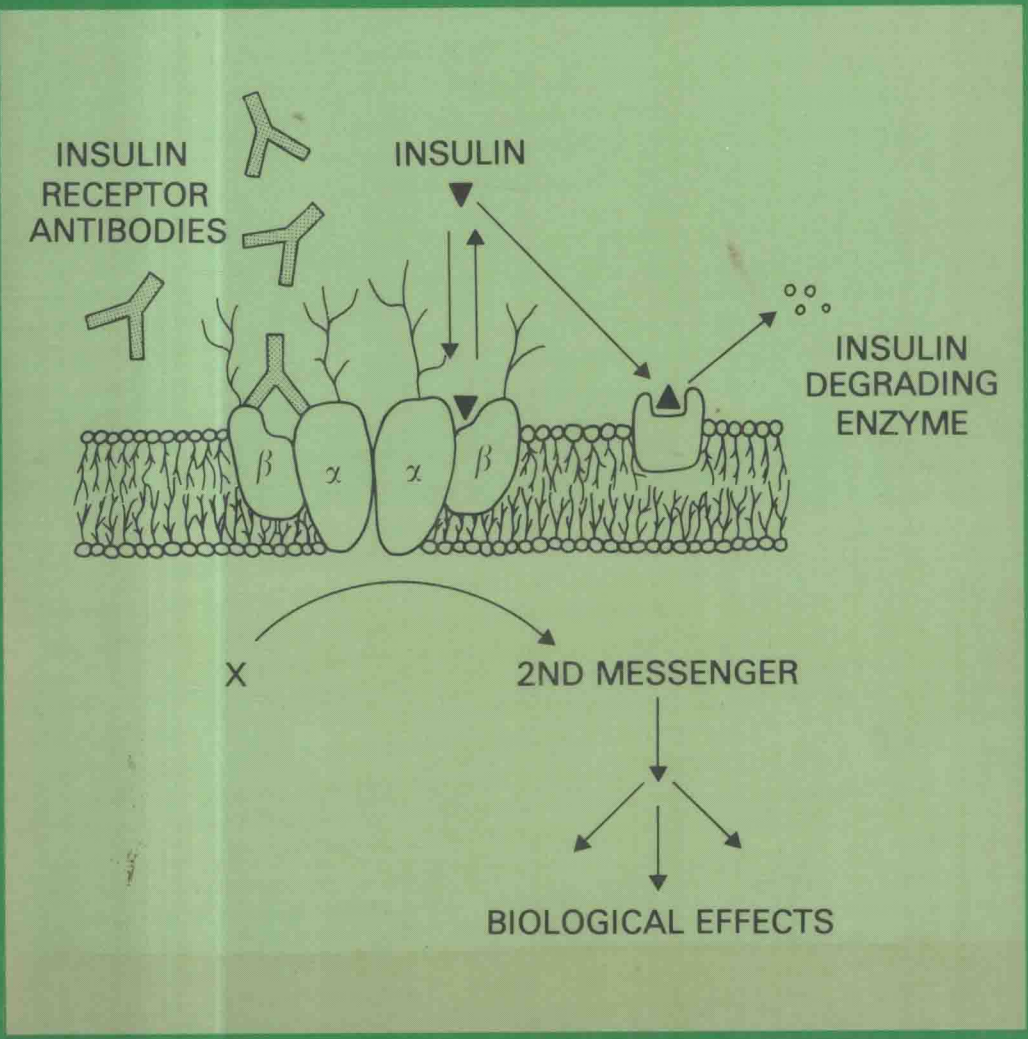
Ciba Foundation Symposium 90

Receptors, antibodies and disease



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Symposium on Receptors, antibodies and disease, held at the Ciba Foundation, London, 27–29 October 1981
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Introduction

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We all know why we are here at this symposium, and we know where this subject of receptor antibodies started. I think it actually began in two places: first, when a group of persevering, patient people in New Zealand started pulling Long Acting Thyroid Stimulator apart. It is therefore particularly good to have John Knight from Duncan Adams' team here. The other place where it began was the Salk Institute, when some rabbits' ears started drooping and a trick was found to get those ears to perk up—I am thinking here of the work of Patrick and Lindstrom. Then we knew we were in business! So that is where we go on from. It has become quite clear that the discovery of antibodies against receptors, and of how important they are in clinical autoimmune disease, is now a central topic in the sense that it has all sorts of implications for cell biology and biochemistry (which are actually the same subject) on one side, and for immunology on the other. The cell biology that we shall hear about will include studies on the structure of receptors—molecular biology proper—and how they mediate transmembrane signalling. It is very interesting to see how these clinically important antibodies turn out also to be probes which provide insight into how transmembrane signalling works and clues about deep questions in endocrinology. On the other hand, in immunology, the fact that an antibody appears is really the *end* of the story. One wants to know how the antibody came to be there to begin with, and also how it can be regulated once it has begun to be produced—how it can be turned on and off. That will take us right into the central questions of regulatory control in the immune system. I suspect we shall hear a great deal about two things: firstly, idiotypes and the idiotype network, because that is one exciting current idea about immune regulation; and secondly, perturbations in the regulatory T cell system, or T cell circuits, which is a second exciting idea at present.

It is a pleasant occasion that brings endocrinologists and immunologists

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together like this, because endocrinology and immunology are subjects that have much in common. They are both very 'conceptual' parts of science, set a little apart from mainstream physiology and biochemistry. We are living in a time of technical revolution for both these subjects. The major revolution is in recombinant DNA, and although we may not talk much about that here, we know it will be looking over our shoulders all the way through this meeting. A second revolution going on in immunology at present is in cloning. In the past year or two, the immune system, which essentially consists of sets of parallel clones of cells all interacting with each other (competing for antigens and suppressing or helping one another), has been systematically pulled apart by cloning. Immunologists are basically divided into T cell people and B cell people, and the two groups got round to cloning at about the same time but more or less independently of one another. The hybridoma, an innovation due to César Milstein, has completely changed the world of antibodies, and equally we know that T cells are also being cloned—sometimes by hybrids; sometimes by viral transformation; sometimes simply by repeated antigenic stimulation, or by using growth hormone; there is a whole range of available techniques. A technical revolution of that sort doesn't immediately change our concepts. What it does is to make much of the work that has already been done semi-obsolete, in that much has now to be repeated, using cloned cells. All the problems in immune regulation that we shall be discussing have to be thought of from that point of view.

Structure–function relationships in adenylate cyclase systems

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Abstract Hormone-sensitive adenylate cyclase systems are composed of hormone-recognition units (R), a nucleotide-regulatory unit (N) for reaction with GTP and divalent cations, and the catalytic unit (C). From the reported sizes of purified R and N subunits and target analysis of functional sizes of these units, the functions of the components for the binding and actions of hormones and GTP require minimally dimers, homologous or heterologous. It is proposed that the catalytic unit exists in the membrane also as a dimer and that its transition to the active state with MgATP as substrate involves corresponding transitions in linked dimers of the hormone-recognition and nucleotide-regulatory units. It is postulated that hormones trigger the activation process by inducing in concert with GTP and divalent cations the appropriate dimer structure of the holoenzyme. In large aggregates of such structures, realignment of only a few occupied holoenzyme units may be sufficient to induce activation of the total aggregate enzyme. This theory serves to explain the synergistic actions of hormones, and how several hormones can activate a common enzyme. It also provides an explanation for 'spare' receptors, and for the efficacy of hormone action.

Most hormones act on their target cells through receptor molecules located on the outer surface membrane. The principal functions of these receptors are to concentrate their specific hormones by a reversible process and to transduce this process into the regulation of cellular responses. The recognition process has been more intensively studied than the transduction process because of the availability of labelled hormones, antagonists, drugs, toxins, cross-linking agents, and receptor antibodies with which to monitor and quantify the presence of receptor molecules. As will be discussed in other papers, through such agents and with the aid of affinity chromatography,

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receptors for acetylcholine and insulin have been purified, the components analysed and, in the case of the cholinergic receptor, even biological function has been reconstituted from purified components. The latter studies indicate that transduction and recognition are achieved by a composite of distinct macromolecules. I have recently reviewed (Rodbell 1980) evidence that hormone receptors involved in the regulation of adenylate cyclase systems are also composed of distinct subunits for hormonal recognition and transduction. Here I will discuss certain aspects of the structure–function relationships in the cyclase components and then suggest how these components may be organized and the enzyme may be regulated by hormones and other regulatory ligands.

Components of adenylate cyclase systems

A large body of evidence has accumulated to show that binding or recognition components (abbreviated R) involved in the hormonal activation of adenylate cyclase are separate molecules from the transduction components that directly induce activation of the catalytic components (C). The transduction process involves proteins (abbreviated variously as N, G/F, or G) that contain sites for the binding of GTP and divalent cations, key elements in the transduction process. The N unit is required also for activation of C by fluoride ion and by cholera toxin. The toxin catalyses ADP-ribosylation of the N unit, a process that with labelled NAD has enabled investigators to identify and purify to near homogeneity a functional N unit (Northup et al 1980). Toxin-labelled proteins with relative molecular masses (M_r) of 42 000 and 53 000 have been detected in purified N units from liver membranes; only the 42 000 M_r protein is seen in certain other membranes. These proteins appear to contain homologous structures but it is not clear that they function identically in the transduction process. In addition to toxin-labelled proteins, the purified N unit contains a 35 000 M_r protein, the function of which remains unknown but which co-purifies in equal quantity with the toxin-labelled proteins. Possibly it is the site of binding and action of divalent cations (Mg or Mn). The functional mass required for hormonal activation of adenylate cyclase is 130 000, suggesting that two or more N units are minimally involved in the transduction process.

The N unit and the C unit are situated on the inner face of the plasma membrane and have sufficiently hydrophobic properties to suggest that they are bound to the membrane through hydrophobic forces, possibly with lipids. The N unit forms complexes with C; these complexes can be activated by fluoride ion or by non-hydrolysable guanine nucleotides such as 5′-guanylylimidodiphosphate (Gpp(NH)p). As judged from the ability of

hormones to promote the binding and degradation of GTP and the ability of the latter to affect the binding of hormones, the N unit also forms complexes with R units (Lad et al 1980). It is likely that such interactions take place in the membrane and that such complexes span the plasma membrane. As with the linkages of N with C, the linkages of N with R are also reversible: the latter are stabilized when R is occupied by hormone. The different properties of RN and NC complexes toward phospholipases, detergents, metal ions, organic mercurials and guanine nucleotide analogues argue for N having differing domains for its interactions with C and R. Studies with cell variants also suggest differing complex-forming domains on N (Schleifer et al 1980). Given the complex subunit structure of N and the possible heterologous structures of both the C and R units, it is not possible at this time to give a detailed view of how the units are organized in the membrane and what forces are involved in their interactions. From what little is known, lipids and SH groups are strong candidates for some of the interacting forces.

The 'coupling' problem

Because R, N and C form reversible associations, it is possible that associations between the units may be rate-limiting steps during the activation process. However, our current inability to quantify the number of functional R, N and C units required for activation, combined with the experimental difficulties of directly measuring interactions in membranes, make it difficult to establish that such interactions are indeed involved in the regulatory process. By the same token, there must be an explanation for findings that different hormones can activate, through distinct receptors, the same enzyme through what appear to be identical N units. Such findings have been cited as arguments for receptors being 'mobile' and capable of interchanging with one another in their interactions with N. Moreover, it has been shown that R or RN complexes can be inserted by fusion techniques into membranes with functional consequences in the hormonal activation process (Schramm 1979). The kinetics of hormonal activation can be accommodated by a concept of a step-wise cascade of events in which R, N and C units interact sequentially in response to the actions of hormones and guanine nucleotides (DeLean et al 1980). Nonetheless, despite what appear to be convincing arguments for ligand-induced associations, there is no convincing evidence against the possibility that the cyclase units are already complexed in the membrane in a form subject to regulation by hormones and guanine nucleotides. A relatively slow equilibrium between complexed and free components might affect the extent and duration of the stimulatory process but not the immediate triggering actions of the activating ligands. If the rate-limiting steps do not

involve associations between the macromolecular components, what other steps or processes have been or should be considered?

The GTPase cycle

In some systems it has been shown that dissociation of tightly bound GDP is a rate-limiting step in the overall activation process by hormones and GTP (Cassel & Selinger 1978). Depending on the system examined, GDP formed by hydrolysis of GTP (presumably at the N unit) can be either a potent inhibitor or a partial activator. In either case, GDP inhibits the activating effects of GTP, the presumed natural activator of the system. In systems in which hormones stimulate release of tightly bound GDP, this action permits activating ligands to enter the site. If it is occupied by GTP, rapid hydrolysis occurs and activation of the enzyme system is dependent on the continued occupation of the R unit by the hormone. This so-called GTPase regulatory cycle can satisfactorily explain the kinetic behaviour of some cyclase systems. However, there are many systems in which GDP is not tightly bound to N and for which there is no evidence that release of GDP formed from hydrolysis of GTP is a rate-limiting step in the activation process.

Transition states

Recently, Neer & Salter (1981) have shown with solubilized N and C units that, while these units form reversible associations, activation by guanine nucleotides does not involve nucleotide-induced associations of these units. Rather, the evidence obtained suggests that the NC complex isomerizes between different states of activity and that the isomerization process is relatively slow compared to the association between N and C. Isomerization between different states of the guanine nucleotide-occupied cyclase system has been postulated (Rodbell et al 1975), based on modelling of the hepatic enzyme system in its response to Gpp(NH)p and Mg^{2+}. It was also suggested that occupation of R by glucagon increases the rate of isomerization between inactive and active states; enhanced occupancy of N by guanine nucleotides was not required to explain the actions of hormones in this model. At that time, the possibility was considered that low and high activity forms of the enzyme reflected differing affinities for inhibitory forms of ATP. However, in view of subsequent evidence that the enzyme systems contains regulatory sites for divalent cations and that these sites require the N unit for the expression of their effects (Londos & Rodbell 1975), the isomerization

process more likely involves changes in the reactivity of the N unit (the 35 000 M_r protein?) with divalent cations; the latter ligands may act in concert with GTP to bring about activation of the C unit. These dual requirements for both GTP and divalent cations (Mg or Mn) could explain the long-known stimulatory effects of hormones on the ability of Mg ions to activate adenylate cyclase.

Properties of the catalytic unit

An important characteristic of the transition between inactive and active forms of C is that C preferentially utilizes MnATP as substrate when it is not activated by hormones, guanine nucleotides, and divalent cations. The physiological substrate, MgATP, is converted to cyclic AMP when the enzyme is activated by these ligands. Interestingly, even though activation of C by fluoride ion also involves the N unit, the fluoride-activated enzyme still prefers MnATP as substrate. Thus, the linkage of N with C is not necessarily the determining factor in the form of the enzyme that uses MnATP or MgATP as substrate. There must be other constraints imposed on the C unit that fluoride ion somehow releases: possibly this represents release of a regulatory subunit.

A soluble form of adenylate cyclase that uses primarily MnATP as substrate is found in the adult testis (Braun & Dods 1975). This form of the enzyme has none of the regulatory characteristics exhibited by the membrane-bound C unit. Moreover, its functional molecular weight (M_r) is about 60 000, or about half that of the functional C unit that uses MnATP as substrate in the membrane (Nielsen et al 1981). Possibly, therefore, the C unit of hormonally regulated systems exists in the form of dimers which, when linked with C, can show transitions between MnATP- and MgATP-states of the enzyme. If C is linked to N as dimers, principles of symmetry dictate that N is also structurally linked to C as dimers. As discussed previously, the functional N unit seems to be minimally composed of heterologous dimers of N. Theoretically, interactions between heterologous N units with C can give rise to a number of possible configurations having differing levels of activities. One could thus imagine that activation by such non-physiological activators as fluoride ion and Gpp(NH)p involves states normally not accessible to the enzyme system. The most obvious role for the hormone and its R unit is to ensure conversion of the NC complex to the most favourable, *reversible* activated state that uses MgATP as substrate. If NC dimers are required for the transition, then it follows that dimers of R units are also involved in the transition process.

Functional structure of receptors

Shorr et al (1981) have reported that the purified β_2-adrenergic receptor in frog erythrocytes contains a 58 000 M_r subunit; the turkey erythrocyte β_1-receptor appears to have subunits of M_r values 41 000 and 37 000 (Atlas & Levitzki 1978). From target analysis of the latter cyclase system (Nielsen et al 1981), the functional mass of the β_1-receptor is about 90 000 M_r whether measured from the binding of antagonists or by the activating effects of an agonist on cyclase activity. From this it would seem that the functional unit of the β_1-receptor is a dimer. Since antagonist binding also required a dimer of R, the differences in the binding and actions of agonists and antagonists probably reside in the structure of the dimer. Possibly the dimer can take two or more transition states, the one induced or stabilized by agonists causing NC to become active. In this case, partial agonists cannot distinguish in an all-or-none fashion between the antagonist and agonist forms of the R dimer. The glucagon receptor in liver membranes has also been tagged with a labelled marker (Johnson et al 1981) and yielded on sodium dodecyl sulphate–polyacrylamide gel electrophoresis primarily a single band with an apparent mass of 62 000. This size is significantly smaller than the target size (about 120 000 M_r) estimated for the glucagon R unit when linked to the activated form of the cyclase system (Schlegel et al 1979). It would appear, therefore, that the functional form of the glucagon receptor is also a dimer of either homologous or heterologous subunits. Different subunits may be involved, since the method of tagging the receptor involved linkage of glucagon via cross-linking agents which may selectively react with but one of two or more putative subunits. Nonetheless, it would appear from this limited number of studies that R units, for either peptide hormones or catecholamines, may require two or more subunits for their participation in the overall activation process. The question at this point is how dimers of R, N, and C units can serve to accommodate the properties of adenylate cyclase systems.

Theory

As a basic assumption, I suggest that the fundamental unit of the enzyme system that expresses activity in response to hormones, guanine nucleotides, and divalent cations is a dimer of RNC units. The R units in the dimer can be either homologous or heterologous with respect to the specificity of hormone binding. Because both R units of a homologous dimer can be occupied by the same hormone, RNC dimers containing homologous R units are more effective in activating the enzyme than RNC dimers containing heterologous units. Efficacy of hormone action in a multi-receptor-containing system is a

function of the number of homologous R units present or formed during hormone action. Since it is likely that the amount of homologous R dimer is proportional to the concentrations of the various R units, it follows that the actions of a hormone with the highest concentration of R units will dominate activity when all hormones are added together at maximal stimulating concentrations. Thus, this postulate provides an adequate explanation for the non-additivity of hormone action on multi-receptor systems. An additional outcome is the possibility that binding of two different hormones to a heterologous dimer might give more activity than when the same dimeric RNC unit is occupied by a single hormone. Synergistic effects of combinations of hormones have been observed (Birnbaumer & Rodbell 1969).

The most difficult problem to solve with any of the theories advanced for hormone activation is the often-observed phenomenon in which minimal occupation of R can yield near maximal or even maximal activation of adenylate cyclase. In the 'collision-coupling' theory (Tolkovsky & Levitzki 1978), a single R unit can interact repeatedly with the same enzyme unit because R serves essentially as a catalyst in the activation process. Bergman & Hechter (1978) suggested a fixed matrix of R units arranged with respect to the enzyme components such that a single hormone molecule triggers, in ricochet fashion, a number of R units within the field with resultant activation of a number of C units in the matrix over a short span of time. In the context of the theory presented here, a similar matrix or cluster of RNC can be postulated, the difference being that the binding of a hormone to one dimeric unit in the cluster might cause neighbouring units to take the requisite transitions to more active states (occupied by GTP and Mg^{2+}). This theoretical triggering action of a hormone can be likened to a propagated change in contractility of an elastic set of intertwined proteins (muscle, for example) in response to a structural modification of only one protein in the complex. A tightly packed arrangement of clustered RNC units might satisfy this type of triggering mechanism, particularly if occupation of the N units by GTP and Mg ions is not dependent on hormone occupation of R—that is, the action of the hormone is primarily that of affecting the rate of transition to the active state rather than the number of units that can be occupied by the activating ligands, GTP and Mg ions.

The idea of a cluster of RNC units is not far-fetched. Target analysis of the hepatic cyclase system indicated that the ground-state structure (prior to activation) is at least four-fold larger in size than the final hormone-activated state of the system (Schlegel et al 1979). Included in this ground-state structure were R and N units. If the C unit is included and a dimer of RNC units represents the holoenzyme in its activated form, then it can be calculated that the ground state of the hepatic system is composed of a tetramer of dimeric RNC units.

Finally, a few comments on how this theory may relate to the phenomenon of desensitization, a process in which the actions of a hormone or a drug on a system can lead to loss of hormone or drug response. The current idea on this process with respect to adenylate cyclase systems is that loss of hormone response is due to uncoupling of the receptor from the system. Since desensitization follows activation one might consider that uncoupling reflects a change in the stability of the organized complex of regulatory and catalytic components when it is converted from a tightly packed matrix of RNC units to a less ordered, metastable structure of the activated state. Since hormones, GTP and divalent cations are required to achieve the activated state, desensitization should depend also on the actions of all these ligands. This dependency has been reported recently for desensitization of the luteinizing hormone-responsive adenylate cyclase system in isolated membranes of corpus luteum (Ezra & Salomon 1981).

Concluding comments

It is apparent from this brief review that rapid strides have been made towards an understanding of the components responsible for the regulation of adenylate cyclase systems. Purification and reconstitution of hormone receptors, GTP-regulatory proteins, the proteins that bind divalent cations, and the catalytic unit now seem feasible ventures. Such efforts should lead to a better understanding of the structure and organization of the system in its membrane environment. The theory presented here suggests that the components are assembled in a highly organized structure. This is based on rather modest evidence gleaned largely from target analysis, a relatively new procedure for assessing the structure of membrane-bound proteins. Nonetheless, the theory at least has the testable feature of predicting a relatively large structure having the specific characteristic of clustered receptors. With appropriately tagged hormones it should be possible to visualize such structures in isolated membranes or intact cells.

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