Binding specificity of receptor chimeras revisited

Meyer B. Jackson
Department of Physiology, University of Wisconsin, Madison, Wisconsin 53706 USA

A powerful method for analyzing ligand binding specificity in receptor chimeras, introduced by Catterall (1), holds great promise for localizing the determinants of ligand binding specificity. Applying this method to data from chimeras of α_2-adrenergic and β_2-adrenergic receptors (2) lead to the conclusion that determinants of specificity reside predominantly on transmembrane segment 7. However, the following considerations, many of which have general importance in the analysis of binding data, lead to different conclusions.

The analysis makes use of agonist dissociation constants (K_d measured in reference 2 by displacement of radiolabeled antagonist) to compute a difference in the binding free energy ($\Delta(\Delta G)$) of two different ligands to the same receptor. Catterall (1) plotted $\Delta(\Delta G)$ values for the drug pair *p*-aminoclonidine (PAC) and isoproternal (ISO) versus the number of segments from the α_2-adrenergic receptor (Fig. 1). However, a point at coordinates [1, 0.08] appears to have been based on EC_{50} measurements of Kobilka et al. (2) rather than K_d measurements. No K_d measurements were made for this chimera (CR6 of reference 2). K_d values and EC_{50} values should not be plotted together because in G-protein-coupled receptors they are generally divergent (3), and for this particular system differed by a factor of up to 50 (2). Furthermore, a dose-response curve for CR6 had only two nonzero points, and showed no saturation (2). Without the point for chimera CR6, the data for the other three chimeras with segment 7 from the α_2-adrenergic receptor do not extend over a sufficiently broad range to justify Catterall's conclusion that the other segments each contribute 0.8 kcal/mol to the difference in binding energy between PAC and ISO. A case for dominance by segment 7 can still be made for the selectivity between PAC and ISO because of the large jump at $n = 5$ of Fig. 1 (2).

A plot of $\Delta(\Delta G)$ for PAC and epinephrine (EPI) leads one to a different conclusion (Fig. 1). Although EPI activates both receptors, the plot exhibits a clear transition from β_2 character to α_2 character. The transition is gradual, with no sharp jump for any one transmembrane segment replacement. Thus, this plot suggests a distribution of determinants of specificity over many regions of the receptor.

A shift in the binding-site saturation behavior of an allosteric protein need not result from a change at the binding site. A change in the free energy difference between the two protein conformations can also shift a binding-site saturation curve (4). Consider the binding-site saturation function given by the Monod-Wyman-Changeux theory for a one-site receptor:

$$S = \frac{C(1 + R_1)}{K_1(1 + R_0) + C(1 + R_1)},$$

where C denotes concentration of ligand, R_0 and R_1 denote the equilibrium concentration ratios of the active and inactive conformations of the unligated and ligated receptors, respectively, and K_1 denotes the dissociation constant for binding to the inactive conformation. What is taken as K_d is then actually $K_1(1 + R_0)/(1 + R_1)$. R_0 and R_1 depend on regions of the protein away from the binding site. Thus, a change in the apparent K_d could result from changes far from the binding site, leaving an ambiguity in how to interpret a change in K_d.

Catterall's proposal of using $\Delta(\Delta G)$ is a step towards overcoming this problem. Taking the difference of two free energies requires taking the ratio of the two apparent K_d's. For a true agonist $R_1 \gg 1$. This gives $1 + R_1 \sim R_1$, which together with the condition of detailed balance reduces the apparent K_d to $K_2(1 + R_0)/R_0$ (K_2 denotes the dissociation constant for the active receptor conformation). Since R_0 depends only on protein and not agonist, taking the ratio of two apparent K_d's for the same protein cancels out the R_0 containing terms to leave a ratio of K_2's. For antagonists, $R_1 \sim 0$, and a similar cancella-

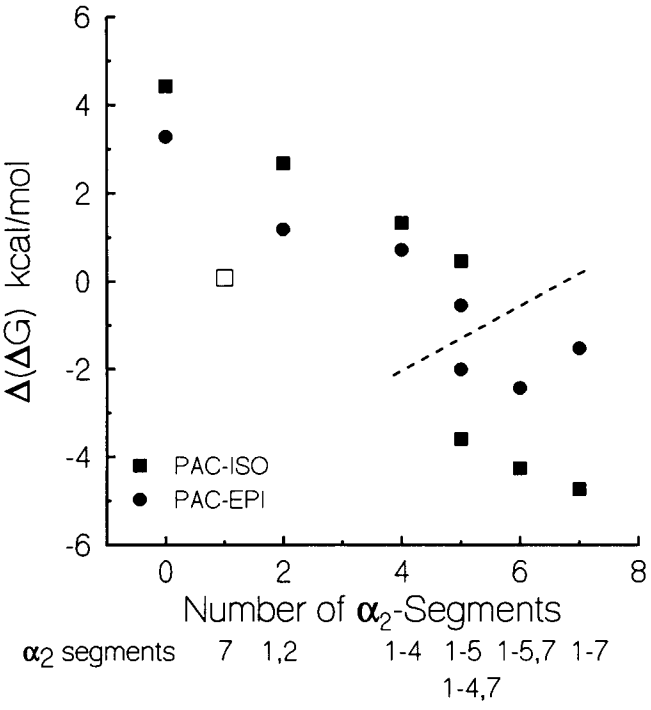

FIGURE 1 $\Delta(\Delta G)$ plotted versus the number of transmembrane segments from the α_2-adrenergic receptor. Gibbs free energies of ligand binding were calculated from the K_d values reported by Kobilka et al. (2). The difference between binding free energies was then computed for the drug pairs PAC and ISO (*squares*) and PAC and EPI (*circles*). A point at $n = 1$ was based on EC_{50} measurements for PAC and ISO activation of chimera CR6 (*open square*). The dashed line divides the K_d data into two groups of chimeras, with segment 7 either from the β_2 receptor (*above, left*) or from the α_2 receptor (*below, right*). The receptors from which the various segments were taken is indicated below the *x*-axis.

0006-3495/92/11/1443/02 $2.00

tion occurs to leave a ratio of K_I's. Thus, in comparisons among agonists or among antagonists, $\Delta(\Delta G)$ is a useful quantitative index of binding site function. These considerations should help investigators use binding data to localize function in molecular variants of receptors.

Received for publication and in final form 8 June 1992.

REFERENCES

1. Catterall, W. A. 1989. Analysis of ligand binding specificity of receptor chimeras. *Science (Wash. DC)*. 243:236–237.
2. Kobilka, B. K., T. S. Kobilka, K. Daniel, J. W. Regan, M. G. Caron, and R. J. Lefkowitz. 1988. Chimeric α_2-, β_2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science (Wash. DC)*. 240:1310–1316.
3. Ross, E. M. 1989. Signal sorting and amplification through G protein-coupled receptors. *Neuron*. 3:141–152.
4. Monod, J., J. Wyman, and J. P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118.