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SCIENTIFIC CORRESPONDENCE

Agonist binding to G-protein coupled receptors

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In a recent article Colquhoun (1998) showed how measurements of agonist affinity at receptors were difficult to interpret owing the conformational change that occurs in the receptor upon agonist binding. It is often assumed, however, for the G-protein coupled receptors that measurements of agonist affinity in the presence of guanine nucleotides (which uncouple receptor and G-protein) represent ground state affinities. As I have argued elsewhere (Strange 1998, 1999) this is unlikely to be true and agonist binding, even to the uncoupled receptor, may involve a conformational change. This can be represented in the scheme shown in Figure 1 where the agonist binds with different affinities to different states of the receptor, R and R*, the latter being the species that couples to the G-protein (Samama *et al*., 1993). Agonist affinities observed (K_d) will not be ground state affinities and will depend on K_A, K_{A*} and K_R as in equation 1. It is important to determine values for the different equilibrium constants and in principle this is possible if mutant receptors are available locked in to the R* or R states.

$$K_d = \left(\frac{1 + K_R}{K_A + K_R K_{A*}}\right) \quad (1)$$

Mutants favouring the R* state have been reported for several receptors although only in the case of the β_2-adrenergic receptor have these been fully characterized (Lefkowitz *et al*., 1993; Samama *et al*., 1993). These show increased agonist affinities independent of G-protein coupling, increased functional potencies for agonists to activate signalling systems and increased agonist-independent activation of signalling systems. There is also some increase in the ability of the receptor to couple to G-proteins so this mutation is not entirely clean. In these mutant receptors K_R (Figure 1) will be favourable and K_d will approximate to $1/K_{A*}$.

No explicit description of a mutant receptor locked in to the R state has been made but mutants in the conserved aspartate about two-thirds down TMII may provide such a model. This amino acid residue is one of the most conserved in all the GPCR's (Van Rhee & Jacobsen, 1996) suggesting an important structural role or a role in the activation of the receptor. The residue has been mutated to Ala or Asn in several receptors (references in Van Rhee & Jacobsen, 1996 and Chung *et al*., 1988; Fraser *et al*., 1990; Horstman *et al*., 1990; Neve *et al*., 1991; Wang *et al*., 1991; 1993). In the mutant receptors agonists are unable (except in two cases) to activate signalling systems and, where this has been measured, coupling of receptor to G-protein is impaired and the regulation of agonist binding by sodium ions prevented. These characteristics are those of a receptor that is unable to form the activated state and indeed it has been suggested that the TMII Asp is important in receptor activation for forming a salt bridge with the Arg at the base of TMIII released from the AspArgTyr sequence following the protonation of the TMIII Asp (Ballasteros *et al*., 1998). This mutant receptor is then a candidate for a receptor that cannot undergo the R/R* transition and so should exhibit a decrease in agonist affinity independent of G-protein coupling i.e. K_R is unfavourable and K_d reduces to K_A (Equation 1). The effects seen may be quite small if the extent to which R* is favoured in the presence of the agonist is not great and this may account for some of the variability in results reported (see below). Nevertheless, if this speculation about the role of the TMII Asp is correct then the effects on agonist affinity of the mutation of the TMII Asp should be a rough index of the extent to which conformational effects can contribute to the effects of other mutations of the receptor.

There are difficulties in interpreting some of the data on the TMII Asp mutants in the literature as the characterization of the mutants is not extensive, usually involving only one or two agonists. In some cases the mutation causes a modest decrease in agonist affinity under conditions where G-protein coupling has been suppressed, in other cases there is no effect and sometimes the affinity is slightly increased. This has been characterized in two studies for the β_2-adrenergic receptor (Strader *et al*., 1987; 1988; Chung *et al*., 1988) and the affinity for the full agonist, isoprenaline, is lower ($\sim$9 fold) in the mutant in the absence of G-protein coupling as predicted by the theory proposed above. For this receptor, it is possible to estimate values of K_A, K_{A*}, K_R, K_{R*} as follows. As stated above, in the TMII Asp mutant the R* state will be disfavoured and agonist affinity will approximate K_A. In the constitutively active mutant receptors R* will be strongly favoured and agonist affinity will approximate K_{A*}. These values may then be used in Equation 1 with the affinity for agonist at the native receptor to determine values for the different equilibrium constants. For the full agonist isoprenaline (average native K_d 165 nM (Chung *et al*., 1988; Samama *et al*., 1993); average effect of TMII Asp mutation 9.06 fold reduction in affinity (Strader *et al*., 1987; 1988; Chung *et al*., 1988); effect of constitutively active mutation 25 fold increase in affinity (Samama *et al*., 1993)) these values are: K_A ($6.7\ 10^5\ M^{-1}$), K_{A*} ($1.5210^8\ M^{-1}$), K_R (0.037), K_{R*} (8.39). The value for K_d for the native receptor is 165 nM (equivalent to $6.05\ 10^6\ M^{-1}$) so that there is a discrepancy of $\sim$9 fold between the affinity of the native receptor and the affinity of the ground state.

This calculation shows that for the β_2-adrenergic receptor the observed K_d for a full agonist may not be a true measure of the affinity of the agonist for binding to the ground state of the receptor and that there is a contribution from the R/R* transition. The extent of this problem for other GPCR's is unclear at present and further work needs to be done on this. If

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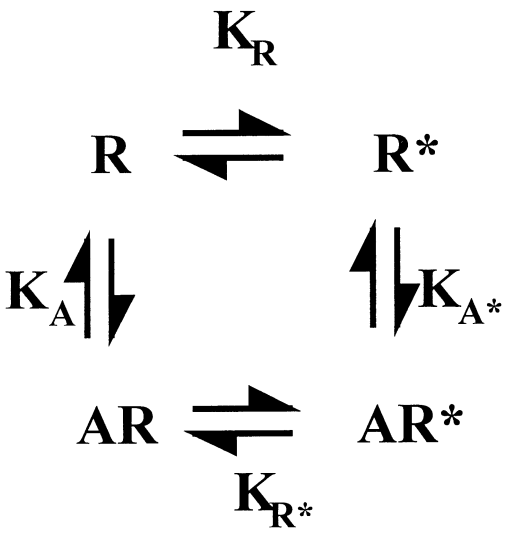

Figure 1 Agonist binding to G-protein coupled receptors under conditions where coupling to G-proteins is suppressed. Receptor exists in a ground state (R) and a partially activated state (R*), that can normally couple to the G-protein to form R*G as in Samama *et al*., 1993. K_A and K_{A*} are association constants for binding of the agonist to R and R* and the observed equilibrium dissociation constant for agonist binding (K_d) is given by equation 1.

discrepancies are found between determinations of K_d and ground state affinities in other GPCR's then this has implications for the interpretation of the results of mutations of residues that are thought to interact directly with ligands. In the case of the mutation of a residue that may form a hydrogen bond to the ligand the energetic effect of the deletion of the hydrogen bond is expected to be a reduction of up to $\sim$20 fold in affinity (Strange, 1996). If the mutation were to impair the R/R* transition then this effect could contribute up to $\sim$10 fold to affinity changes based on the above calculation. Given that the ligand/receptor interaction and R/R* transition are likely to be linked events it will be difficult to disentangle the individual contributions to the overall affinity change. If, however, the effects of the mutation were evaluated in a receptor locked in to the R* state (using the mutations considered earlier) then the size of the effect would reflect deletion of the hydrogen bond alone. Interpretation of mutation of a hydrophobic interaction may be easier as such a mutation should reduce binding affinity by up to 10^4 fold (Strange, 1996). In this case effects on the conformation of the receptors will be small by comparison.

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