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The Role of Antibodies and Physiological Receptors in Cardiovascular Diagnosis, Therapy, and Research*

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ANTIBODIES are now an accepted tool in the measurement of physiological substances and drugs, particularly when these are present in low concentration in various body fluids. The radioimmunoassay has been widely exploited in both fundamental and clinical investigations. It has answered problems which were previously unapproachable by other techniques. In this part of the symposium, the speakers will concentrate on the application of radioimmunoassay techniques to the measurement of drugs and hormones modulating or controlling the cardiovascular system.

If one were to view an antibody as simply being a very specific reagent which is capable of binding a desired hormone or drug with very high affinity, it becomes relatively easy to extrapolate the application of antibodies from the measurement of physiological substances to specific interference with their action. Butler (3) discussed the application of digoxin antibody to the reversal of its physiological effect *in vivo*. In this instance, an exogenously produced antibody was employed to compete with a physiological receptor for a drug. The potentials for investigation and therapy of this general approach seem very great.

Immunoassays have not found application to all compounds of interest. In some instances, antibodies of either sufficient specificity or affinity cannot be raised; in others the range of discrimination of the antibody may be inappropriate. Small changes in structure which inactivate a hormone may not cause an appreciable change in binding energy to antibody. The relationship between antibody binding and physiological action of a wide variety of amino acid sequence variants on the structure of bradykinin was reviewed recently (7). Physiological inactivation implies a change in binding characteristics to the receptor of the hormone, and it occurred to several investigators that the isolated receptor might be utilized for an isotope displacement assay *in vitro*. The application of the specific adrenal receptor for adrenocorticotropin in a radio displacement assay has permitted not only some increase in sensitivity over radioimmunoassay but also a new range of discrimination. When a variety of congeners of this hormone were tested, binding was proportional to biological activity (9). In an analogous fashion, Brooker and Jelliffe (2) devised a radioreceptor assay for digitalis glycosides utilizing a crude preparation of brain Na, K-activated adenosine triphosphatase (ATPase). Unlike the radioimmunoassay which will differentiate among glycosides on the basis of small structural differences within the steroid structure, this enzyme binds these compounds with approximately equal affinity. For example, there is little

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difference in binding between digoxin and digitoxin, whereas antibodies may be raised which discriminate between these glycosides by at least one to two orders of magnitude in binding constant. As in the example of the adrenocorticotropin receptor, the enzyme appears to bind the drug in relation to its pharmacological effect. Variations in structure which affect binding to this enzyme receptor must reflect changes in pharmacological efficacy.

In a subsequent paper in this symposium, Lefkowitz (10) presents the potential use of the *beta*-adrenergic receptor protein as a method for measuring plasma concentrations of catecholamines. A radioimmunoassay has not been developed for these compounds. A possible reason may be that the catecholamine molecule is of insufficient size or complexity to permit the development of antibodies of sufficient specificity or affinity. The receptor protein is able to do what antibodies cannot, namely to discriminate between the physiologically active catecholamines, epinephrine and norepinephrine, and their metabolites.

The greatest investigative potential of antibodies lies in their capacity to specifically interdict a physiological process either by binding a hormone or by binding its receptor. An example of such an application has been an attempt at a definition of the role of the renin-angiotensin system both in the normal maintenance of blood pressure and its elevation in renovascular hypertension. A major tool in these investigations has been antibody to angiotensin II. While the physiological questions have not yet been answered, it has been clearly demonstrated that either the active immunization of animals with conjugates of angiotensin II (4–6, 12) or passive immunization by infusion of antisera (1, 8) results in blockade of the pressor response of intravenously injected angiotensin II.

In a most interesting recent application, Na, K-ATPase was isolated and purified from canine kidney. The enzyme proved to be a good antigen in rabbits. The resultant antibody was capable of inhibiting the activity of the purified enzyme but not of magnesium-dependent ATPase. However, despite marked inhibition of this enzymatic activity in either human or canine purified enzyme or in microsomal particulate preparations, experiments with canine renal slices and human red cells showed no specific effect of the antibody on active monovalent ion transport in the intact cell. These experiments suggest that the antibody response is directed against antigenic determinants inaccessible to the macromolecules of the outer cell surface (11).

We now stand at the beginning of an exciting era not only in the diagnosis of cardiovascular disease but also in research on the major problems of cardiovascular control. It is generally accepted that any drug or hormone present in low concentrations, which needs to be measured, can be measured by the techniques discussed here. Of far greater interest, however, is the utilization of some of these methods for enhancing our understanding of how these physiologically active substances work. Finally we may one day see the application of antibodies in human therapy in situations where it may be desirable to reverse the action of a drug or a hormone.

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