CHAPTER 5

Antibodies to Beta-Adrenergic Receptors

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1. Introduction

Contemporary biochemists and molecular biologists strive to understand the relationship between the function and the detailed chemical structure of macromolecules. Whereas chemical and direct physical analyses are employed to probe molecular structure, specific antibodies to proteins have been invaluable reagents in the determination of the fine-structure of the antigen as well as the immunologic relationship of the antigen to other proteins. Often a crowning achievement to many years of arduous work purifying and characterizing a cellular protein is the production of specific antibodies to the protein. The availability of specific antibodies then propels the direction of research into investigations of entirely new areas of protein structure, function, and regulation that could not be approached by any other route.


181
Historically, the use of antibodies to explore important questions of protein structure and regulation was first applied to the study of enzymes involved in intermediary metabolism. Methods of purification developed for many of these enzymes, which are cytosolic, globular proteins, provided a solid foundation for the successful production of specific antibodies. In the last two decades antibodies have been prepared to many structural proteins, enzymes, and cell-surface antigens. Recent advances in several converging technologies now make possible the production of highly specific antibodies to membrane-bound receptors for hormones and drugs. First, the widely applied methodologies of affinity chromatography have facilitated our ability to purify membrane-bound receptors (Cuatrecasas, 1972). These receptor proteins are often of very low abundance but can be rapidly purified by taking advantage of a specific interaction between the receptor and a high-affinity ligand immobilized on an insoluble matrix. Utilized in tandem with ion-exchange, hydrophobic, and high-performance liquid chromatography systems, affinity chromatography has been employed as a means to purify membrane proteins that often constitute less than 10^-4% of cellular protein (see Chapter 2 this vol.; Bahouth et al., 1988). Second, advances in microsequencing of proteins (Hunkapiller and Hood, 1983) as well as solid-phase peptide synthesis (Marglin and Merrifield, 1970) now make it possible to provide primary sequence information from picomole quantities of purified proteins as well as the automated synthesis of peptides (ranging up to 30 amino acid residues in length) for use as defined antigens. Molecular cloning and the application of the polymerase chain reaction (PCR) to isolate and amplify regions of DNA displaying sequences of homology are but two prime examples of how molecular biology has provided powerful new tools for the identification of low-abundance membrane proteins based upon scant protein or DNA sequence information. Finally, hybridoma-monoclonal antibody techniques (Kohler and Milstein, 1975) permit the production of many different, yet individually-homogeneous antibodies to single antigenic sites (epitopes) of proteins and synthetic peptides. In concert, these techniques have revolutionized the immunochemical approaches employed to generate antibodies capable of probing the fine-structure and function of membrane proteins.