Making agonists of antagonists

Cell-surface receptors can be divided into three classes, depending on whether they transmit information by allosteric conformational change, by receptor dimerization, or by receptor aggregation. So far, only the first class of receptors has proven readily accessible to chemical techniques; this is no accident, but times are changing.

Chemistry & Biology November 1994, **1**:125–129

Cell surface receptors typically transmit information across the plasma membrane by one of three principal mechanisms: allosteric conformational change, receptor dimerization, and receptor aggregation. Although seemingly arbitrary, classifying receptors according to their signaling mechanism exposes a deeper partition. Each class of receptors has a characteristic or frequently encountered three-dimensional organization; similarly, the different classes differ in ligand size, complexity, and affinity, and even in their characteristic susceptibility, for the purposes of medicinal intervention, to the traditional methods of the pharmaceutical and biotechnological industries.

Structure and mechanism are often entwined

Frequently encountered representatives of the class of allosterically-activated receptors include members of the rhodopsin family of integral membrane proteins [1,2], which bear seven membrane-spanning α-helical segments and a ligand-binding site located in or close to the plane of the membrane which allows the reorganization of the relative positions of the membrane-spanning segments to be conveniently transmitted to the cytoplasm upon ligand binding (Fig. 1). Ligand-gated ion channels, although more heterogeneous as a group and more likely to consist of multi-chain complexes, frequently feature a similar organization, at least insofar as the ligand-binding elements are concerned.

Members of the dimerization-mediated activation class [3,4] are often encountered among receptors for growth or differentiation factors and frequently consist of one or a few associated polypeptide chains with a conventional type I integral membrane protein organization — that is, bearing an amino-terminal extracellular domain, a single membrane-spanning domain, and a carboxy-terminal cytoplasmic tail. In the case of the growth/differentiation factor receptors the cytoplasmic tail is often of substantial size, and may have intrinsic enzymatic activity. Examples of the latter include protein tyrosine or serine/threonine kinase activity, and may include protein tyrosine phosphatase and guanylate cyclase activities.

The third class, the aggregation-activated receptors, is frequently encountered in the immune system and draws its members from both simple single-chain and complex multichain receptor structures [5–8], in which any given receptor chain more often than not traverses the membrane only once. The members of this receptor class frequently bear short cytoplasmic domains which act to bind or recruit other cellular factors following the aggregation of their extracellular domains.

Ligand structure, complexity and affinity

The structural and mechanistic divisions among the receptor classes extend to the characteristic ligands for each type of receptor. The ligands of the allosterically activated receptors are frequently small molecules, either products of secondary metabolic pathways, such as the catecholamines, or short peptides, such as the neuropeptides. The ligands of the dimerization-mediated activation class are typically larger polypeptide hormones, including the cytokines, insulin and various growth factors. The aggregation-activated receptors frequently recognize cell-associated ligands, which may themselves be integral membrane counter-receptors. Among this class are the lymphocyte T and B cell antigen receptors and a variety of immunologically important receptors such as CD4, CD8, CD28, B7 and the family of NGFR/TNFR-related receptors and their ligands. Thus the complexity of the ligand correlates with receptor mechanism and function, effectively spanning the range from simple low-molecular-mass compounds to intact cells.

The receptor classification scheme described above also, to some extent, divides the receptors according to the type of agent likely to affect their activity, and even according to whether the agents currently available are likely to block or induce receptor signaling. Drugs developed for the small allosterically-activated receptors are frequently antagonists, while those developed for the dimerization-activated receptors have been, to date, naturally occurring (or minimally modified) agonists. Agents being developed for the aggregation-activated category appear to be about evenly mixed between agonists and antagonists at the present time. In part these divisions reflect the roles of the receptors in various disease states, and in part the present feasibility of creating the desired agents. For example the very high affinity of cytokines for their receptors considerably complicates the task of devising a suitable low-molecular-weight antagonist.

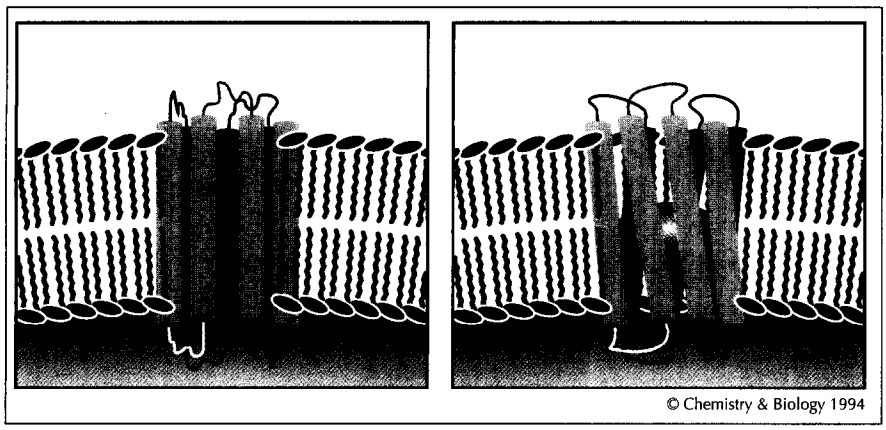

Fig. 1. Signal transduction by a seven-transmembrane receptor. On binding to ligand (grey), the relative positions of the helices alter, allowing signaling molecules in the cytoplasm (such as trimeric G proteins) to detect the ligand binding event.

Differences in ligand complexity underlie another, somewhat less obvious aspect of the receptor classification, which is the differing industries that have grown up around the exploitation of the receptors for medicinal purposes. The members of the allosterically-activated receptor class are typically the targets of established pharmaceutical companies, while the members of the dimerization- and aggregation-activated receptor classes are usually the targets of biotechnology firms. This partition reflects the expertises of the different industries, and their rooting in chemistry and biology respectively. For example, among the common targets of drug discovery, only the allosterically-activated receptor class has yielded pharmacologically effective agents from the large-scale screening of libraries of small synthetic compounds. Particularly telling is the fact that, despite decades of effort, no small organic molecule that is capable of mimicking the action of insulin has been described. (Vanadate or its coordination compounds, known to be nonspecific inhibitors of protein tyrosine phosphatases, are unique among low molecular weight compounds in that they induce a euglycemic state in diabetic animals by apparently mimicking the consequences of insulin receptor activation; however, the mechanism of action is unknown and is presumably intracellular, acting downstream of the receptor kinase activity [9,10]). One of the purposes of this article is to draw attention to the potential for targeting new low-molecular-weight synthetic compounds to act upon the class of receptor activated by ligand-mediated dimerization, possibly including the insulin receptor itself.

Two models for dimerization- (and aggregation-) activated receptor signaling

Two basic mechanisms for receptor activation contingent on molecular association have been suggested. In the structural transition mechanism, receptor association facilitates a weak interaction between chains, which results in the formation or stabilization of a tertiary structure that is recognizably different from the structures the individual chains are capable of adopting. The resulting new conformation prompts signal initiation either by modulating an intrinsic or associated enzymatic activity, or by forming a substrate for other signal transduction pathway enzymes. This type of mechanism may account for the activation of JAK family kinases bound to the intracellular domains of the growth hormone, erythropoietin and related receptors ([3,11,12]; see Fig 2). In the second type of activation pathway, the substrate proximity mechanism, aggregation allows a receptor-associated (or integral) enzyme to direct its action against other chains that are brought near the enzyme as a consequence of dimerization or aggregation. This mechanism is thought to account for the activation of at least some of the integral membrane receptor kinases, which undergo intersubunit phosphorylation following ligand-mediated dimerization of the extracellular domains ([3]; see Fig 3). The intermolecular phosphorylation activates the tyrosine kinase activity, which then goes on to act on other substrates, initiating a complex signal propagation cascade. Frequently, intersubunit phosphorylation recruits other signal-transducing molecules to the phosphotyrosine residues created by receptor activation; this interaction, mediated by Src-homology 2 (SH2) domains, allows the recruited proteins to distinguish between different sites of phosphorylation on the receptor intracellular domains.

Synthetic antagonists for dimerization-activated receptors

Various experimental and formal considerations support the notion that it should be possible to identify small synthetic compounds that interfere with the action of the dimerization-activated receptors. The formal basis for this expectation is the proven ability of large-scale random screening and directed synthesis to identify small organic molecules that have a high affinity for the relatively small binding pockets of receptors of the allosterically activated class. Two factors are likely to be important in the successes that medicinal chemists have achieved: first, the binding cleft of the target receptors is in general relatively hydrophobic, increasing the magnitude of the free energy change associated with the displacement of water from the ligand binding site; and second, the contact surface that is needed to ensure high affinity binding is commensurately small, permitting chemically less complex compounds to effectively block the agonist-binding surface.

Because the dimerization and aggregation-activated receptors recognize large proteinaceous ligands, the chances for effective occlusion of the ligand-binding

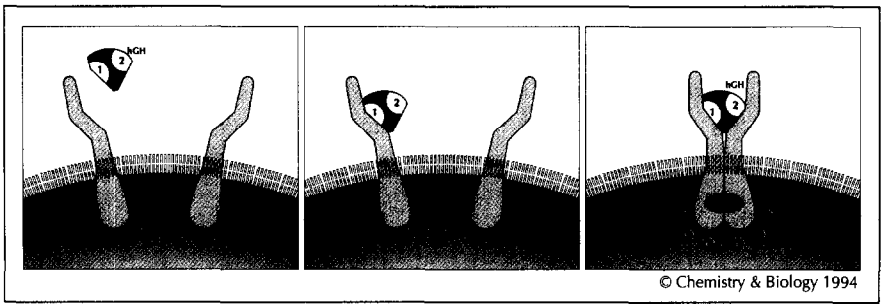

Fig. 2. Signaling by human growth hormone (hGH) requires receptor dimerization. Growth hormone has two sites for its receptor; when two receptors are crosslinked by a molecule of hGH, the JAK tyrosine kinases associate with the cytoplasmic tails of the receptor, initiating signaling.

surface would appear to be dim [2]. But unlike the aggregation-activated receptors, the dimerization-activated receptors typically bind their ligands with very high affinity, with dissociation constants frequently five or more orders of magnitude lower than those of the allosterically-activated receptors. This suggests that a comparably effective binding surface, as measured by the decrease in free energy of the complex per unit surface of contact, could exist for both classes of receptor. For example the difference in dissociation constant between that of a cytokine receptor, typically in the range of pM to tens of pM, and that of a rhodopsin family receptor, typically on the order of μM, is sufficient to account for a difference in free energy of binding of ~20 kcal mol^{-1}. Roughly speaking, if the free energy decrement per unit of contact surface is constant, this corresponds to an approximate doubling of the effective area contributing to the binding.

Although the available surface area of a cytokine or polypeptide hormone can be very much greater than twice that of a small secondary metabolite, it has not been clearly elucidated how much of the contact area of a polypeptide is important for interaction; in at least one case it appears likely, based on mutational analyses, that only a fraction of residues contribute in an important way to the contact surface [3]. Clearly the nature of the contact surface is very important for determining the overall strength of the interaction. As mentioned above, hydrophobic surfaces present a particularly favorable opportunity for increasing the strength of interaction, whereas the binding between hydrophilic ligands and their receptors, as exemplified by the interactions between carbohydrates and their cognate lectins, is typically much weaker [13]. Fortunately, it appears that the mating of complementary hydrophobic surfaces is an important theme in the determination of binding specificity, which offers the possibility that small synthetic molecules with appropriate contours may meet or exceed the local binding energy of the natural ligand, which is to say the binding energy considered thermodynamically as an intensive variable. Moreover, as the example of the rhodopsin family receptors shows, it is frequently easier to make a high-affinity antagonist than it is to make a high-affinity agonist. This is because the agonist must effect a particular structural change and hence has a structurally-limited contact surface with which to interact. An antagonist, by comparison, merely needs to impede access to the ligand binding site, which does not impose the same structural constraints. Moreover, an antagonist can exploit features of the target receptor that are unimportant for ligand binding, such as exposed residues well outside the ligand binding site, which may present favorable surfaces for high-affinity interaction. This is an important reason why antagonists, in addition to enjoying a greater structural diversity than agonists, can also have much higher affinities for their receptors.

Agonists from antagonists

For these reasons it appears likely that it should be possible to find small molecule antagonists which have the ability to bind and at least partially impede the adhesion of hormones to their target receptors. As mentioned above, the very much higher affinity of most cytokines for their receptors compared to that of the small-molecule agonists of the allosterically-activated receptors, will make it difficult to fully block the action of a physiologically-released hormone. But once even moderately effective small-molecule antagonists exist, the peculiar dependence of the signaling of the dimerization-activated receptors on the physical juxtaposition of two receptors, presumably under two-fold rotational symmetry, should make it possible to create agonists out of antagonists. A suitably constructed dimer of any small molecule that binds reasonably tightly to the receptor, with an appropriate spacer portion to allow mutual

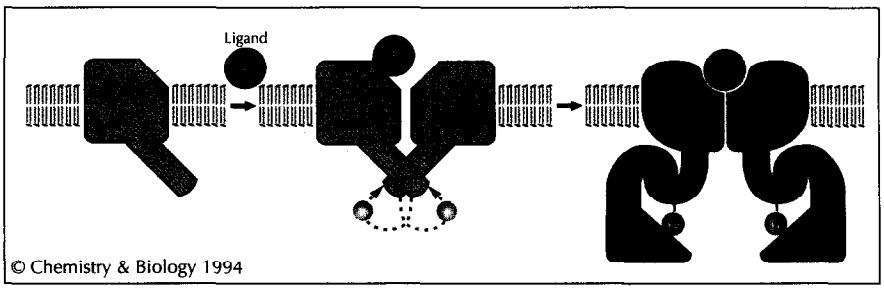

Fig. 3. Integral receptor tyrosine kinases need only be brought into proximity to each other to initiate signaling. Phosphorylation of the receptor tail then provides new binding sites for SH2-containing proteins, initiating a signaling cascade.

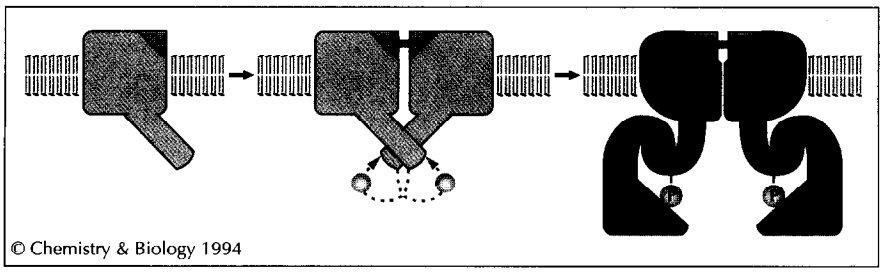

Fig. 4. Dimeric antagonists might act as agonists. If antagonists can be found that bind tightly to a dimerization-activated receptor, suitably constructed dimers of an antagonist should initiate signaling in the same way as the natural ligand (see Fig. 3).

contact of the active moieties with their receptors, should possess agonist activity (Fig. 4). Moreover, the strength of the overall binding interaction should be intensified by the addition of the second receptor-binding element, allowing the dimeric agonist to have a higher effective affinity, and presumably higher biological potency, than the monomeric antagonist. Under favorable circumstances, the free energy of binding of a dimer could approach twice the free energy of binding of the monomer, this situation being achieved if the unfavorable entropic contributions associated with receptor dimer formation are counterbalanced by favorable receptor–receptor interactions.

In addition to possibly providing orally active drugs to replace parenteral biologicals, dimeric synthetic agonists could be expected to shed light on the mechanism of receptor action — in particular, on the specific geometric requirements for dimerization-mediated activation. It can be anticipated, for example, that the precise spacer length will be less critical if receptor activation follows a substrate proximity mechanism as opposed to a structural transition mechanism.

Mixing and matching receptor pairs

As well as producing mimics for hormones and cytokines, small dimer agonists might allow the creation of novel cross-receptor activities, by using heterofunctionalized molecules to link the extracellular domains of one type of receptor to another. For example if substrate proximity underlies the activation of integral membrane receptor kinases, crosslinking kinases with dissimilar extracellular domains but shared enzymatic activities might lead to cross-phosphorylation and concomitant initiation of signal transduction. If they are active as agonists, such molecules could be used to increase target-cell specificity — that is, they could be expected to be active only on cells bearing appropriate pairs of receptors, which in turn might afford a more precise control over a particular cellular compartment, such as a specific stage in hematopoietic maturation. This approach is less likely to be successful if the precise spacer geometry is critical. It might be interesting to attempt in the case of the hematopoietin and γ-interferon receptors, however, which appear to share common binding sites for the JAK kinases but show different patterns of cellular activation [11].

Heterofunctionalized molecules which crosslink pairs of incompatible receptors should have an action equivalent to that of 'dominant negative' receptors, that is, receptors in which the natural activation following ligand binding is frustrated by pairing with an unreactive partner. Such receptor antagonists can be expected to be useful only if the increased affinity attributable to agents having two binding sites can approach that of the natural ligands, or if some other feature of the paired receptor, such as a propensity to be internalized, can be exploited to reduce the cell surface density of the targeted receptor.

In all of the above it can be argued that it is not, strictly speaking, necessary to begin with an antagonist; that is, any agent which forces the dimerization of a receptor in the appropriate manner should be acceptable. While this is true in principle, two arguments suggest that antagonists should be highly effective lead compounds. The first is structurally grounded, and based on the observation that the binding site is either known to be, or likely to be a cleft between faces of the receptor juxtaposed in the required orientation for initiation of signal transduction. Agents that impede access of the hormone or cytokine are therefore more likely to be on the appropriate surface and within range of a second binding site. The second argument is based on the hypothesis that binding clefts are more likely to support hydrophobic interactions than exterior surfaces. There is little evidence to support either of these arguments directly at present, so they remain largely teleological. But for either argument, the availability of a ligand competition assay greatly facilitates large-scale random screening and increases the probability that an appropriate lead will be found.

Increasing the scope of chemistry

All of this is to suggest that the lessons of the past may need to be unlearned. The failure to find fruitful agonists for the insulin receptor, while accurately reflecting the difficulty of finding a low-molecular-weight compound that mimics the action of a relatively large polypeptide hormone, is not due to the necessity of effecting an allosteric change contingent on full occupancy of the binding site. With improvements in structure-based rational synthesis programs, the prospects for identification and refinement of small dimer antagonists will increase the scope of chemists interested in mechanistic aspects of biological signal transduction.

These ideas are discussed further in the following pages [14], which also note a second approach to understanding these signaling events. This is to force the dimerization of receptor intracellular domains by appending to them novel binding sites that are susceptible to the action of

membrane-permeant low-molecular-weight dimerizers. For those who want their answers now, this approach allows the effects of signaling to be studied and exploited in the absence of a natural ligand, using compounds that are either known today, or readily identified prospectively.

References

1. Coughlin, S.R. (1994). Expanding horizons for receptors coupled to G proteins: diversity and disease. *Curr. Opin. Cell Biol.* **6**, 191–197.
2. Lameh, J., *et al.*, & Sadee, W. (1990). Structure and function of G protein coupled receptors. *Pharm. Res.* **7**, 1213–1221.
3. Wells, J.A. & de Vos, A.M. (1993). Structure and function of human growth hormone: implications for the hematopoietins. *Annu. Rev. Biophys. Biomolec. Struct.* **22**, 329–351.
4. Schlessinger, J. & Ullrich, A. (1992). Growth factor signaling by receptor tyrosine kinases. *Neuron* **9**, 383–391.
5. Reth, M. (1992). Antigen receptors on B lymphocytes. *Annu. Rev. Immunol.* **10**, 97–121.
6. Cambier, J.C., Pleiman, C.M. & Clark, M.R. (1994). Signal transduction by the B cell antigen receptor and its coreceptors *Annu. Rev. Immunol.* **12**, 457–486.
7. Chan, A.C., Desai, D.M. & Weiss, A. (1994). The role of protein tyrosine kinases and protein tyrosine phosphatases in T cell antigen receptor signal transduction. *Annu. Rev. Immunol.* **12**, 555–592.
8. Ravetch, J.V. & Kinet, J.P. (1991). Fc Receptors. *Annu. Rev. Immunol.* **9**, 457–492.
9. Heyliger, C.E., Tahiliani, A.G. & McNeill, J.H. (1985). Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science* **227**, 1474–1477.
10. Meyerovitch, J., Rothenberg, P., Shechter, Y., Bonner-Weir, S. & Kahn, C.R. (1991). Vanadate normalizes hyperglycemia in two mouse models of non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **87**, 1286–1294.
11. Sato, N. & Miyajima, A. (1994). Multimeric cytokine receptors: common versus specific functions. *Curr. Opin. Cell Biol.* **6**, 174–179.
12. Carter-Su, C., Argetsinger, L.S., Campbell, G.S., Wang, X., Ihle, J. & Witthuhn, B. (1994). The identification of JAK2 tyrosine kinase as a signaling molecule for growth hormone. *Proc. Soc. Exp. Biol. Med.* **206**, 210–215.
13. Weis, W.I., Drickamer, K., and Hendrickson, W.A. (1992). Structure of a C-type mannose-binding protein complexed with an oligosaccharide. *Nature* **360**, 127–134
14. Austin, D.J., Crabtree, G.R. & Schreiber, S.L. (1994). Proximity versus allostery: the role of regulated protein dimerization in biology. *Chemistry & Biology* **1** 131–136

Brian Seed, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA.