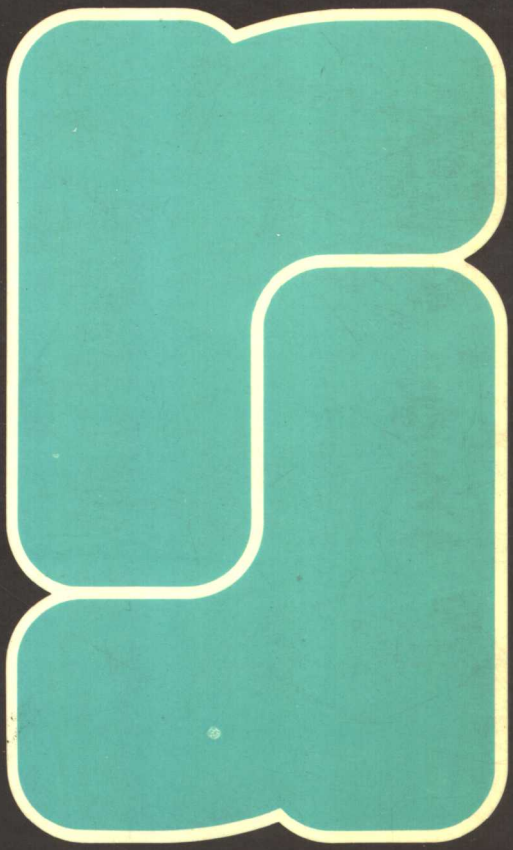
6 Receptors and Recognition

Series A



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6 Receptors

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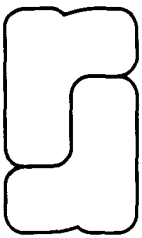
Receptors and Recognition



Preface

As in previous volumes of this series of 'Receptors and Recognition', Volume 6 seeks to demonstrate and promote the value of comparing and integrating recognition events in widely different biological systems in the search for common mechanisms or evolutionary links. The topics presented in this volume illustrate the realization and potential of cross-fertilization of methodologies and concepts.

The chapter by Eytan and Kanner describes imaginatively the current state of the art in the exciting area of membrane reconstitution. The methodology for incorporating isolated or purified membrane proteins into artificial lipid model systems while retaining or regaining the original function of that protein (ie. reconstitution) is becoming an indispensable tool in membrane biochemistry. This approach can provide a functional assay for membrane proteins, an especially important tool for proteins (eg., promoters of solute transfer proteins, hormone receptors, etc.) whose functionality is lost or disturbed upon solubilization. In addition, it is possible by this approach to examine systematically the effects of changing the membrane milieu (ie. membrane composition) on the function of a given protein. Such studies are also providing insights into the normal mode of membrane assembly *in vivo*.

O'Brien's chapter is a lucid and critical review of one of the most important model systems in membrane biology, that of the light-sensitive glycoprotein, rhodopsin. Like the erythrocyte, which has taught us profoundly important lessons about oxygen transport and the chemistry of heme proteins, the study of rhodopsin illustrates how broad can be the research opportunities of an apparently narrow, specialized problem. O'Brien describes vividly how photoreceptor outer segments may be the most convenient preparation of excitable membranes and provide a nearly ideal system for study. Apart from providing insights into the molecular events of vision research, the outer segments are an excellent model for studying the role of phospholipids in the permeability of membranes. Rhodopsin is a remarkably interesting protein – it is a lipoprotein, a glycoprotein, and a conjugated protein with a chromophore that absorbs in the visible portion of the spectrum, and it is an

integral membrane protein that spans the membrane and may even itself be an ion pore under conditions of illumination.

The chapter by Fain represents a most perceptive and imaginative exposition of the broad field of hormones, membranes and cyclic nucleotides. Perhaps the most crucial questions and problems in this field have been identified and addressed in a fashion that brings together devergent facts and separates fancy. A number of extremely important and exciting topics are raised in a critical and interesting manner. Among those are included the nature of hormone receptors, the possible mechanisms for receptor-adenylate cyclase recognition and coupling, agonist-specific desensitization and tachyphylaxie and the possible roles of calcium, adenosine and phosphotidylinositol breakdown. The complexities and highly integrated nature of interactions in these systems are brought out forcefully, and an interesting case (the activation of glycogen phosphorylase in rat liver by hormones and cyclic nucleotides) is presented which illustrates these points convincingly. Despite the enormous problems and difficulties of these biological systems, progress into the molecular bases of some fundamental phenomena are now occurring rapidly!

May 1978

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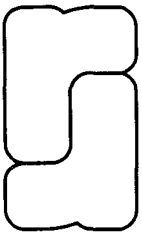
M.F. Greaves



Contents

1 Hormones, Membranes and Cyclic Nucleotides

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Abbreviations

Cyclic AMP	Adenosine 3′, 5′-monophosphate
Cyclic GMP	Guanosine 3′, 5′-monophosphate
EGTA	Ethylene glycol bis (β-aminoethylether)–N, N-tetraacetic acid
GppNHp	Guanyl-5′-yl imidodiphosphate
CDP diglyceride	Cytidine diphosphate diglyceride

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1.1 INTRODUCTION

Many hormones interact with receptors on the outer surface of the plasma membrane of cells. The hormone–receptor complex alters the enzymatic activity of membrane-bound enzymes. Adenylate cyclase is probably the best example of a membrane-bound enzyme which is activated by hormones and other agents. Adenylate cyclase catalyses the formation of cyclic AMP which serves as an intracellular messenger to activate cytoplasmic enzymes.

Cyclic AMP was discovered twenty years ago by Sutherland and Rall (Rall and Sutherland, 1958; Sutherland and Rall, 1958). They found that the activation of glycogen phosphorylase in slices of dog and cat liver by catecholamines and glucagon involved an unknown heat-stable compound which turned out to be cyclic AMP. Sutherland and Rall were able to obtain cell-free liver homogenates in which activation of phosphorylase could be readily demonstrated upon the addition of hormones. The response of the liver homogenate occurred in two steps. In the first step, a particulate fraction of the liver homogenates (which contained fragments of the plasma membrane) produced a heat-stable factor (cyclic AMP) in the presence of glucagon or catecholamines. In the second step, the addition of cyclic AMP activated phosphorylase in the supernatant fraction of the homogenate.

Most effects of hormones on intact cells which are thought to involve changes in the activity of membrane-bound enzymes have been difficult to demonstrate in cell-free systems. However, adenylate cyclase is an exception since it responds to a large array of hormones added to membrane preparations derived from a wide variety of cells.

A formidable problem in all biological research is the development of suitable assay procedures for substances present in very low concentrations. Cyclic AMP was no exception. For ten years the only available assay was the activation of dog liver phosphorylase introduced by Sutherland and Rall. Their laboratory was virtually the only one in which this assay could be reliably performed. However, the recent development of sensitive, accurate, fast and relatively inexpensive assays has resulted in a tremendous expansion of cyclic nucleotide research. The radioligand binding assays using protein kinase introduced by Gilman (1970) and antibodies against cyclic AMP by Steiner, Parker and Kipnis (1972) are

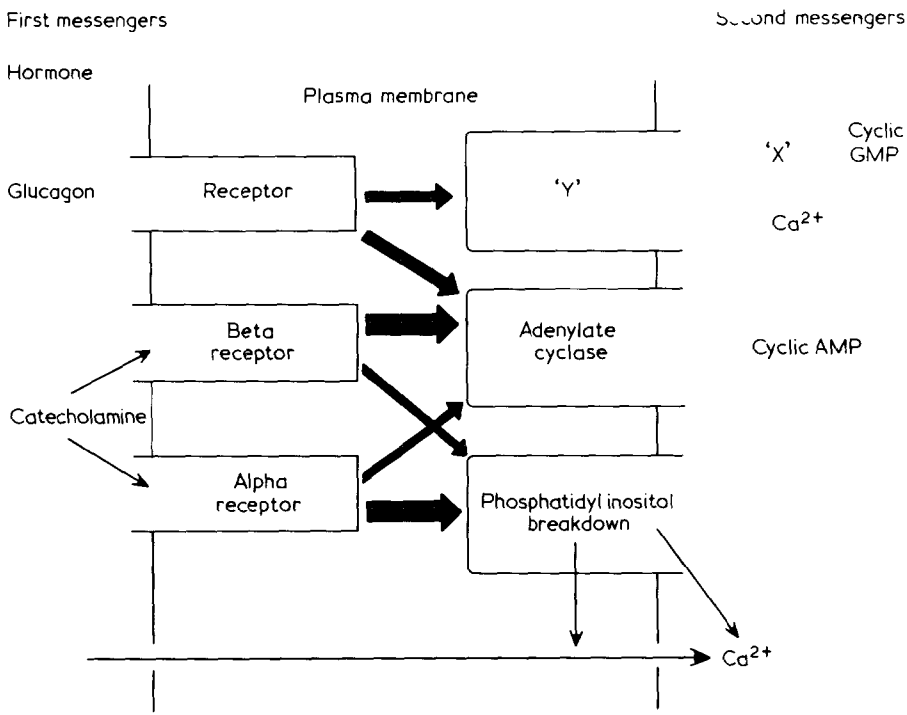

Fig. 1.1 Representation of the interaction of hormones (first messengers) with the cell membrane and the release of intracellular signals (second messengers). Cyclic AMP accumulation is accelerated by the interaction of glucagon or β-catecholamines with receptors fixed on the outer surface of the plasma membrane. The hormone–receptor complex rapidly activates adenylate cyclase located on the inner surface of the plasma membrane. There are receptors in the membrane for α-catecholamines which alter an unknown enzyme 'Y' resulting in an increase in the intracellular accumulation of 'X'. The two leading candidates for 'X' are Ca^{2+} and cyclic GMP. Possibly 'X' is intracellular Ca^{2+} which is responsible for the increase in cyclic GMP. The model postulates that the glucagon–receptor complex is able to activate other enzymes besides adenylate cyclase such as 'Y'. The difference between α and β effects of catecholamines is that β predominantly activate adenylate cyclase while α activate 'Y'. The breakdown of phosphatidylinositol may be an example of the 'Y' activation by α adrenergic agonists which is related in some unknown fashion to the release of 'trigger' Ca^{2+} and the entry of extracellular Ca^{2+}.

now universally used and have supplanted all other assays for cyclic nucleotides. The antibody procedure can be used to detect other cyclic nucleotides such as cyclic GMP by using the proper antisera.

The original concept of Sutherland and his associates (Robison *et al.*, 1971) was that hormones are first messengers which carry information to the plasma membrane of cells where they interact with receptors (Fig. 1.1). The receptors are localized in the plasma membrane and do not carry the hormone into the cytosol or nucleus of the cell. If the hormone–receptor complex does not enter the cytosol then a mechanism is required to transfer information into the cell. Possibly some hormones regulate cellular function by activating membrane-bound proteins such as those involved in the regulation of hexose and Ca^{2+} transport. However, in many cases, there is a need for a second messenger to transfer information to the cell's internal machinery. Robison *et al.*, (1971) suggested that cyclic AMP was not the only second messenger. At the moment cyclic GMP and Ca^{2+} are the leading candidates to join cyclic AMP as second messengers.

There has been an unfortunate tendency to assume that if a hormone or other agent alters the level of a cyclic nucleotide then all of the effects of that hormone are secondary to alterations in cyclic AMP or GMP. There are fads and fashions in science as in all other aspects of human endeavour. At the moment, cyclic nucleotides are in style and we are being deluged with reports on measurements of cyclic nucleotides in every possible system. Investigators have reported correlations between cyclic nucleotides and almost every known physiological and pharmacological response. However, data which do not agree with cyclic nucleotide involvement in a given response have often been ignored in the past.

A prior article in this series by Sonenberg and Schneider (1977) emphasized biophysical approaches to hormone action at the plasma membrane. Cuatrecasas and Hollenberg (1976) have reviewed the role of membrane receptors in hormone action. I have reviewed the role of cyclic nucleotides in the hormonal regulation of fat cell metabolism (Fain, 1973a, 1977, 1978). The present chapter emphasizes the effects of hormones on the coupling of the hormone–receptor complex to adenylate cyclase in the plasma membranes of mammalian cells. Particular attention is given to the hypothesis that activation of adenylate cyclase is not the sole effect of hormones such as glucagon, catecholamines and thyrotropin.

1.2 CRITERIA USED FOR INVOLVEMENT OF CYCLIC AMP IN HORMONE ACTION

The original criteria established by Sutherland and his associates (Robison *et al.*, 1971) for involving cyclic AMP in a given effect of a hormone were as follows:

(1) Adenylate cyclase activity of broken cell preparations should be stimulated by the hormone. Hormones which do not give the particular response should be without effect on adenylate cyclase.

(2) Intracellular cyclic AMP should be elevated by concentrations of the hormone which are capable of producing the physiological response. The log-dose response curve for the physiological response and for elevation of cyclic AMP should be identical. Furthermore the elevation in cyclic AMP should precede the physiological response rather than follow it. Hormones which do not produce the given response should also be inactive with respect to elevating cyclic AMP.

(3) The response to the hormone should be potentiated by inhibitors of cyclic AMP phosphodiesterase.

(4) Exogenous cyclic AMP should mimic the action of the hormone.

In addition there are now several other criteria which can be added:

(5) There should be protein kinase activity of extracts from the particular cell which is activated by cyclic AMP.

(6) The addition of cholera toxin should mimic the effect of the hormone if cyclic AMP is elevated by the toxin.

(7) If the enzyme responsible for the response to the hormone (lipase in the case of lipolysis or phosphorylase for glycogenolysis) is known it should be activated by the addition of cyclic AMP and protein kinase.

The effects of many hormones meet these criteria and can be explained by activation of adenylate cyclase. However, it is possible that some hormones exert effects independent of cyclic AMP. The concentration of hormone required to give a half-maximal activation of cyclic AMP accumulation is often far greater than that required to give a half-maximal response to the hormone. Few studies have shown a good correlation between levels of cyclic AMP and the physiological response under a wide variety of conditions. Most reports presented as proof of this criteria measured cyclic AMP in the presence of methyl xanthines and the response to the hormone in the absence of methyl xanthine.

The lack of correlation between cyclic AMP and the given response may sometimes be more apparent than real if there is a high basal

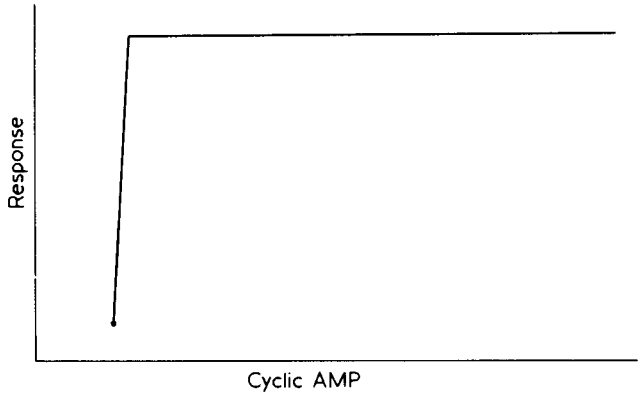

Fig. 1.2 The theoretical relationship between total cyclic AMP accumulation and a particular biological response when the pool of cyclic AMP is a small part of the basal pool. This model assumes that doubling the physiologically important pool of cyclic AMP is sufficient to give a maximal response. The large excess capacity for cyclic AMP accumulation may have other functions unrelated to the short-term response.

concentration of cyclic AMP. Basal cyclic AMP values may represent the total cyclic AMP content of several different cell types and intracellular compartments. If the active cyclic AMP pool is a small fraction of the total, it could be doubled without any detectable elevation in total cyclic AMP. This may be the explanation for the almost all-or-nothing effect of hormones on cyclic AMP illustrated in Fig. 1.2 which is frequently seen in many cells. The accumulation of large amounts of cyclic AMP usually requires an unphysiologically high concentration of hormone and the presence of methyl xanthine. Neither are characteristic of *in vivo* conditions. A concentration of hormone sufficient to give a maximum biological response *in vivo* seldom elevates cyclic AMP by more than a factor of two.

Another possibility is that there are other second messengers formed in the presence of hormones which potentiate the action of cyclic AMP. This hypothesis suggests that cyclic AMP is not the primary signal, but rather is responsible for a prolonged and sustained response to hormones.

In rat liver cells, an increase in cytosol Ca^{2+} activates glycogen phosphorylase. An attractive hypothesis is that low concentrations of hormones increase the Ca^{2+} pool in contact with glycogen phosphorylase while higher concentrations elevate cyclic AMP. The action of Ca^{2+} may be more transient than that of cyclic AMP which activates protein kinases that phosphorylate regulatory enzymes. Possibly Ca^{2+} or other unknown

messengers might increase the sensitivity of protein kinase to cyclic AMP by binding to and inactivating inhibitors of protein kinase.

1.3 ADENYLATE CYCLASE

This review is largely concerned with the regulation of adenylate cyclase which is located in the plasma membrane of animal cells. Adenylate cyclase catalyzes the conversion of a molecule of ATP to cyclic AMP and pyrophosphate. Mg^{2+} is required for the reaction and the substrate is probably a Mg^{2+}–ATP complex.

Under physiological conditions, the formation of cyclic AMP is virtually irreversible due to the very high level of pyrophosphatase. The formation of cyclic AMP utilizes the equivalent of two high energy phosphate bonds per molecule of cyclic AMP which is formed. The phosphodiester bond of cyclic AMP is a high energy bond since the free energy of hydrolysis is about 12 kcal mol^{-1}. This does not appear to have any particular role in cyclic AMP activation of protein kinase. Rather it may be involved in ensuring that the conversion of cyclic AMP to 5′-AMP by cyclic AMP phosphodiesterase is an irreversible reaction.

Adenylate cyclase is widely distributed throughout the animal kingdom and is found in all nucleated cells (Robison *et al.*, 1971). The location of adenylate cyclase in the plasma membrane was first demonstrated by Davoren and Sutherland (1963). In the early studies of Sutherland on adenylate cyclase, the tissues were homogenized under conditions in which the plasma membrane was present as large pieces which sedimented with nuclei in the 600 *g* precipitate. Subsequent studies have clearly demonstrated the presence of adenylate cyclase in the plasma membrane (Perkins, 1973). The plasma membrane appears to be the only location of this enzyme in mammalian cells.

Adenylate cyclase is probably located on the inner surface of the plasma membrane. The addition of ATP to intact cells results in its cleavage by membrane-bound ATPase but no formation of cyclic AMP occurs (Robison *et al.*, 1971). Treatment of whole cells with proteolytic enzymes reduced the activity of ATPase but not that of adenylate cyclase (Oye and Sutherland, 1966).

The isolation and purification of adenylate cyclase from membranes has proven to be a difficult problem. Cyclase activity can be solubilized rather readily with detergents. However, in most studies solubilization results in an irreversible loss of hormone sensitivity. With few exceptions,