to the receptor releases a significant amount of 'productive' energy that can be used to achieve some

reduction in the activation energy necessary to bring about the necessary conformational change in the receptor protein.

A key feature of this model, based on the contribution of binding energy to receptor excitation, is that productive binding energy, by reducing the activation energy requirement for the conformational transition in the receptor, accelerates the rate at which the new equilibrium, favouring the excited state of the receptor, is achieved. The ability of hormones to accelerate the rates at which excited receptors are formed can be expected to be important in receptor systems in which major conformational changes and complex protein–protein interactions are known to occur, for example, the many adenylate cyclase-linked receptors. The binding energy model would predict, therefore, that the efficacy of partial agonists capable of stimulating such receptor systems is a reflection of the rate at which excited receptors are formed as well as the final equilibrium.

*The role of secondary ligands in receptor systems*

Guanosine triphosphate (GTP) and its non-hydrolyzable analogue, 5′-guanylylimidodiphosphate, have the ability to modify the affinities of several distinct receptors including $\alpha$-receptors, adenylate cyclase-linked receptors and the muscarinic receptor, for their agonists but not for antagonists. The involvement of GTP in the regulation of adenylate cyclase has been intensively studied. Howlett *et al.* [9] found, as others have done, that GTP *decreases* the affinity of the $\beta$-receptor in cell free preparations for the $\beta$-agonist isoproterenol, while at the same time the nucleotide increases the efficacy of isoproterenol as a stimulant of adenylate cyclase.

Irrespective of the detailed complexities of the interactions between receptors, GTP-binding protein and adenylate cyclase, it can be proposed, in terms of the binding energy model, that the interaction of GTP with its binding protein produces a conformational change in that protein 'paid for' out of the free energy of binding. Cooperative interactions between the receptor protein, GTP-binding protein and adenylate cyclase enable part of the free energy released on the binding to GTP to enhance the productive binding of the agonist to the receptor, presumably as a result of a conformational change affecting the agonist recognition site in the receptor protein. Since the affinity of agonists for their receptors is decreased by GTP, it is suggested that the intrinsic free energy of agonist binding is repartitioned to yield a greater proportion of productive binding. The GTP-induced changes are therefore characterized by an increased agonist efficacy and a decreased affinity for the receptor.

In the progression through partial agonists to competitive antagonists without intrinsic activity, it is suggested that the ability of GTP to increase the availability of 'productive' energy from the ligand–$\beta$-receptor interaction progressively approaches zero. This would account for the inability of GTP to modify the dissociation constants for agonistically inert competitive $\beta$-adrenergic antagonists.

*Intrinsic free energy of binding*

The intrinsic free energy of binding, as distinct from the observed free energy derived from the equilibrium constant, is not directly measureable. However, as Jencks [1] and Page [10] point out, a partial solution to the problem of intrinsic binding energy is available through the observation of the change in binding energy when a substituent is added to a molecule. The free energy of binding of a group B may be estimated by measuring the free energies of binding of the parent molecule, A, and its substituted derivative A–B. Provided that B is not unduly large so as to sterically hinder the binding of A–B in comparison with A and provided that B does not have a large inductive effect that would significantly modify the binding of the rest of the molecule, it is reasonable to conclude that the difference in the binding energies of A and A–B represents an approximation of the intrinsic binding energy of B. This is because the unfavourable intrinsic entropy change that occurs on binding (equation 1) is essentially the same for A and A–B. It is important to realize that the unfavourable entropy change here refers to the loss of translational and rotational entropy that occurs on the tight binding of the ligand to its receptor and it excludes entropy changes that are associated with the displacement of any bound water from the binding site and entropic changes associated with subsequent conformational changes in the receptor. Furthermore, the difference method approach to intrinsic binding energies of substituents will not detect the utilization of binding energy to induce conformational changes in the receptor protein.

Jencks [1] concluded that free energies of binding determined by the difference method can be expected to be additive for different substituent groups, provided the binding of one substituent does not directly or indirectly affect the binding of another. This difference approach has been used recently by Wolff *et al.* [11] to assess the contributions of substituents to the free energy of binding of a range of steroid agonists to the glucocorticoid receptor derived from cultured rat hepatoma cells. Wolff *et al.* [11] showed that addition of the binding energies of several substituents gave a rough approximation of the difference in binding energies between a parent steroid and a multisubstituted molecule. They concluded that the increments in binding energy were not fully independent of each other and that the increments due to a particular substituent varied according to the molecule into which they were inserted. As Jencks [1] has pointed out, the intrinsic binding energy is an idealized parameter that cannot be directly measured. The best that can be expected is an approximation of the type attempted by Wolff *et al.* [11].

*Entropy changes in ligand binding and receptor activation*

The entropy term in equation 1, $T\Delta S_{int}$, also cannot be measured directly by determining the temperature dependence of observed dissociation constants, since these measurements will be markedly influenced by solvation effects. Even the gross entropy changes for the binding interaction alone may be difficult or impossible to determine if ligand binding induces conformational changes in the receptor protein. In this event, the conformational changes may cause

major entropic changes in the associated water or in the phospholipids in the case of membrane-bound receptors. These effects are likely to obscure entropy changes due to the ligand–receptor encounter reaction. Wolff *et al.* [11] found that there was a positive, favourable entropy change when glucocorticoid agonists bound to the cytoplasmic receptors derived from hepatoma cells. These workers interpreted their results as being typical of 'entropy-driven', hydrophobic interactions between molecules. However, it should be pointed out that their binding studies were performed over 14–18 hr, during which time agonist-induced conformational changes in the steroid receptor would almost certainly have occurred in addition to any non-specific changes caused by denaturation and protein degradation. In another thermodynamic study, Weiland *et al.* [8] investigated the enthalpic and entropic changes that occurred on the binding of a range of β-adrenergic agonists, partial agonists and antagonists to the β-receptor of the turkey erythrocyte. The binding of full agonists was associated with a negative entropy change that was compensated by large favourable changes in enthalpy. Antagonist binding caused a large positive entropy change with little change in enthalpy. The thermodynamic changes produced by the binding of partial agonists were intermediate, except that two antagonists with significant β-stimulant activity, practolol and pindolol, caused large positive changes in entropy, i.e. characteristic of antagonists such as propranolol that are devoid of intrinsic agonism. These examples are quoted to indicate that it is not possible to generalize about the gross thermodynamic changes elicited when hormones and their analogues interact with receptors. What can be said is that the initial binding interaction necessitates an intrinsic unfavourable entropy change, due mainly to the loss of translational and rotational freedom of the binding ligand, and that this loss of entropy is 'paid for' from the intrinsic free energy of binding. In turn, of course, the free energy of binding will reflect any immediate favourable entropic contribution resulting from the displacement of bound, structured water from the binding group(s) on the ligand and from the ligand binding site on the receptor.

*Conclusion and prospects*

In this commentary I have drawn attention to the important implications for molecular pharmacology of the concept proposed by Jencks [1] for the utilization of the free energy of binding in facilitating enzyme catalysis and conformational changes in proteins. The efficacy of hormones and pharmacological agonists can be interpreted in the light of the suggestion that the free energy of binding is partitioned between productive binding energy which can be used to induce biologically effective conformational changes in the receptor, and non-productive binding which is expressed as tightness of binding. The observed free energy of binding is derived both from productive binding energy not required for inducing the conformational change and from non-productive binding. The observed free energy of binding is therefore less than the *intrinsic* free energy of binding because of the utilization of some of the productive binding to induce conformational changes in the receptor. It is proposed that in the case of biological stimulation involving a major conformational change in the receptor with a high activation energy requirement, productive binding energy is used to lower the activation energy barrier and thus accelerate the conformational change and to establish a new equilibrium in favour of the excited state of the receptor. On this model, full agonists are seen as ligands with structures that yield optimum productive binding energy, whereas competitive antagonists with zero efficacy are considered to bind in a non-productive mode. The efficacy of partial agonists depends, therefore, on the level of productive binding they achieve with the receptor.

The contribution of substitutents of ligands to the free energy of binding can be approached by observing the change in binding energy caused by small non-interacting substituent groups. In addition to conventional binding studies by the radio-ligand displacement technique, apparent binding energies can be derived from dose–response curves to agonists. This latter method cannot be justified if there is an amplification mechanism between the activation of the receptor and the observed biological response. However, in the case of a closely coupled response, such as the activation of adenylate cyclase, a correlation between the changes in apparent binding energies caused by substituent groups of agonists may provide useful information on the contribution of such groups to productive binding, especially if compared with data derived from radioligand displacement measurements.

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