Reply to technical comment of M. Jackson

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Analysis of the binding specificity of receptor chimeras through use of $\Delta(\Delta G)$ values for pairs of agonist or antagonist drugs allows a linear comparison of the relative contributions of different receptor domains to receptor binding specificity (1, 2). This method was first applied to the data set of Kobilka et al. (3) on chimeras between β-adrenergic and α_2-adrenergic receptors, one of the first reports to use the chimeric-receptor approach to analyze the contributions of multiple transmembrane domains to receptor binding specificity. The data set of Kobilka et al. (3) was not ideal for this analysis because it included both comparisons of K_i values for competitive inhibition of ligand binding and EC_{50} values for activation of adenylate cyclase. Jackson (2) is correct in pointing out that these two values can differ substantially for agonists activating adenylate cyclase and therefore should not generally be mixed together in a quantitative analysis. However, $\Delta(\Delta G)$ values are calculated from the ratio of two K_i values or two EC_{50} values; for agonists of similar efficacy, these ratios will be similar. Indeed, the consideration of $\Delta(\Delta G)$ values, which depend only on ratios of apparent K_d values as previously proposed (1), substantially simplifies the assumptions that must be made in analyzing changes in relative binding specificity as pointed out by Jackson (2).

In applying this method to the data set of Kobilka et al. (3) for relative affinity of isoproterenol (ISO) and *p*-amino clonidine (PAC) for receptor chimeras, the same tentative conclusion is reached whether ratios of K_i values from competitive binding data are considered alone or together with ratios of EC_{50} values for chimeric receptor 6: transmembrane segments 1 through 5 each make an important contribution of ~0.8 kcal/mol to the value of $\Delta(\Delta G)$, but transmembrane segment 7 makes a much more substantial contribution of ~3.7 kcal/mol (1). These published data suggest that transmembrane segment 7 has a unique role in determining the binding specificity for these two ligands (1, 3). This conclusion would be substantially strengthened by a more complete analysis of the binding affinities of these two ligands for additional chimeras in which each transmembrane segment is exchanged in a stepwise manner. Data for such an analysis are not available at present. However, a recent report (4) shows that a single residue in the seventh transmembrane segment determines the binding specificity of yohimbine and related α_2 receptor antagonists in agreement with the conclusions of the previous work.

Epinephrine is a physiological ligand for both α_2- and β-adrenergic receptors. Although it binds with higher affinity to α_2 receptors, it is not nearly as selective as ISO or PAC. Jackson (2) shows by analysis of the results of Kobilka et al. (3) using $\Delta(\Delta G)$ values that each transmembrane segment contributes approximately equivalently to the binding specificity for epinephrine and ISO. One may expect that the determinants of binding selectivity for different classes of ligands will reside in distinct chemical interactions with different functional groups in or near the agonist binding sites of receptors. Evidently, the binding interactions which determine the selectivity of epinephrine and PAC for these two receptors are distinct, as one would predict from their different structures and specificity ratios.

Analysis of $\Delta(\Delta G)$ values for binding of receptor-specific ligands to chimeras of β_1 and β_2-adrenergic receptors synthesized in a bacterial expression system led to the conclusion that all seven transmembrane segments contribute to the binding specificity of these two adrenergic receptors and that the contribution of individual transmembrane segments varies depending on the specific ligand (5). Altogether, the experimental results analyzed by comparison of $\Delta(\Delta G)$ values for ligand binding to the family of adrenergic receptors argue that all transmembrane segments are involved in determination of binding specificity. Transmembrane segment 7 has a special role in binding ligands of specific structural classes. The use of $\Delta(\Delta G)$ values as a parameter for comparison (1) helps to simplify and clarify these complex analyses.

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