Cell Regulation by Intracellular Signals

Edited by
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and
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PREFACE

In 1980, the IVth International Cyclic Nucleotide Conference was held in Brussels. As this meeting attracted many investigators involved in cyclic nucleotides and calcium role in intracellular regulation, it was thought that this opportunity could be used to organize, prior to the Congress, an in-depth introductory course on the subject. This was carried out as a NATO Advanced Study Institute. The participants included Ph.D. students and M.D.s engaged in a research training, but also fully trained and well known researchers who wanted a refresher course on the whole subject. During the course, most of the participants and lecturers asked to be provided with a text summarizing the basic lectures of the course. This book was therefore conceived as a basic textbook on the regulation and action of intracellular signal molecules, concentrating mainly on cyclic nucleotides and calcium. It was deliberately kept at a basic level. We would therefore be happy if it could be used as an introduction for interested M.D.s or Ph.D.s working in other fields or entering this field and as a general refresher for researchers interested in the subject. For this reason, very general schemes have been asked of the authors, along with reading lists of available reviews rather than extensive bibliographies.

The editors should like to thank the NATO Scientific Affairs Committee for having supported the course and Mrs. Gh. Wilmes who prepared the manuscripts.

S. SWILLENS
J.E. DUMONT

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INTRODUCTION : CELL CONTROLS AND SIGNAL MOLECULES

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All cells are submitted to a variety of specific and unspecific extracellular signals (Fig. 1.): photons, ions, metabolites, neurotransmitters, and local or general hormones. At all moments, the number, size, and activity of the cells result from their complex present and past network of interactions with these signals. The action of these signals may be defined by several of their characteristics : their nature, their kinetics, their biochemical mechanisms. Signals may control the level of activity of the target cell, either stimulating this activity (positive control) or inhibiting it (negative control). They can influence the nature of the cell activity, e.g., by changing the program of the cell; this is a differentiating action. They may also increase the number of functional units (e.g. organelles) per cell or the number of cells (hypertrophy and hyperplasia) or decrease it; these are positive or negative trophic actions. The kinetics of the action of signals varies from a few microseconds to days. The biochemical mechanisms of action also vary from the simple opening of an ion channel to the complex activation of sets of genes. It is obvious that these three characteristics are related; the opening of an ionic channel is almost immediate and can directly influence very defined enzymatic or transport functions. Differentiation which implies the alteration of the expression of sets of genes, and the consequent modification of the cell protein pattern, will necessarily take at least hours and will involve controls at the level of transcription.

Different extracellular signals acting on the same cell may interact at several levels : the primary site of interaction, the generation or action of intracellular signals, the concentration of receptors or intracellular signal-generating systems. Sometimes they activate the same biochemical system (e.g., adenylate cyclase),

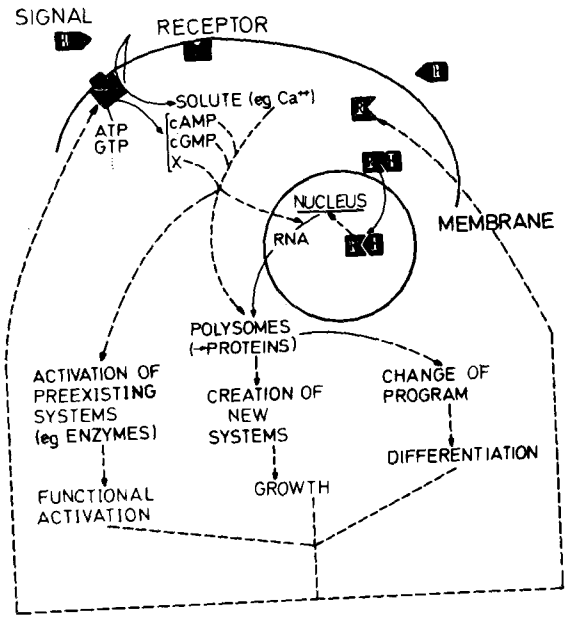

Fig. 1. Mechanisms of action of extracellular signals.

in which case their actions will only differ in amplitude and duration. They can also act in opposite ways on the same system (e.g., the α-adrenergic inhibition of the β-adrenergic-activated adenylate cyclase). It was hypothesized a few years ago that cyclic AMP on the one hand and cyclic guanosine monophosphate (cyclic GMP) and Ca^{++} on the other hand would act similarly in cells submitted to one type of external control, positive or negative (unidirectional system), whereas they would have opposite effects in systems submitted to both positive and negative controls (bidirectional systems). In fact, when they act on different biochemical systems, these different intracellular signals may cause similar, antagonistic, or complementary effects or combinations of such effects. A modification of the response to a signal may be called a modulation. On the long term, signals may modulate their own action or the action of other signals by increasing or depressing the level of specific

receptors. This type of action may involve the downward (e.g. insulin) or upward regulation of their receptors in their target cells, the induction or the repression of receptors to other hormones (e.g. steroid induction of insulin receptors), or even the regulation of the level of intracellular target enzyme systems.

In general, it must be emphasized that, whereas the nature of the extracellular signals and for the most part the accessory signals controlling a cell and the sign of the effect (positive or negative) of intracellular or extracellular signals may vary from species to species, the biochemical mechanism of action of these signals (e.g. cyclic AMP through protein kinase) and the overall pattern of control remain constant. All thyroids are activated by thyrotropin and inhibited by iodide.

Among the various regulation systems, this book is concerned only with extracellular signals molecules acting on the plasma membrane of the target cell to elicit intracellular signal molecules which, by affecting their effector proteins, induce their terminal physiological or pharmacological actions. The two best known systems at the moment, involve cyclic AMP and Ca^{++} as intracellular signal molecules. It is interesting that these systems have many properties suggesting that a general framework may apply to these and to other still to be discovered intracellular signals (Fig. 2). It may be useful to summarize these concepts in the introduction before the chapters describing the different elements of those systems.

The concept of the intracellular signal molecule or "secondary messenger" was proposed and developed by Sutherland, Rall, Butcher, Hardman, Krebs and their coworkers on the basis of the work which led to and followed their discovery of cyclic AMP. The model proposed can now be applied to a great number of hormones, local hormones and neurotransmitters. As it stands now this model can be described as follows :

1) Activation of adenylate cyclase. The extracellular signal molecule binds to specific receptor proteins floating in the two dimensional plane of the membrane. This binding leads to a conformational change in the receptor which allows it to activate a transducing protein (N, G/F, GTPase) which will then activate a catalytic unit that will convert ATP to cyclic AMP. Whether the three units of adenylate cyclase are bound together or more probably floating independently in the membrane remains controversial. Catabolism of cyclic AMP involves its hydrolysis to 5'AMP by specific phosphodiesterases.

2) Action of cyclic AMP on effector protein. Cyclic AMP dependent protein kinase is constituted of 2 catalytic units C and 2 regulatory units R_2. The regulatory units block the activity of the catalytic units. By binding to R_2 as $R_2(cAMP)_4$, cyclic AMP causes the release of the 2 catalytic units which thus become operational.

The protein kinases catalyse the phosphorylation of their specific protein substrates by ATP.

3) Activated proteins and effects. The phosphorylation of the target enzyme of the kinase may turn "on" or "off" its activity. This positive or negative effect will more or less directly determine the known physiological or pharmacological effects of the extracellular and intracellular signal molecules. Termination of the action is due to disappearance of the extracellular signal, loss of response to this signal (desensitization), and the action of cyclic nucleotide phosphodiesterases and protein phosphatases. Direct negative control of the cyclase by extracellular signal molecules through receptors and transducing proteins has also been demonstrated.

At about the same time that Sutherland developed his model, neurochemists working on the action of acetylcholine on nicotinic receptors showed that this agent acts directly on the membrane ionophores. The consequence of this effect in muscle was to elicit release of calcium from the sarcoplasmic reticulum and thus to induce contraction (stimulus-contraction coupling). This model was later extended to other types of muscles and to secretory tissues (stimulus-secretion coupling). As it now stands the model can be described as follows:

1) Opening of calcium channel. The extracellular signal molecule binds to a receptor protein in the plasma membrane. The activated receptor turns on an ionophore for calcium, thus allowing extracellular calcium to flow down its chemical and electrical gradient in the cytosol.

2) Effector protein. Calcium combines to a specific protein calmodulin (very similar to skeletal muscle troponin) which is then able to bind to specific enzymes and to activate them.

3) Activated protein-effect. The binding of calcium containing calmodulin turns on specific enzymes, the activity of which, through a more or less extended sequence, causes the physiological or pharmacological response of the target cell. In one such sequence, calmodulin activates specific protein kinases which, by catalyzing the phosphorylation by ATP of specific protein substrates, thus activates or inhibits them. Termination of the effect results from desensitization of the receptor, release of the extracellular signal molecule and from calcium removal mechanisms : plasma membrane extrusion pumps and sequestration pumps.

The Ca^{++} and cyclic AMP system thus both involve plasma membrane exterior receptors, membrane catalytic units (cyclase, ionophore), intracellular signal molecules and their effector proteins (regulatory

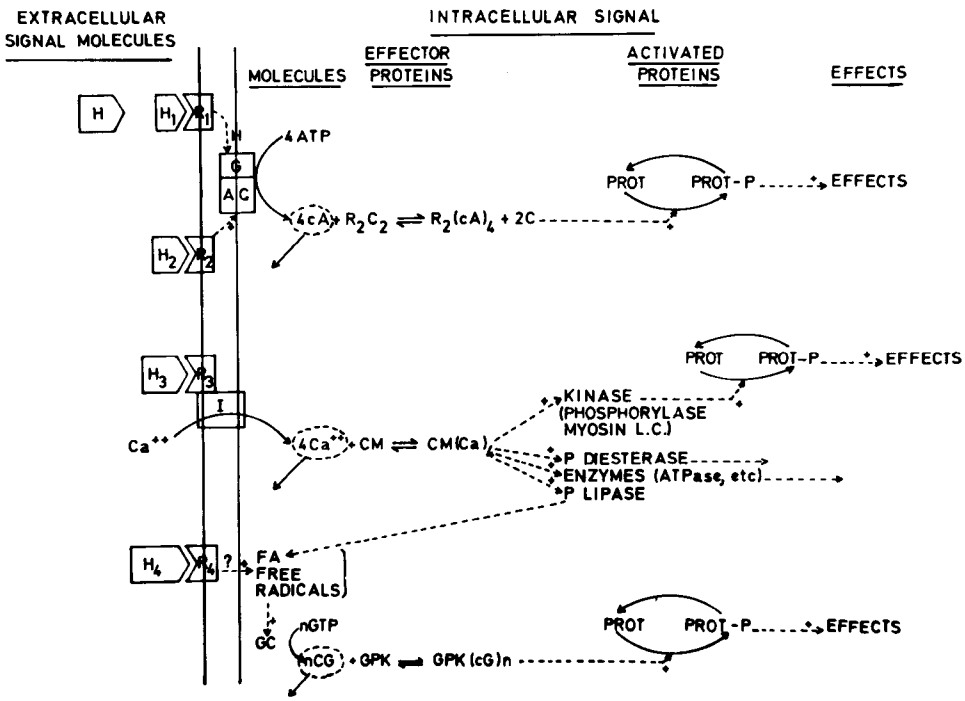

Fig. 2. Cellular control by intracellular signal molecules

H = extracellular signal molecules
R = receptor
G = G/F, N, unit of adenylate cyclase (GTPase)
AC = catalytic unit of adenylate cyclase
I = ionophore
CA = cyclic AMP
R_2C_2= cyclic AMP dependent protein kinase (R : regulatory
C : catalytic units)
Prot, Prot P = dephospho and phosphorylated protein
CM = calmoduline
LC = light chains of myosin
P diesterase = phosphodiesterase
P lipase = phospholipase
FA = fatty acids
GC = guanylate cyclase
CG = cyclic GMP
GPK = cyclic GMP activated protein kinase
$-\overset{+}{\underset{-}{}}\rightarrow$ = positive control
$--\rightarrow$ = negative control

unit of the cyclic AMP protein kinase, calmodulin) and activated proteins. In both cases activated protein kinases turn on or off the target enzymes by phosphorylation, i.e. by a covalent post-translational modification.

The cyclic GMP system is less well known but certainly shares some common features with the other two, such as a specifically activated protein kinase. Other intracellular signal molecules have been discovered such as oligoisoadenylate (2-5 A) which mediates some of the effects of interferon. On the other hand, the intracellular mediators of such well known hormones as insulin, prolactin remain unknown. The strategy to discover such postulated intracellular signal molecules could very well be adapted from the strategy used by Sutherland for cyclic AMP. Finally, the discovery of specific protein kinases coded by transforming genes may provide a link between physiological hormonal control and disordered control in malignant cells.

REGULATION OF THE RESPONSIVENESS OF ADENYLATE CYCLASE TO CATECHOLAMINES

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INTRODUCTION:

Cells possess within the plasma membrane a variety of proteins involved in the reception, transduction and amplification of extracellular signals. Neurotransmitters and many hormones serve as intercellular signal molecules that alter target cell function upon interaction with cell surface, structure-specific receptors. Beta adrenergic receptors (BAR) can be included in this class of plasma membrane proteins that mediate transmembrane processes.

The idea that target cell response is a function of the extracellular concentration of the signal molecule is well accepted and needs no elaboration here. However, the observation that cells, tissues and animals can exhibit refractoriness or tachyphylaxis to the effects of administered neurotransmitters, hormones, or their analogs, indicates that variation in the cellular response to similar doses of these agents is possible. Similarly, states of "super responsiveness" can be induced in tissues of animals after surgical or pharmacological manipulation of neural pathways or hormone-secreting organs. Thus, it seems reasonable to conclude that the capacity of cells to respond to molecular signals is not a static but a dynamic process and that such processes must be regulated.

One aspect of such regulation is observed when certain target cells are exposed to catecholamines. Not only does such a challenge lead to the activation of adenylate cyclase but it also sets in motion a complicated series of events designed to down-regulate responsiveness of the cell to any subsequent challenge with a catecholamine.

The hormone-sensitive adenylate cyclase system is known to be composed of at least three separate moieties: a hormone receptor, a catalytic protein and a guanine nucleotide binding protein, the latter being involved in coupling the effects of hormone binding to enzyme activation. The response of adenylate cyclase to hormones could thus be regulated at any of the steps that are involved in the sequence of interaction of these proteins. For example, there is now good evidence that the number of hormone receptors can be regulated independently of the other components of the adenylate cyclase system. Such changes appear to involve chronic adaptive or developmental changes in cells and have been observed during ontogenesis and as a result of chronic alterations in the exposure of target cells or tissues to receptor agonists. In addition to changes in receptor number, changes in the functional properties of receptors theoretically could be expected to alter their capacity for interaction with the guanine nucleotide binding protein. Other cell types exhibit a tachyphylaxis to the effects of hormones that involves nonspecific changes in adenylate cyclase; under such a condition basal, NaF-stimulated, and guanine nucleotide-stimulated activities, as well as hormone-stimulated adenylate cyclase activity are all reduced. Losses in enzyme responsiveness that are nonspecific in nature suggest that changes occur in components of the adenylate cyclase system other than in hormone specific receptor sites.

Our discussion will focus on the mechanisms of 1st messenger-induced decreases in the responsiveness of adenylate cyclase, these include agonist-specific and non-specific processes.

THE STRUCTURE AND FUNCTION OF ADENYLATE CYCLASE

Since the discussion will focus on the regulation of the response of adenylate cyclase to hormones it will be useful to have in mind some idea of the structure and function of this complex enzyme system. Furthermore, the regulatory phenomena we will discuss involve relationships derived for the most part from studies of the catecholamine-sensitive adenylate cyclase; thus, regulation of the BAR-linked adenylate cyclase will be emphasized. Excellent and more exhaustive reviews have recently appeared (Maguire et al., 1977; Stadel et al., 1980; Ross and Gilman, 1980; also see Neer and Swillens in this volume).

In Figure 1 a model of the catecholamine-sensitive adenylate cyclase is illustrated. This formulation is based on the "steady-state" model of Cassel and Selinger and incorporates the idea put forth by Lefkowitz and Williams that the receptor can exhist in a form that has relatively low affinity for agonists, (R), or a form that has relatively high affinity for agonists, (RN). The basic premise of the steady-state model is that a GTP-liganded coupling

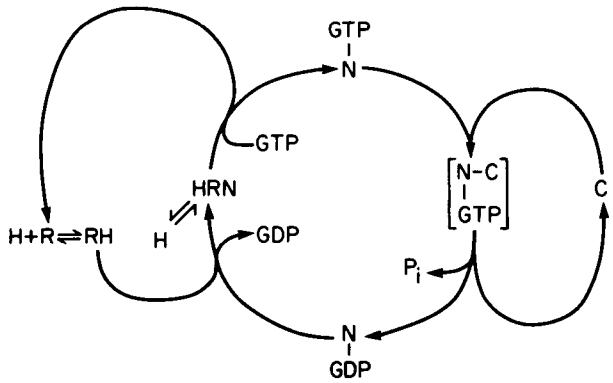

Fig. 1. This model indicates the hypothetical interactions of a catecholamine (H), the β-adrenergic receptor (R), a guanine nucleotide binding protein (N), the catalytic protein (C), and guanine nucleotides (GDP and GTP). The model indicates that RH is able to bind to N, resulting in the release of free GDP and the formation of HRN. The formation of HRN is rate-limiting in the activation process and, in the presence of GTP, HRN is rapidly converted to N-GTP and HR; thus, the role of the hormone-receptor system is to effect the conversion of N-GDP to N-GTP. Once formed, N-GTP interacts with C to form the enzymically active complex C-N-GTP. The lifetime of the active complex is determined by the activity of a GTPase (probably an integral part of N) which hydrolyzes the bound GTP to release P_i, with the subsequent regeneration of C and N-GDP. In the absence of GTP, addition of H leads to the formation of HRN in amounts sufficient to change the apparent K_a of the system for H. Thus, in the absence of GTP, agonists (H) exhibit binding characteristic of interreaction at two sites (R and RN). In the intact cells, or upon addition of GTP to membranes, the amount of HRN would be small because its rate of formation is postulated to be the limiting step in the intact system. Under these conditions, agonists (H) would exhibit binding characteristic of the reaction R + H⇌RH, namely low affinity binding to a single type of site. Modified from Su et al., 1980.

factor (N-GTP) is the actual activator of adenylate cyclase (C); inactivation results from hydrolysis of the bound GTP by a GTPase that is intrinsic to N. Catecholamines stimulate enzyme activity by accelerating the formation of N-GTP either by increasing the rate of dissociation of GDP from N-GDP as shown, or by directly increasing the association of N and GTP by forming HRN. Any perturbation of the system resulting in an increase in the steady-state level of (GTP-N-C) would increase enzyme activity. Thus, activation can be effected by: 1) catecholamines, as indicated above, 2) cholera toxin, which inhibits the GTPase activity of N, and 3) non-hydrolyzable analogs of GTP (Gpp(NH)p, GTPγS) which effectively eliminate the inactivation of the system by the GTPase reaction. As shown in the model, catecholamines act through association with the BAR to accelerate the formation of the activated state of the enzyme; but, the BAR apparently is not directly associated with the activated form of adenylate cyclase. The non-hydrolyzed GTP analogs can effect an accumulation of the active form of the enzyme by a pathway that circumvents involvement of the BAR; however, catecholamines acting through the receptor will increase the rate of formation of (Gpp(NH)p-N-C) albeit, not the extent. The effect of cholera toxin, which apparently involves ADP-ribosylation of N, is to markedly reduce GTPase activity; thus, the kinetics of activation of the enzyme by GTP in the presence of cholera toxin become similar to those of the analog Gpp(NH)p, i.e., pseudo-irreversible.

It is clear from the model that a guanine nucleotide is required for activation of the system by catecholamines. Such absolute dependence on GTP has been shown experimentally under rigorously defined experimental conditions. Purine nucleotides also decrease the apparent affinity of catecholamines for the BAR as measured by direct binding techniques. It was initially considered paradoxical that GTP would on the one hand be required for stimulation of enzyme activity by catecholamines, but on the other hand markedly decrease the apparent binding affinity of these agonists. The shift in the binding affinity caused by GTP is observed only with agonists, not antagonists, which suggests an effect of the activation process on the receptor binding reaction. The model accounts for such observations by proposing that the RH complex, which readily dissociates H, can combine with N to form a complex, HRN. The agonist-selective reaction, $RH + N \rightleftharpoons HRN$, could account for the increased agonist affinity.

However, in the presence of GTP the HRN complex is dissociated to form N-GTP + RH. If the formation of NRH is slow relative to the rate of its dissociation in the presence of GTP, then the BAR would exist primarily in the form (RH) that exhibits lower affinity for H. In the absence of GTP, the addition of catecholamines would shift the equilibria such that a significant amount of the high affinity form (HRN) would be present. The capacity of nucleotides to decrease agonist binding thus reflects association of the BAR and