Review Letter

HORMONE-RECEPTOR–ADENYLATE CYCLASE INTERACTIONS

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1. Introduction

Since publication of the first review letter [1] on hormone receptor interactions in January 1976 progress in the field has been rapid. Recent advances now make it possible to discuss hormone-receptor–adenylate-cyclase interactions and their control in more definitive terms. This has encouraged us to present some further thoughts on the same topic. A comprehensive coverage of the literature was not intended and certainly the selection of topics is strongly biased by the authors interests.

A large number of hormones and neurotransmitters activate adenylate cyclase (EC 4.6.1.1) in the course of eliciting their physiological response. In each case the hormone or the neurotransmitter interacts with a specific receptor at the outer surface of the cell which, in turn, interacts with the catalytic moiety of adenylate cyclase that faces the inside of the cell and causes its activation:

$$\text{ATP} + \text{Mg}^{2+} \xrightarrow[\text{receptor and adenylate cyclase}]{\text{hormone or neurotransmitter}} \text{cAMP} + \text{PP}_i$$

Sutherland and his colleagues have already suggested that the receptor–cyclase system is a regulatory enzyme and have pointed out the analogy between adenylate cyclase and aspartate transcarbamoylase with the receptor as regulatory unit in the case of adenylate cyclase [2]. Recent evidence by Schramm and others indeed demonstrates that receptor and cyclase moiety are separate proteins [3–7]. Moreover, it became apparent since the original studies of Rodbell and his colleagues [8–11] that an additional regulator, the transducer, plays a decisive role in the processing of the hormonal signal. Although binding of the hormone or the neurotransmitter is an obligatory event [12–14] the nucleotide GTP must also be present for hormone-induced activation of adenylate cyclase to occur. The requirement for GTP was not recognized for quite a while, because the substrate ATP used in the cyclase assay is often contaminated with enough GTP to saturate the regulatory site [15]. When ATP free of GTP is used in the cyclase assay, the dependence of cyclase activity on GTP can readily be demonstrated in most cases.

Abbreviations: DOPC, dioleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; Gpp(NH)p, guanosine 5′-(β,γ-imino) triphosphate; GTPγS and GDPβS, guanosine 5′-(3-thio) triphosphate and guanosine 5′-(2-thio) diphosphate, respectively; cAMP, adenosine 3′,5′-cyclic monophosphate

2. The problems

The three problems in the forefront today are therefore the role of GTP, the mode of coupling between receptor and adenylate cyclase and the regulatory control over these processes.

3. The role of GTP

It is now generally accepted that GTP functions as an intracellular effector which interacts with another separate regulatory moiety and activates the enzyme synergistically with the hormone (for references, see [16]). GTP analogues such as Gpp(NH)p, GTPγS

and Gpp(CH_2)p activate adenylate cyclase in a quasi-irreversible fashion and, in the presence of hormone, induce the formation of a stable, high activity state (cf. [16] and [17–30]). Detailed kinetic analysis of the β-adrenergic receptor-dependent adenylate cyclase activation [12–14,31,32] indicated that the β-adrenergic agonist facilitates activation by the guanyl nucleotide. The efficacy of the β-agonist has the following order:

l-isoproterenol = l-epinephrine = l-norepinephrine

> dopamine > phenylephrine >> metanephrine

Antagonists such as l-propanolol have no effect. Aside from a role of GTP in the activation of adenylate cyclase, effects of guanyl nucleotides on the interaction of receptors with agonists have also been described. Thus, GTP and its analogues were found to reduce the affinity of glucagon for its receptor [33]. Similarly, GTP was shown in two cell types to reduce the affinity of β-adrenergic receptors towards β-agonists but not towards β-blockers [34–36]. These effects of guanyl nucleotides were attributed to a receptor-linked nucleotide site which may be different from the activating site [37]. The evidence for the existence of such a second site, however, is as yet circumstantial.

3.1. *The GTP binding protein*

The hypothesis [16,17] that the guanyl nucleotide regulatory site represents a separate protein was recently verified [18,38]. GTP binding proteins could be separated by affinity chromatography from the cyclase catalytic unit by treating Lubrol PX-solubilized pigeon erythrocyte membrane proteins with a GTP–Sepharose matrix. These nucleotide-binding proteins can be dissociated specifically from the GTP-matrix by Gpp(NH)p or GTP. Upon addition to a cyclase preparation deprived of guanyl nucleotide binding proteins, Gpp(NH)p- and NaF-stimulated adenylate cyclase activity is regenerated. The guanyl nucleotide binding proteins isolated from pigeon erythrocyte membranes are capable of activating rabbit myocardial adenylate cyclase preparations depleted of guanyl nucleotide binding proteins, suggesting that this regulatory protein is a universal component of adenylate cyclase systems and might be able to couple with the catalytic unit from other species. The evidence available at present indicates that in pigeon erythrocyte membranes, among several GTP binding proteins, one with mol. wt 42 000 is the guanyl nucleotide regulatory unit which is involved in cyclase activation [18,38]. That this protein is the GTPase responsible for the catecholamine-activated GTPase activity discovered by Cassel and Selinger [39] in turkey erythrocyte membranes was made probable by the action of cholera toxin on adenylate cyclase activity in avian erythrocyte membranes. However, the final proof will require the complete purification of the protein.

4. The action of cholera toxin

A variety of mammalian cells and membranes derived therefrom exhibit increased adenylate cyclase activity subsequent to treatment with cholera toxin and NAD^+ [40,41]. It was suggested [42] and subsequently shown [43] that cholera toxin inhibits the GTPase step, thus increasing the steady state concentration of the activated form of adenylate cyclase in the presence of hormone and GTP (fig.1). A molecular basis for this effect was recently provided when it was shown that cholera toxin-induced activation of adenylate cyclase is correlated with ADP-ribosylation of the 42 000 mol. wt guanyl nucleotide binding protein [44,45] and inhibition of the GTPase step [43]. Consequently GTP becomes as effective as nonhydrolyzable GTP analogs. On reversal of ADP-ribosylation, GTP activation is likewise reversed [44].

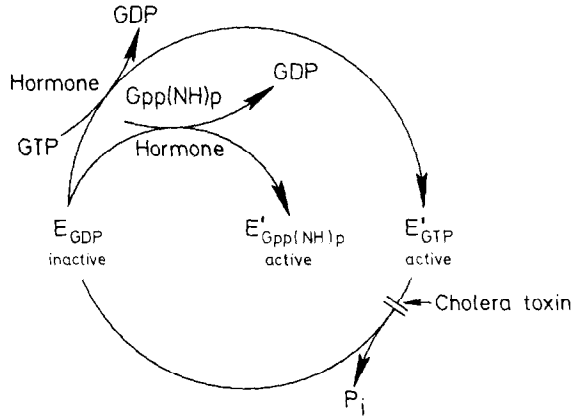

Fig.1. Activation–deactivation of adenylate cyclase: the GTP-cycle according to Cassel and Selinger [43].

5. Mechanism of adenylate cyclase activation

Conversion of adenylate cyclase to its activated state requires simultaneous binding of agonist and guanyl nucleotide to their respective sites. Termination of hormonal activation occurs when GTP is hydrolyzed at the guanyl nucleotide regulatory site to GDP and P_i. In turkey [39] and pigeon erythrocytes (Cassel and Pfeuffer, unpublished) the β-receptor-dependent GTPase activity could be measured directly. Indeed, the hydrolysis step per se does not require the continued presence of the hormone at the receptor [46,47], but enzyme activation occurs only when a new molecule of GTP enters the guanyl nucleotide binding site and the GDP produced in the hydrolysis step is discharged from this site. Thus GTP⇌GDP exchange only occurs when the receptor is occupied by the hormone [48]. This sequence is interrupted when a non-hydrolyzable GTP analogue is bound at the guanyl nucleotide site because the hydrolytic step does not take place [13,14,49] and consequently the enzyme remains in the active state E′. It is important to note therefore that the only function of the hormone is to facilitate GTP⇌GDP exchange [48] and that the GTP form of the binding protein associates with the catalytic moiety whereas the GDP form can not form active holoenzyme [38] (see fig.1).

When hormone and GTP are saturating, the system at steady state is described by the equation:

$$E \underset{k_{off}}{\overset{\text{GTP, hormone, } k_{on}}{\rightleftharpoons}} E' \qquad (1)$$

Thus, the total enzyme concentration in the membrane $[E_T]$ is:

$$[E_T] = [E] + [E'] \qquad (2)$$

and at steady state:

$$k_{on}\,[E] = k_{off}\,[E'] \qquad (3)$$

Inserting (2) into (3):

$$[E'] = [E_T]/[1 + k_{off}/k_{on}] \qquad (4)$$

Thus, the fraction of the total cyclase pool in its active form is determined by the ratio k_{off}/k_{on} [46,47]. For the turkey erythrocyte β-adrenergic-dependent adenylate cyclase, k_{on} and k_{off} have been measured directly: k_{on} was determined under conditions where $k_{off} = 0$ by following the rate of adenylate cyclase activation at saturating concentrations of l-epinephrine and Gpp(NH)p [12,13]. Since Gpp(NH)p cannot be hydrolyzed, all the cyclase molecules are eventually converted to the activated form and $[E'] = [E_T]$. This occurs in the range of $k_{on} = 0.4$–$1.0\ min^{-1}$ at 37°C. k_{off} was also measured directly in the turkey erythrocyte system by two independent means: one method involved the measurement of GTP hydrolysis at the regulatory site [39]; the second method involved the measurement of the rate of decay of the activated state E′ to the inactive state E. In the latter case the enzyme was preincubated with hormone, GTP, and non-radioactive ATP; and decay was initiated by simultaneous addition of [α-^{32}P]ATP and of an excess of β-antagonist [46,50] to block the reconversion (activation) of E to E′. Under these conditions c-[^{32}P]AMP will be produced until E′ is exhausted. The time course of c-[^{32}P]AMP formation therefore is the time course of decay of E′ to E from which k_{off} can be derived [47,50]. When a suitable antagonist is not available, the experiment can be performed with GDPβS [51] which replaces GDP at the regulatory site but because of its tight binding, prevents exchange with GTP. The latter quenching method can be applied to any adenylate cyclase system, including preparations where measurements of specific hormone-dependent GTPase activity over the background of nucleoside triphosphatase activity are not feasible. Using eq. (4) one can compare theoretical and experimentally observed activity ratios. Such a comparison has actually been carried out for the turkey erythrocyte β-receptor system, and the correspondence was satisfactory, [14,46,47,52] affirming that the simple two state system described in eq. (1–4) is adequate.

6. The mode of coupling

Little is known about the organization of the multi-component adenylate cyclase system – which is composed of at least three separate components: the

receptor, the GTP-binding protein and the catalytic unit – within the membrane. In this context the stoichiometry and the mode of coupling between the components are of main interest. In principle, four modes of coupling are plausible: (see [25,53] and for further references [52]). In the precoupled model (I), enzyme and receptor are permanently coupled like regulatory and catalytic subunits in aspartate transcarbamoylase. In this case enzyme activation can be described by the upper scheme of fig.2. The model predicts non-cooperative β-agonist and β-antagonist binding. It also predicts that conversion to the permanently active state, in the presence of hormone and Gpp(NH)p is first order. In the dissociation model (II), enzyme and receptor are only attached to each other in the absence of a β-agonist. Subsequent to hormone binding, receptor and enzyme separate concomitantly with enzyme activation. This is analogous with cAMP-dependent protein kinase activation [54]. This model predicts negatively cooperative β-adrenergic ligand binding and non-first order kinetics of enzyme activation in the presence of β-agonist and Gpp(NH)p. In the floating receptor model (III), hormone, receptor and enzyme are in equilibrium, and the fraction of enzyme combined with the receptor is dependent on agonist concentration. This model, like model II, predicts negatively cooperative hormone binding and complex non-linear kinetics of enzyme activation. The collision coupling model (IV), is described in the bottom scheme of fig.2 and predicts first order kinetics of enzyme activation by hormone and Gpp(NH)p and non-cooperative ligand binding. One of us (A.L.) has recently presented evidence (for technical details and additional references consult [52]) with the turkey erythrocyte β-receptor-dependent cyclase, indicating that coupling between receptor and enzyme can be described by the collision coupling model. The evidence is as follows: binding of β-antagonists and β-agonists is non-cooperative and the kinetics of enzyme activation in the presence of Gpp(NH)p is first order. Although binding as well as kinetic data could be equally well accounted for by either model I or IV, a distinction between these models was possible on the basis of the following argument: model I predicts that in the presence of Gpp(NH)p ($k_{off} = 0$), the rate constant of enzyme activation (k_{on}) is independent of receptor concentration whereas the maximal number of activated catalytic units is proportional to the receptor concentration.

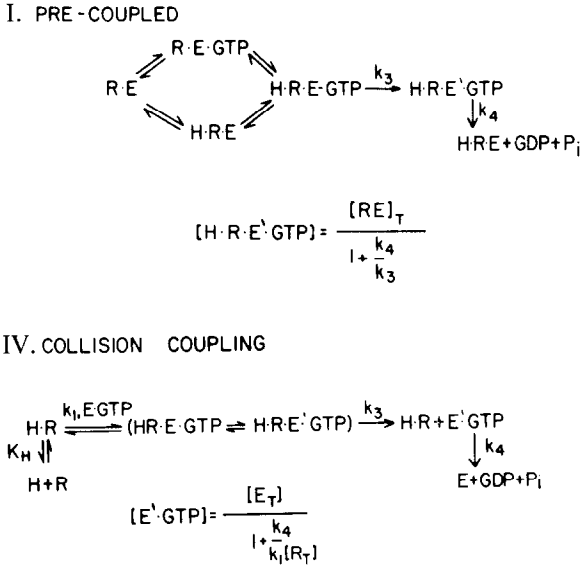

Fig.2. The precoupled model and the collision coupling model for receptor to adenylate cyclase coupling. R, receptor; E, enzyme; E', activated enzyme; $[E_T]$, the total enzyme concentration; RE, the total receptor–enzyme concentration. The formulas for each of the models describe the maximal concentration of active cyclase in the presence of saturating concentrations of hormones and GTP. Further explanations are given in the text.

The 'collision coupling' model (IV) predicts the opposite: in this case the rate constant of enzyme activation is proportional to the receptor concentration whereas the maximal number of catalytic units that can eventually be activated is independent of receptor concentration. This prediction was scrutinized experimentally with an irreversible β-adrenergic blocker. The results were compatible with the 'collision coupling' mechanism in the case of β-adrenergic receptor, whereas in the case of the adenosine receptor the precoupled model described the data more adequately (cf. [52]).

7. The influence of membrane fluidity [55–68]

Two effects of an increase in membrane fluidity have recently been described by us [59,60] and

[61–63]. In membranes of Chang liver cells in culture made more fluid by fusion with DMPC and DOPC vesicles β-receptors disappeared at 37°C (above the phase transition) and the specific activity of isoproterenol-stimulated adenylate cyclase (in the absence of Gpp(NH)p) declined. At 17°C (below the phase transition) no changes were observed in the DMPC-enriched membrane: but as was to be expected, receptors and activity were still decreased in the DOPC-enriched membrane at 17°C which is still above phase transition for this membrane. The decline of activity as a consequence of membrane fluidity was most pronounced with the receptor-dependent isoproterenol-stimulated adenylate cyclase and with (Na^+, K^+)-activated ATPase. Functions of adenylate cyclase independent of the receptor, such as Gpp(NH)p- and NaF-dependent cyclase activities declined much less as a function of membrane fluidity. We therefore concluded [59,60] that lateral diffusion of membrane proteins must be restricted and order maintained in fluid membranes to allow spatially organized enzyme systems which catalyze vectorial reactions to couple in a two dimensional space. Thus, when the coupling efficiency of the remaining receptors which were not dislodged or 'sunk' in the fluidized membrane was measured, it was found to be equal to or greater than that in the normal membrane. Thus, so long as spatial order is maintained, fluidity actually increases the rate of coupling between receptor and adenylate cyclase. The latter observations were therefore in agreement with the observation [61–63] that insertion of *cis*-vaccenic acid into turkey erythrocyte membranes [66] caused a progressive increase in membrane fluidity and a concomitant increase in the rate constant (k_{on}) of cyclase activation by β-agonist (but not by adenosine). Recently Schlessinger et al. could directly observe with a sensitive video–microscopic system aggregation and internalization of insulin– and epidermal growth factor–receptor complexes in 3T3 fibroblasts [68a]. It should therefore be feasible also to study the fate of β-receptors by a similar experimental approach. Such information might give some insight into the molecular order of multicomponent systems in membranes which undergo association–dissociation reactions in the course of activation–deactivation [68,38]. Nevertheless, both these independent observations, with the Chang liver cells in culture and with turkey erythrocytes, are consistent with a collision coupling mode for β-receptor–adenylate cyclase coupling. It should be noted that the commited step which leads to activation in the collision coupling model is the encounter of HR and E, since $k_5 >> k_4$:

$$H + R \underset{K_H}{\rightleftharpoons} HR + E \cdot GTP \overset{k_3}{\underset{k_4}{\rightleftharpoons}} [HRE\ GTP \rightleftharpoons HRE'\ GTP]$$

$$\xrightarrow{k_5} HR + E' \cdot GTP \xrightarrow{k_6} E + GDP + P_i$$

Under physiological conditions in the presence of saturating concentrations of GTP, the activated form E′ will decay to its inactive form concomitantly with the hydrolysis of GTP at the regulatory site. In the presence of Gpp(NH)p, $k_6 << k_5$ and k_6 becomes rate limiting preventing the decay of E′→E.

8. Future directions

There are consequences of the collision coupling mode for hormonal activation of adenylate cyclase worth discussing. Firstly, only a fraction of the total adenylate cyclase pool may be in the active state when the physiological activator GTP is present. Secondly, the apparent first order rate constant of activation (but not the maximal activity attainable in the presence of Gpp(NH)p) is linearly dependent on total receptor concentration. It is thus tempting to speculate that physiological mechanisms come into play either to control the collision rate for example by modulating membrane fluidity and/or to control the GTPase reaction. In the former case, mechanisms like those catalyzed by methyltransferases which convert phosphatidylethanolamines into phosphatidylcholines [69] and control of membrane fluidity by cytoskeletal contractile elements are attractive candidates [70–72]. In the latter case a physiological covalent amplification cascade deserves attention since covalent ADP-ribosylation by the unphysiological ADP-ribosyltransferase of cholera toxin has actually been demonstrated to inhibit this step [43,44]. The ADP-ribosyltransferase in turkey erythrocyte cytosol recently discovered by Moss and Vaughan might play a physiological role [73].

In any event, further progress will require continuing efforts towards purification of the adenylate

cyclase system. The 5000-fold purification of canine myocardial adenylate cyclase by hydrophobic and affinity chromatography achieved by Homcy et al. [74] and the identification of the β-receptor subunit [75] are a hopeful beginning.

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