Cell receptors as drugs
Ligand binding domains of receptors as drugs: a potential new class of therapeutic agents

ABSTRACT—Modern technology applied to the study of receptor proteins has revealed that distinct structural domains of these molecules subserve different functions. With many receptors it is known that a soluble, extracellular portion of the molecule is responsible for the recognition and binding of endogenous or exogenous ligands. This binding is the initial event in signal transduction for cell-to-cell communication. Some of these soluble, extracellular ligand binding domains of various receptors have been genetically cloned, expressed and pharmacologically evaluated. The preclinical data available to date suggest that these portions of receptor molecules represent potentially useful new therapeutic agents.

The concept of a drug binding to a specific receptor on the cell surface has been of seminal importance to pharmacological sciences for more than 80 years. It evolved from Ehrlich's and Langley's speculations on the presence of chemically receptive substances on cell surfaces [1] and has now been confirmed by demonstrating the existence of distinct receptor protein molecules as products of single genes. Exploitation of this concept by pharmacology, medicinal chemistry and molecular biology continues to provide a heuristic focus for the discovery of many therapeutic agents. The object of the present commentary is to illustrate where, in a circumscribed arena, the application of recombinant DNA technology to the study of receptor structure–function relationships holds out the promise of a new class of therapeutic agents. Attention is particularly drawn to efforts to clone, express and ultimately evaluate in the clinic certain of the components of the receptor protein molecules themselves as new drugs. These components are specifically the soluble extracellular ligand binding domains of receptor molecules. The present discussion does not attempt to summarise the very large body of data dealing with efforts to identify, purify and more recently to clone, express and evaluate other molecules said to have inhibitory or receptor antagonistic activities against numerous endogenous ligands. (For a few recent examples see references 2–6.)

This molecular biology based endeavour is, in reality, a new variation on an old theme. By way of background, consider that the initial and, in many cases, the most pharmacologically accessible step in complex signal transduction pathways for cell-to-cell communication is the binding interaction of a circulating or synaptically released hormone or transmitter with its specific receptor(s). This binding event has been and continues to be a prime target in strategies for the discovery or design of new therapeutic agents which augment or inhibit this interaction. From a very substantial body of information accumulated over the past decade it appears that many cell surface receptors, which to date have been cloned and reconstituted in various expression systems, exhibit certain common structural and associated functional features.

Thus, regardless of the class of receptor (eg ligand gated voltage channels, G-protein coupled receptors, growth factor or cytokine tyrosine kinase coupled receptors, guanylate cyclase coupled receptors, cell adhesion molecule receptors), the binding event is mediated by distinct and identifiable domains of a single chain protein molecule or associated receptor proteins or subunits. (See references 7–9 for succinct reviews of this concept as it applies to various receptor classes.) These structural ligand binding domains of receptors provide the molecular basis or substrate for the well developed pharmacological concept of ligand or drug affinity. Other domains of receptor molecules, predominantly the transmembrane and cytosolic segments, are concerned with the initial sequelae of transduction and amplification of the binding event into an effector signal which initiates cellular responses. As such, the latter domains are the structural loci which provide the molecular substrate for the pharmacological concept of ligand or drug efficacy. With an understanding of these structure–function relationships, several laboratories have cloned and expressed soluble ligand binding domains of receptors with the object of exploring the therapeutic potential of these proteins.

Some relevant examples

Human immunodeficiency virus

The extensive and ongoing efforts to assess the therapeutic potential of soluble recombinant and, more recently, immunoadhesion forms of the CD4 molecule
can be cited as one of the first applications of a general ‘receptors as drugs’ hypothesis. The CD4 molecule is the major target receptor on the cell surface of T-lymphocytes for the initial binding of the gp120 glycoprotein present on the HIV, and for infectivity to occur this receptor–ligand interaction must take place. Smith et al [10] were the first to report the cloning and expression in CHO cells of a truncated form of soluble CD4 (sCD4) which lacked the transmembrane and cytoplasmic portions of the complete receptor molecule and secreted into the incubation medium. They also showed that this molecule would bind to the HIV gp120 envelope protein and inhibit HIV infectivity. Tests to date of its therapeutic efficacy indicate that extended or truncated forms of sCD4 protein molecules by themselves offer little promise, primarily because of their short plasma half-lives [11]. However, as will be discussed later, the concept of small molecule ‘receptor mimetics’ as it relates to the CD4 receptor in particular and the ligand binding domains of other receptors in general may represent the final, or at least the best, hope for the clinical potential of the general hypothesis under discussion.

Graft rejection

Dower et al [12] reported in 1989 on the cloning and expression of a soluble interleukin binding protein (sIL-1R). This work was based on the expression in HEK293 cells of a cDNA derived sequence coding for the extracellular region of the murine T-cell IL-1 receptor with sIL-1R secreted into the culture medium. The secreted protein molecule exhibited binding properties similar to those of the full length receptor protein. A collaborative and coincident report by Curtis et al [13] showed that sIL-1R, which constituted only a portion of the complete IL-1 receptor, was not sufficient for IL-1 signal transduction as measured in several IL-1 effector models. Of relevance to a possible therapeutic potential for sIL-1R is a recent report from the same organisation [14] which demonstrated in vivo effects on the immune system. Thus, injections into mice of low doses of sIL-1R prolonged the survival of heterotrophic heart allografts and blocked lymph node hyperplasia resulting from the injection of allogenic cells (irradiated C57BL/6 cells) into syngeneically compatible BALB/c mice.

Complement

Weisman et al [15] recently published a report on the cloning, expression and biological evaluation of a soluble form of a human receptor component of the complement system. The targeted protein, termed CR1, is an enzyme inhibitor of the convertases that proteolytically activate C3 and C5 proteins in the complement activation pathway. The authors were able to express a soluble portion of the protein, sCR1, from CHO cells transfected with a modified human cDNA encoding the A allotype of complement receptor 1. In vitro this sCR1 molecule, lacking the transmembrane and cytoplasmic domains of the parent protein CR1, was shown, at nanomolar concentrations, to bind the relevant ligands C3b and methylamine treated C4, thereby disrupting the activation of the complement pathway. An important observation was the finding that in vivo pretreatment with sCR1 significantly reduced myocardial ischaemia in rats subjected to temporary coronary artery ligation, suggesting that this novel approach to suppression of complement activation may have therapeutic application.

Tumour necrosis factor

As a final example, Gray et al [16] have reported the expression in and secretion from monkey COS-7 cells of the soluble extracellular binding domain of one of the human receptor proteins for tumour necrosis factor (TNF). This recombinant derivative was shown to bind TNFα and to inhibit the cytotoxicity of TNFα in an appropriate in vitro bioassay. In vivo data on the activity of this ligand binding domain protein have yet to be reported.

Prospects

The notion that protein molecules consisting of the ligand binding domains of receptors could themselves emerge as useful therapeutic agents has considerable merit but at the same time is compromised by other considerations. It would appear logical to assume that throughout the pharmaceutical industry widespread efforts are underway to clone, express and evaluate the biological activities of receptor proteins (in whole or in part), not only as substrates for drug design but as biopharmaceutical drug candidates themselves. However, aside from the present lack of clinical information on these particular recombinant molecules, problems with regard to immunogenicity and bioavailability are intrinsic to many high molecular weight proteins (inter alia antibody-based therapeutic agents). These problems may also prove to be a major barrier to the clinical success of soluble receptor proteins. For this reason, the concept of small, low molecular weight peptides, natural products or synthetic organic molecules which faithfully mimic the ligand binding avidity of receptor protein binding domains, ie ‘receptor mimetics’, may emerge as a driving force in screening or molecular modelling efforts to discover new types of drugs. An example of apparent success in this direction has recently been reported by Finberg et al [17]. With prior knowledge from mutational analysis of CD4 which implicated the Phe43 residue as the critical site for the binding of gp120 to CD4 [18–20], Finberg and associates have demonstrated that derivatives of small phenylalanine containing dipeptides (termed CPFs) could block gp120 binding to CD4 and inhibit HIV infectivity. It is important to note that these small
molecules do not interfere with the binding of CD4 to class II major histocompatibility complex molecules, thereby preserving CD4-dependent T-cell function. Thus, these CPFs, on the basis of their apparent affinity for gp120 (which may be considered as the ligand in the gp120/CD4 interaction), may represent the first example of small molecules functioning as a receptor mimetic. There do not appear to be any theoretical or conceptual barriers to the possibility that, for many classes of receptors, other small molecules can be found which act in a similar fashion.

Finally, the challenges that arise in all fields of therapeutics associated with the multiplicity of receptor subtypes differentially localised amongst cells and tissues may prove more susceptible to attack through the use of highly selective recombinant binding proteins. This will be the case if and where it can be shown that the binding domains of receptor subtypes contribute to selective receptor subtype activation by virtue of their structural (ie distinct amino acid sequences) as well as anatomical diversity. The converse and more pessimistic perspective is that the structural domains of any given class of receptors (eg IL-1α–IL-1β, D1–D3 receptors, M1–M3 receptors) which confer and mediate high affinity binding to an endogenous ligand may be similar, if not identical. If this proves to be the case, then soluble ligand binding domains of receptors may turn out to act in effect as circulating molecular ‘chelators’ with little discriminative powers among receptor subtypes.

Conclusions

The first generation of biotechnology based therapeu- tic successes derived from cloning and expression of mammalian proteins is well established. Relevant examples include insulin, growth hormone, erythro- poietin and perhaps IL-2. Returning to the seminal receptor concept, these molecules subserve agonist functions to stimulate presumed suboptimal cell-to-cell signalling in various pathological conditions. It may be that a new, second generation of agents that influence signal transduction is emerging from genetically engineered receptor protein molecules. Specifically, they are the soluble portions of receptor molecules which recognise and bind endogenous and exogenous ligands.

Substantial and intensive efforts have been made to solubilise and purify receptors and to clone and express them. A major objective of these efforts has been to provide pure substrates for structure–function analysis and to assist in the design or discovery of molecules that specifically interact with these proteins. These efforts will no doubt continue unabated. It is worthy of note, however, than an outcome of this endeavour, namely the possible use of the ligand binding domains of receptor protein molecules as drugs, may portend important and widely applicable therapeutic gains.

References

1 Taylor P, Insel PA. Molecular basis of drug action. In: Pratt WB, Taylor P, editors. Principles of drug action. New York: Churchill Livingstone, 1990.
2 Sekinger P, Zhang JH, Hauptmann B, et al. Characterization of a tumor necrosis factor α (TNF-α) inhibitor: evidence of immunological cross-reactivity with the TNF receptor. Proc Natl Acad Sci USA 1990;87:5188-92.
3 Fernandez-Botran R, Vitteta ES. A soluble, high-affinity, interleukin-1-binding protein is present in the biological fluids of mice. Proc Natl Acad Sci USA 1990;87:1929-32.
4 Hannum CH, Wilcox CJ, Arend WP, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990;343:336-40.
5 Carter DB, Deibel MR Jr, Dunn CJ, et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 1990;344:633-8.
6 Eisenberg SP, Evans RJ, Arend WP, et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 1990;343:341-6.
7 Lowe JA. Progress in the characterization of peptide receptors. In: Bristol JA, editor. Annual Reports in Medicinal Chemistry. San Diego, California: Academic Press Inc. 1990;25:281-7.
8 Brackenbury R. Cell adhesion molecules. Curr Biol. 1990;2:235-44.
9 Triggle DJ, Langs PW, Schooley J, et al. Purine nucleoside phosphorylase and other enzymes of the purine salvage pathway as potential drug targets. Annu Rev Med 1990;41:281-304.
10 Smith DH, Byrn RA, Marsters SA, et al. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science 1987;238:1704-7.
11 Mitsuya H, Yarchoan R, Broder S. Molecular targets for AIDS therapy. Science 1990;249:1533-44.
12 Dower SK, Wignall JM, Schooley J, et al. Retention of ligand binding activity by the extracellular domain of the IL-1 receptor. J Immunol 1989;142:4314-20.
13 Curtis BM, Gallis B, Overell RW, et al. T-cell interleukin-1 receptor DNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1. Proc Natl Acad Sci USA 1989;86:3045-9.
14 Fanselow WC, Sims JE, Sassenfeld H, et al. Regulation of allerrootivity in vivo by a soluble form of the interleukin-1 receptor. Science 1990;248:739-42.
15 Weisman HF, Bartow T, Leppo MK, et al. Soluble human complement receptor type 1: in vivo inhibitor of complement suppression post-ischemic myocardial infarction and necrosis. Science 1990;249:146-31.
16 Gray PW, Barrett K, Chantry D, et al. Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein. Proc Natl Acad Sci USA 1990;87:7380-4.
17 Finberg RW, Diamond DC, Mitchell DB, et al. Prevention of HIV-1 infection and preservation of CD4 function by the binding of CPFs to gp120. Science 1990;249:287-90.
18 Arthos J, Deen KC, Chaikin MA, et al. Identification of the residues in human CD4 critical in the binding of HIV. Cell 1989;57:469-81.
19 Chao BH, Costopoulos DS, Curiel T, et al. A 113-amino acid fragment of CD4 produced in Escherichia coli blocks human immunodeficiency virus-induced cell fusion. J Biol Chem 1989;264:5812-6.
20 Bowman MR, Macferrin KD, Schreiber SL, et al. Identification and structural analysis of residues in the V1 region of CD4 involved in interaction with human immunodeficiency virus envelope glycoprotein gp120 and class II major histocompatibility complex molecules. Proc Natl Acad Sci USA. 1990;87:9052-6.

Address for correspondence: Dr G. G. Yarbrough, Panlabs Inc., 11804 North Creek Parkway South, Bothell, Washington 98011, USA.