Hypothesis

Agonist induction, conformational selection, and mutant receptors

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Received 10 October 2003; revised 17 November 2003; accepted 26 November 2003

First published online 8 December 2003

Edited by Robert B. Russell

Abstract Current models of receptor activation are based on either of two basic mechanisms: agonist induction or conformational selection. The importance of one pathway relative to the other is controversial. In this article, the impossibility of distinguishing between the two mechanisms under a thermodynamic approach is shown. The effect of receptor mutation on the constants governing ligand–receptor equilibria is discussed. The two-state model of agonism both in its original formulation (one cycle) and including multiple active states (multiple cycles) is used. Pharmacological equations for the double (two cycles) two-state model are derived. The simulations performed suggest that the double two-state model of agonism can be a useful model for assessing quantitatively the changes in pharmacological activity following receptor mutation.

Key words: G protein-coupled receptor; Agonist induction; Conformational selection; Signal transduction; Mutant receptor; Multiple state

1. Introduction

Agonist induction and conformational selection are two pharmacological mechanisms widely used in receptor theory. Agonist induction involves the generation of an active (R*) receptor state as a consequence of agonist binding to the inactive (R) state whereas conformational selection implies the selective binding of the agonist to different receptor conformations present in the system [1]. Although the importance of one mechanism relative to the other has been debated before [2,3], some recent studies claiming the primacy of agonist induction over conformational selection [4–6] indicate that the question remains open. As an example, Hunyady et al. stated [4], from experiments with N111G mutant AT_1 angiotensin receptor, that conformational selection is not sufficient to explain the mechanism of receptor activation and that agonist induction should be considered as a general mechanism of G protein-coupled receptor activation. This assumption agrees with a previous proposal [5] remarking that agonist–receptor interaction for AT_1 angiotensin receptor is not passive (conformational selection) but rather that the agonist coordinates the transition of R to R* (agonist induction) through the formation of agonist-induced pre-active states. In line with these ideas, the sequential binding model [6] for the activation of the β_2 adrenergic receptor suggested that binding of agonist does not occur directly to R* but sequentially, resulting in a series of conformational states that are intermediates between R and R*. However, it may not be possible to distinguish between the two mechanisms in the case of constitutively active receptors, under a thermodynamic approach. To illustrate this assessment, the two-state model of receptor activation both with a single [7] and with multiple [8] active states will be used because of the simplicity of the resulting equations. It will be assumed hereinafter that R* is the conformation able to activate G proteins.

2. Receptor selection and agonist induction within the two-state model of agonism

Scheme 1 displays the equilibrium cycle for a wild-type receptor under the two-state model with a single active state [7].

R and R* are the inactive and active free receptor, respectively, and AR and AR*, the corresponding ligand-bound species. The equilibrium constants are defined as:

$$X = \frac{[R^*]}{[R]};\ Z = \frac{[A][R]}{[AR]};\ T = \frac{[A][R^*]}{[AR^*]};\ Y = \frac{[AR^*]}{[AR]} \tag{1}$$

Because only three from the $\{X, Z, T, Y\}$ set of constants are independent, the definition of either of them can be related to the others [9]. For instance, Y can be expressed as $Y = XZ/T$. The degree of agonist induction (Y) depends on the extent of basal response (X) and on the selectivity (Z/T) of the agonist for the receptor states.

The functional response of a receptor is related to the concentration of receptors in the active form ([R*]+[AR*]) which, expressed as a fraction (fR*) of the total receptor concentration ($[R_t]$ = [R]+[R*]+[AR]+[AR*]) gives [7]:

$$f_{R^*} = \frac{T + [A]}{Ta + b[A]};\ \text{where } a = 1 + \frac{1}{X} \text{ and } b = 1 + \frac{T}{XZ} \tag{2}$$

$$\text{Basal response} = \frac{1}{a}$$

$$f_{R^*}^{max} = \frac{1}{b}$$

$$A_{50} = \frac{f_{R^*}^{max}}{\text{basal response}} \cdot T$$

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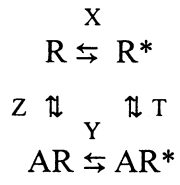
0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01404-2



Scheme 1. The single two-state model for a wild-type receptor.

using the conformational selection concept and

$$f_{R^*} = \frac{\frac{XZ}{Y} + [A]}{\frac{XZ}{Y}a + b[A]}; \text{ where } a = 1 + \frac{1}{X} \text{ and } b = 1 + \frac{1}{Y} \qquad (3)$$

$$\text{Basal response} = \frac{1}{a}$$

$$f_{R^*}^{max} = \frac{1}{b}$$

$$A_{50} = \frac{f_{R^*}^{max}}{\text{basal response}} \cdot \frac{XZ}{Y}$$

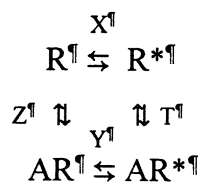
using the agonist induction concept. Eqs. 2 and 3 are algebraically equivalent. A mechanistic distinction between them would be possible if we could separate the molecular from the molar scenario. At a given time, a particular AR* molecular complex is formed either via conformational selection (from R*) or via agonist induction (from AR). However, the route followed by each individual molecule is hidden to an external observer who will perceive only the molar relationships defined by the aforementioned equilibrium constants (see [10] for a discussion between molecular and molar models).

3. Mutant receptors and equilibrium constants for receptor activation

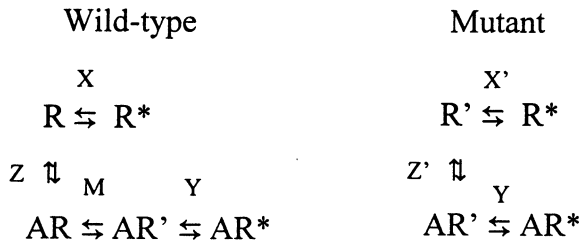
Experiments with mutant receptors can shed new light on signal transduction processes. In fact, the necessity for an explicit equilibrium between inactive and active receptor states was proposed [11] on the basis of findings from mutation experiments. However, a word of caution is needed when translating the properties of a mutant receptor to its parent wild-type. It is likely that mutation of a residue will change the molecular features of the receptor states. Accordingly, an effect on some or all the equilibrium constants is expected.

Scheme 2 displays the equilibrium cycle for a mutant receptor under the two-state model.

In analogy to the case of the wild-type receptor, the relationship $Y^{¶} = X^{¶}Z^{¶}/T^{¶}$ is obtained. I have assumed that, in theory, any constant involved in these equilibria may change after mutation. This assumption is not new, inasmuch as the



Scheme 2. The single two-state model for a mutant receptor.

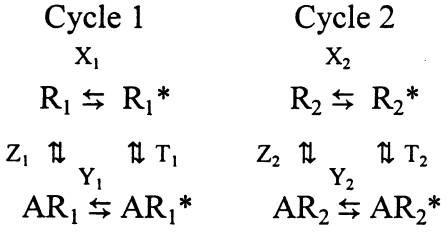


Scheme 3. Agonist induction mechanism with pre-active species.

hypothesis that the active conformation of a mutant receptor may not be the same as the one of the wild-type has been considered previously [9].

The response profile of a mutant receptor expressed as $f_{R^{*¶}}$, using either the conformational selection (Eq. 2) or the agonist induction (Eq. 3) approaches, will differ from the wild-type if any of the equilibrium constants changes. Thus, a ligand can be a full agonist in the mutant receptor and partial in the wild-type and vice versa. The exclusion of conformational selection and the proposal of agonist-induced pre-active states was suggested [5] from the different behavior of [Sar^1,Ile^4,Ile^8]Ang II, an analogue of hormone Ang II, in wild-type and N111G AT_1 receptors. [Sar^1,Ile^4,Ile^8]Ang II fully activated the mutant receptor, but, although it bound to the wild-type it did not activate it. This finding cannot be explained by Scheme 1 if the receptor active state, R*, is required to be the same for the wild-type and the mutant receptors. Consequently, an agonist-induced pre-active state AR′, which coincides with the conformation of the mutant receptor, was suggested [4,5] and receptor selection (direct binding of A to R*) was ruled out. Scheme 3 reflects a possible model corresponding to the authors' hypothesis. This scheme also contains the sequential binding model [6] mentioned above.

The model expressed by Scheme 3 is, in thermodynamics terms, incomplete: a chemical equilibrium between {A, R*} and AR* with its corresponding equilibrium constant is missing. Scheme 2, which includes a new active state for the mutant receptor, overcomes this apparent conflict without the necessity of postulating agonist-induced pre-active receptor states. Scheme 2 can explain some published [4] pharmacological results: (i) Ang IV is more potent in the mutant receptor than in the wild-type (WT): if $T^{¶} < T$ then $A_{50}^{mutant} < A_{50}^{WT}$; (ii) [Sar^1,Ile^4,Ile^8]Ang II is an antagonist in the wild-type receptor: if $T = Z$ then $f_{R^*}^{max}$ = basal response; (iii) [Sar^1,Ile^4,Ile^8]Ang II is an agonist in the mutant receptor: if $T^{¶} < Z^{¶}$ then $f_{R^*}^{max} >$ basal response.



Scheme 4. The double two-state model.

4. The double two-state model of agonism

The two-state model of agonism is the simplest model able to explain receptor constitutive activity or inverse agonism pharmacological properties. In the present paper this model allows us to show the functional equivalence between conformational selection and agonist induction mechanisms with algebraic simplicity. However, the two-state model appears to be insufficient in some complex pharmacological situations, for instance, those situations requiring the inclusion of multiple active receptor states (see [12–15] for a review). It can be seen, however, that this property is, in part, implicit in Scheme 2. Comparison of Schemes 1 and 2 shows that physiological response arises from two states of the receptor: R* (wild-type) and R*¶ (mutant). In other words, G proteins are flexible enough to recognize several active receptor states. Let us address the simplest case of multiple receptor states in which two inactive (R_1, R_2) and two active (R_1*, R_2*) states are already present in the wild-type receptor. Within the framework of the two-state model, two cycles can be constructed to form the double two-state model (Scheme 4).

The equilibrium constants for each cycle are defined as:

$$X_i = \frac{[R_i^*]}{[R_i]};\quad Z_i = \frac{[A][R_i]}{[AR_i]};\quad T_i = \frac{[A][R_i^*]}{[AR_i^*]};\quad Y_i = \frac{[AR_i^*]}{[AR_i]}$$

$$\text{with } i = 1 \text{ or } 2 \tag{4}$$

The equilibrium constants connecting both cycles are defined as:

$$a_{12} = \frac{[R_2]}{[R_1]};\quad b_{12} = \frac{[AR_2]}{[AR_1]};\quad c_{12} = \frac{[R_2^*]}{[R_1^*]};\quad d_{12} = \frac{[AR_2^*]}{[AR_1^*]} \tag{5}$$

Scheme 4, which is an extension of the classical two-state model (Scheme 1), is the simplest case of the multi-two-state model presented earlier [8]. It is worth noting that the multi-two-state model is essentially equivalent to the multistate probabilistic model of receptor activation [16,17], in which it is assumed that the receptor can explore a very large number of conformations and that function arises as a macroscopic result of the distribution of these microscopic states. Here, the equation for the agonist concentration–effect relationship and the corresponding geometric parameters are derived for the particular case of two cycles (Scheme 4). Pharmacological activity now comes from two receptor sources: R_1*+AR_1* (Cycle 1) and R_2*+AR_2* (Cycle 2). Cycles 1 and 2 are not independent. The relation between them is modulated by four (a_{12}, b_{12}, c_{12}, d_{12}) equilibrium constants. If we use the receptor selection mechanism, the fraction of active receptors

$$f_{R^*} = \frac{[R_1^*] + [AR_1^*] + [R_2^*] + [AR_2^*]}{[R_1] + [R_1^*] + [AR_1] + [AR_1^*] + [R_2] + [R_2^*] + [AR_2] + [AR_2^*]}$$

may be written as:

$$f_{R^*} = \frac{T + [A]}{Ta + b[A]};\quad \text{where } T = T_1T_2\cdot\frac{X_1 + X_2a_{12}}{X_1T_2 + X_2T_1a_{12}};$$

$$a = 1 + \frac{1 + a_{12}}{X_1 + X_2a_{12}};$$

$$\text{and } b = 1 + \frac{T}{(X_1 + X_2a_{12})\cdot\dfrac{Z_1Z_2}{Z_2 + Z_1a_{12}}} \tag{6}$$

$$\text{Basal response} = \frac{1}{a}$$

$$f_{R^*}^{max} = \frac{1}{b}$$

$$A_{50} = \frac{f_{R^*}^{max}}{\text{basal response}}\cdot T$$

Eq. 6 contains the general pharmacological expressions for the double two-state model. The contribution of one cycle relative to the other is determined by the link constant a_{12} (this is an arbitrary election, we could have chosen b_{12} or c_{12} or d_{12} instead) whose value may change after mutation. The agonist response of a particular ligand depends on its selectivity towards inactive and active receptor conformations within each cycle and on the ratio of one cycle relative to the other (a_{12}). Thus, a_{12} translates in molar terms the molecular rearrangement caused by receptor mutation. Two limit cases may be considered: a wild-type receptor in which Cycle 1 is majority ($a_{12} \ll 1$) and a mutant receptor in which Cycle 2 is majority ($a_{12} \gg 1$). These limit cases correspond to Schemes 1 and 2 for wild-type and mutant receptors under the single two-state model of agonism. Between these limit situations a continuum of system states are possible according to the a_{12} value. To illustrate the potential utility of the double two-state model of agonism, let us address some typical pharmacological issues related to mutant receptors.

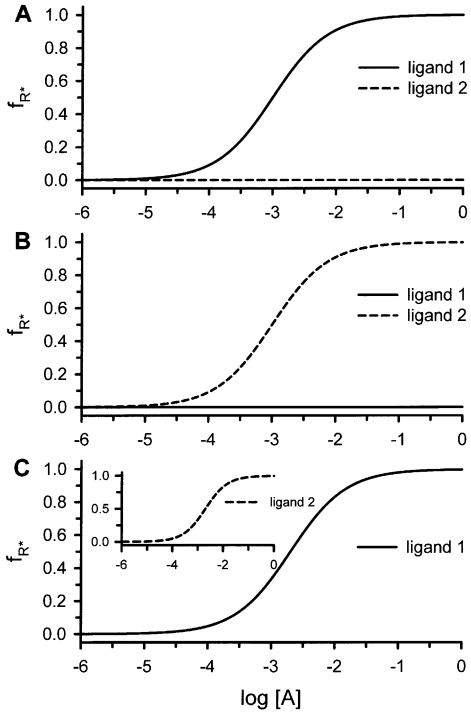


Fig. 1. Simulation of concentration–effect curves resulting from the double two-state model (Scheme 4; Eq. 6). For simplicity, the fraction of receptors in the active state (fR*) is considered to represent the observed effect. The values for the parameters included in Eq. 6 are the following: $X_1 = X_2 = 10^{-6}$; Ligand 1: $T_1 = 10^{-9}$, $Z_1 = 1$, $T_2 = 1$, $Z_2 = 1$; Ligand 2: $T_1 = 1$, $Z_1 = 1$, $T_2 = 10^{-9}$, $Z_2 = 1$. Panel A represents a wild-type receptor in which Cycle 1 is majority ($a_{12} = 10^{-6}$); panel B represents a mutant receptor in which Cycle 2 is majority ($a_{12} = 10^{6}$); and panel C represents a system in which both cycles are balanced ($a_{12} = 1$).

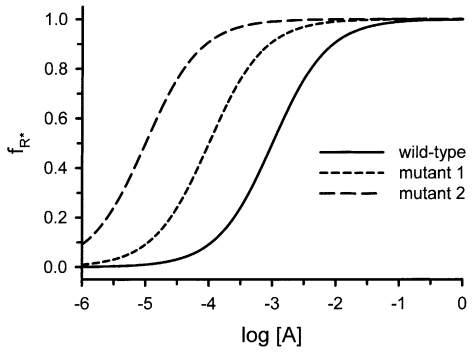


Fig. 2. The increment in basal response after receptor mutation and its effect on concentration–effect curves for wild-type ($a_{12} = 10^{-6}$, $X_1 = 10^{-6}$, $X_2 = 10^{-5}$, solid line), mutant 1 ($a_{12} = 10^{6}$, $X_1 = 10^{-6}$, $X_2 = 10^{-5}$, short dashed line) and mutant 2 ($a_{12} = 10^{6}$, $X_1 = 10^{-6}$, $X_2 = 10^{-4}$, long dashed line) receptors. The equilibrium constants for the ligand–receptor interactions have been considered to be the same for both cycles ($T_i = 10^{-9}$, $Z_i = 1$, with $i = 1,2$). Eq. 6 has been employed in the simulations.

4.1. The agonist–antagonist identity of a ligand depends on the system

Fig. 1 shows the concentration–effect curves for two ligands under the double two-state model of agonism (Eq. 6). Panel A corresponds to a particular wild-type receptor ($a_{12} = 10^{-6}$; Cycle 1 is majority) whereas panel B represents a particular mutant receptor ($a_{12} = 10^{6}$; Cycle 2 is majority). In addition, panel C characterizes a system in which the two cycles are equally weighted ($a_{12} = 1$). The values for the rest of parameters included in Eq. 6 are shown in the figure legend. The equilibrium between R and R* receptors is assumed to be the same in both cycles ($X_1 = X_2$). Ligand 1 is defined as a full agonist ($T \ll Z$) in Cycle 1 and as a neutral antagonist ($T = Z$) in Cycle 2. In contrast, Ligand 2 is defined as a neutral antagonist ($T = Z$) in Cycle 1 and as a full agonist ($T \ll Z$) in Cycle 2. The simulation shows that Ligand 1 behaves as a full agonist in the wild-type and as a neutral antagonist in the mutant receptor whereas the opposite occurs for Ligand 2. However, both ligands behave as full agonists in panel C, giving exactly the same concentration–effect curves. The maximum responses yielded by both ligands in panel C would decrease if the affinities for the receptor species from the cycles where they act as neutral antagonists increase. This change will render Ligand 1 and Ligand 2 partial agonists.

4.2. The correlation between basal response and agonist potency

Other pharmacological outcomes can be assessed properly by the double two-state model of agonism, for instance, the increment in basal response after receptor mutation leading to constitutively active mutant receptors. Fig. 2 shows the concentration–effect for a ligand in a wild-type receptor ($a_{12} = 10^{-6}$) with relatively low basal response ($f_{R*} = 10^{-6}$) and in two mutant receptors ($a_{12} = 10^{6}$) in which the basal response increases (Mutant 1, $fR^* = 10^{-5}$ and Mutant 2,

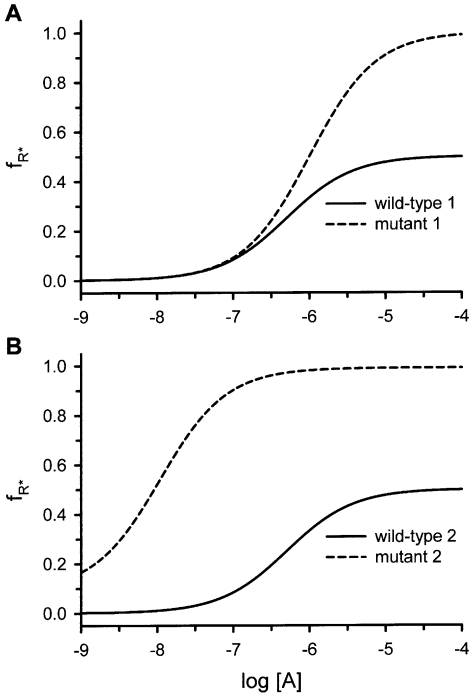


Fig. 3. A: Partial agonism depends on the affinity ratio of the ligand for the inactive and active receptors. Cycle 1: $X_1 = 10^{-3}$, $T_1 = 10^{-9}$, $Z_1 = 10^{-6}$; Cycle 2: $X_2 = 10^{-3}$, $T_2 = 10^{-9}$, $Z_2 = 10^{-3}$. Wild-type 1: $a_{12} = 10^{-6}$, solid line; Mutant 1; $a_{12} = 10^{6}$, dashed line. B: Partial agonism depends on the value of the equilibrium constants for inactive and active receptors in the absence of agonist. Cycle 1: $X_1 = 10^{-3}$, $T_1 = 10^{-9}$, $Z_1 = 10^{-6}$; Cycle 2: $X_2 = 10^{-1}$, $T_2 = 10^{-9}$, $Z_2 = 10^{-6}$. Wild-type 2: $a_{12} = 10^{-6}$, solid line; Mutant 2: $a_{12} = 10^{6}$, dashed line.

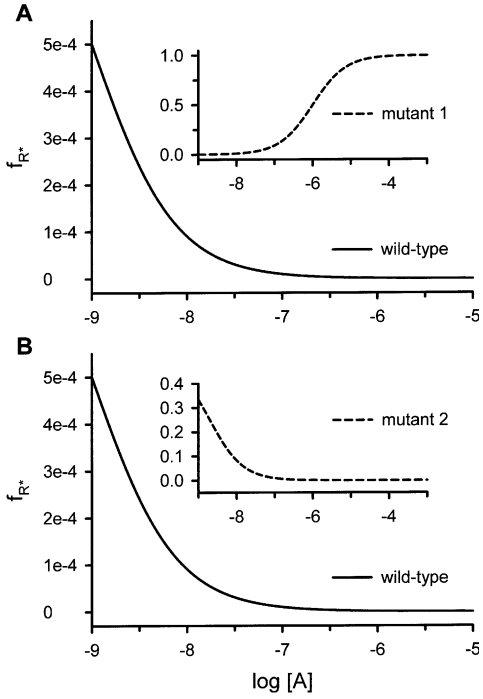


Fig. 4. A: An inverse agonist can be converted into a full agonist by changing the affinity ratio for the inactive and active receptors. Cycle 1: $X_1 = 10^{-3}$, $T_1 = 1$, $Z_1 = 10^{-9}$; Cycle 2: $X_2 = 10^{-3}$, $T_2 = 10^{-9}$, $Z_2 = 1$. Wild-type 1: $a_{12} = 10^{-6}$, solid line; Mutant 1: $a_{12} = 10^{6}$, dashed line. B: An inverse agonist remains as that although with higher basal activity if the value of the constant for the equilibrium between R and R* increases. Cycle 1: $X_1 = 10^{-3}$, $T_1 = 1$, $Z_1 = 10^{-9}$; Cycle 2: $X_2 = 1$, $T_2 = 1$, $Z_2 = 10^{-9}$. Wild-type 2: $a_{12} = 10^{-6}$, solid line; Mutant 2: $a_{12} = 10^{6}$, dashed line.

fR* = 10^{-4}). It can be seen that changing the value of the equilibrium constant X between R and R* receptor conformations ($X_1 = 10^{-6}$ rules for the wild-type and $X_2 = 10^{-5}$ and $X_2 = 10^{-4}$ for the Mutant 1 and Mutant 2 receptors, respectively) has a profound impact on the observed concentration–effect curves: the ligand, which has not changed its affinity constants for the different forms of the receptor, appears to be more potent in either of the mutants than in the wild-type receptor. Moreover, the increment in the potency of the agonist is correlated with the shift of the equilibrium between R and R* towards R* as measured by the constant X.

4.3. Partial agonists can be converted into full agonists

Within the two-state model [7], an agonist is a ligand which presents more affinity for the active than for the inactive receptor ($T < Z$). The ratio between these two constants determines that the agonist is full or partial. Fig. 3 shows two ways in which a partial agonist is converted into a full agonist after receptor mutation ($a_{12} = 10^{-6}$ for the wild-type and $a_{12} = 10^{6}$ for the mutant receptor). See Eq. 6. In panel A, the dissociation constant of the ligand for the inactive receptor is higher in Cycle 2 than in Cycle 1 ($Z_2 = 10^{-3} > Z_1 = 10^{-6}$). In panel B, the ligand displays the same values for the ligand–receptor equilibrium constants in both cycles ($T_i = 10^{-9}$; $Z_i = 10^{-6}$). However, in Cycle 2 the basal response is augmented ($X_2 > X_1$).

4.4. Inverse agonists can be converted into full agonists

Fig. 4A shows the concentration–effect curves of a ligand, which is defined as an inverse agonist for Cycle 1 and as a full agonist for Cycle 2. Assuming that Cycle 1 is majority in the wild-type receptor ($a_{12} = 10^{-6}$) and Cycle 2 is majority in the mutant receptor ($a_{12} = 10^{6}$) allows the ligand to perform opposite roles. In Fig. 4B the ligand is intrinsically an inverse agonist with identical ligand–receptor equilibrium constants for both cycles ($T_i = 1$; $Z_i = 10^{-9}$). However, in Cycle 2 the constant for the equilibrium between R and R* increases ($X_2 = 1 > X_1 = 10^{-3}$). This change does not alter the definition of the ligand, which is still an inverse agonist. Yet, the basal response is higher in the mutant than in the wild-type receptor.

5. Concluding remarks

A number of simulations have been performed with the double two-state model of agonism. This model assumes that the functional response of a receptor is determined by the sum of two interdependent cycles. Receptor mutation may alter the value of the constant that measures the importance of one cycle relative to the other, and this allows the pharmacological profile of a system to be simulated before and after mutation. The present model is closely related to the three-state receptor model of agonist action [18,19], where one inactive and two active conformations of the receptor were proposed to exist. Although the latter model was used to simulate multiple transduction pathways rather than the effects of receptor mutation, the kinds of variation of ligand pharmacology predicted by both models are coincident.

With respect to the main issue of the present paper, the discussion between conformational selection and agonist induction mechanisms, it can be seen that by substituting $T_i = (X_i Z_i)/Y_i$ with $i = 1$ or 2 in Eq. 6, new relationships for the agonist induction mechanism are obtained, which are equivalent to those produced by the conformational selection approach. It should be noted that the parameters in the foregoing equations involve concentrations at equilibrium of the ligand, the receptor and the ligand–receptor complexes. Because of the thermodynamic nature of the model, the conformational pathways followed by the molecular entities between the different steady states are ignored. Thus, it may be concluded that it is not possible to distinguish between the above mechanisms within a purely thermodynamic framework. This result agrees with the previous statement [3] that there is no dichotomy between the mechanisms of receptor selection and agonist induction if the energy landscape idea with the presence of multiple active states is accepted. Nevertheless, new insights into this intriguing point can be obtained from research areas where kinetics and molecular mechanisms play crucial roles. Thus, fluorescence studies can provide essential details on the conformational changes undergone by the receptor upon agonist binding [20]. Although fluorescence lifetime spectroscopy experiments on the β_2 adrenergic receptor [21] are consistent with the agonist induction mechanism, further investigations would be needed to reconcile these results with the existence of basal activity. This property requires the presence of a significant concentration of active native receptors, which, in principle, could be affected by the addition of agonist molecules. Interestingly, the use of single-molecule techniques revealed that the native β_2 receptor exists in several conformational substates in equilibrium and that the full agonist isoproterenol seems to stabilize substates that might be rare in the native receptor [22]. The authors hypothesized that one or more of the states having the smaller probabilities represent the active state of the receptor. These findings are compatible with both selection and induction mechanisms. From a different perspective, structure–activity studies can also be helpful for testing hypotheses on receptor activation. Thus, at the stage of rational design of agonists for a particular receptor system, one can devise ligand structures suitable to bind preferably R* (conformational selection) or ligand molecular structures suitable to bind preferably R, but containing the chemical groups necessary for triggering the process of receptor activation (agonist induction). As has been shown [23], because the ligands and the residues from the receptor active site are in chemical equilibrium between different ionic and tautomeric forms, there is not a unique route for receptor activation. In fact, the existence of two ligand conformations (A, A*) in equilibrium, one (A*) recognized by R* and the other (A) recognized by R, is implicit in the two-state model. Thus, it is possible that some agonists are more prone to transmit the signal by binding R* whereas others proceed mainly by binding R and inducing the conformational change to R*. This approach implies the examination of the potential mechanisms of signal transduction in relation to the molecular structures of the ligands. Structure–activity studies focusing on this topic can be useful both for providing a better understanding of the processes of receptor recognition and activation and for introducing more diversity in the generation of ligand molecules.

Acknowledgements: J.G. is grateful to Dr. Jordi Quintana for critical reading of the manuscript. Financial support of EC Vascan 2000 Consortium (QLG1-1999-00084) and Grups de Recerca Consolidats de Catalunya (2001SGR 00193) is acknowledged.

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