Review Letter

HORMONE–RECEPTOR INTERACTIONS

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Hormone–receptor complexes are rather long lived allowing several discrete cycles of activation and deactivation of adenylate cyclase. Regulatory steps are therefore postulated facilitating dissociation of the hormone–receptor complex and modulating coupling between receptor and adenylate cyclase.

1. Hormone receptors and adenylate cyclase

At present the translation of hormone–receptor interactions into biological action is not understood. An important, although not universal action which is elicited by the interaction of the hormone with its receptor is the activation of adenylate cyclase. Some hormones, among them epinephrine, glucagon and ACTH, have been shown to act primarily through the product of the adenylate cyclase reaction, the second messenger cyclic 3′, 5′-AMP, but some biological effects may not be mediated by cyclic 3′, 5′-AMP [1]. In any event, the interaction of hormones with receptors activates in some cases adenylate cyclase but initiates in all cases the biological response. It is therefore necessary to understand the translation of hormone–receptor interactions in order to comprehend the function of hormones.

2. The lifetime of hormone–receptor complexes

We have recently summarized the evidence from the literature on the lifetime of hormone–receptor complexes, cf [2].

The 'on' constants (k_1) of the insulin–receptor complex range between 5×10^7 and 1.5×10^5 mol^{-1} sec^{-1} and the 'off' constants (k_{-1}) range between 1×10^{-3} and 3×10^{-4} sec^{-1}. $K_{ass} = k_1/k_{-1}$ of the insulin–receptor complex and ranges therefore between 5×10^{10} and 5×10^8 M^{-1}, cf [3]. Thus, the insulin–receptor complex is a tight complex with an accordingly long lifetime. Assuming that dissociation of the hormone–receptor complex [H] · [R] → [H] + [R] is first order, $\tau/2 = \ln 2/k_{-1}$ may be calculated; it ranged from 15.6 to 42.8 min at 24°C. Insulin actions on adenylate cyclase are doubtful; but similar data have recently been published on neurohypophyseal hormones and membranes from bovine renal medulla. In contrast to insulin important biological actions of these hormones are transmitted via adenylate cyclase activation and 3′, 5′-cAMP. For vasopressin $k_1 = 3.2 \times 10^6$ mol^{-1} sec^{-1} and $k_{-1} = 7.0 \times 10^{-3}$ sec^{-1} and $K_{ass} = 4.5 \times 10^8$ M^{-1} [4]. (In a more recent study a K_{ass} value of 5×10^9 M^{-1} was reported [5]). Thus, the lifetime of the vasopressin–receptor complex is likewise in the minute range as appears also to be the case for the glucagon–receptor complex [6].

These data raise doubts whether hormone–receptor interactions can control the rate of activation and deactivation, because several discrete cycles of activation and deactivation of adenylate cyclase could occur during the relative long occupancy of the receptor by the hormone. Thus, one should consider means for facilitating and enhancing the dissociation of the complex. One would like to know therefore how the hormone–receptor interaction can be modulated and moreover, what factors might control the coupling reaction between the liganded hormone–receptor complex and adenylate cyclase in the membrane. Evidence for independent regulation of both steps bears also on the structural and functional relationships of hormone receptor and adenylate cyclase. First, the control of the hormone receptor interaction is discussed.

3. Negative co-operativity

There is evidence that some hormone binding sites, i.e.: for insulin [7] and catecholamines [8] may interact with each other, although other plausible explanations of apparent nonlinearity in Scatchard plots need seriously to be considered [9]. The reverse has also been described, namely that non-interacting mutually independent hormone binding sites activate adenylate cyclase in a co-operative manner [5]. Both situations imply nonlinear coupling. But if there is more than one hormone binding site involved in adenylate cyclase activation the possibility should be entertained that hormone receptors form clusters. These clusters could be inherent features of cell surface structures or they might be formed temporarily on binding of hormones to mobile receptors as has recently been suggested for cholera toxin bound to ganglioside G_{M1} [10]. Recent experiments of Dr Pierre De Meyts and colleagues, cf [11], with lymphocytes in culture and liver plasma membranes suggest that the binding domains of insulin and of the receptor responsible for the biological effects of the hormone and those involved in negative co-operative interactions may be different. In a study with several insulin analogues the region of the insulin molecule responsible for the co-operative interaction was mapped. It was concluded that this site is covered by dimerisation of insulin, accounting for the fact that at high concentrations when insulin dimerizes negative co-operativity disappears. Insulin dimers are biologically active hence they still must bind to receptors but the insulin–dimer receptor complexes no longer interact co-operatively*. Concanavalin A does not bind to the activating site on insulin receptors but prevents negative co-operative interactions. This lectin is known to affect the distribution of cell surface components suggesting that the redistribution of hormone receptors could play a role in negative co-operativity.

Growth hormone and the neurohypophyseal hormones, vasopressin and oxytocin act differently:

Roy, Barth and Jard [5] have recently tested a number of vasopressin analogues on pig kidney plasma membranes for their ability to activate adenylate cyclase. Adenylate cyclase activation and hormone binding were measured on the same preparation under identical conditions. While activation with [Lys^8] vasopressin exhibited marked negative co-operativity and a Hill coefficient of n = 0.35, binding did not, or was positively co-operative with a Hill coefficient of 1.42 (See also: [4]). Consequently, the apparent K_M value for adenylate cyclase activation in pig kidney membranes with [Lys^8] vasopressin was 3×10^{-10} M whereas the apparent K_M value for binding was about 100 times greater, i.e.: 2×10^{-8} M. Obviously, with hormones such as vasopressin or analogues therefrom which activate in a negative co-operative manner higher concentrations are required for saturation of binding sites than for adenylate cyclase activation. This may be interpreted to mean occurrence of spare receptors. But analogues exhibiting different degrees of negative co-operativity activated adenylate cyclase to the same maximal extent and all active analogues tested completely displaced [Lys^8] vasopressin. Thus, this possibility was considered unlikely and it was concluded that this hormone binds reversibly to a homogenous population of binding sites all of which are involved in adenylate cyclase activation. No binding sites linked to other physiological functions aside from adenylate cyclase activation were detected. Oxytocin binds at least 100 times less tightly than vasopressin [5]. Maximal activation of adenylate cyclase by oxytocin was about 80% of that with [Lys^8] vasopressin. The Hill coefficients for adenylate cyclase activation and binding of oxytocin approached 1.0 and the apparent K_M values for activation and binding were nearly identical.

This comparative study suggests that low affinity peptide hormones activate in a linear fashion whereas those with high affinity for receptor sites activate in a nonlinear co-operative manner. The latter need only to occupy a part of the total available receptor sites in order to elicit maximal adenylate cyclase activation.

Negative co-operative interactions [13,14] among hormone binding sites on the cell surface would be biologically important because they facilitate dissociation of tight hormone–receptor complexes with increasing hormone concentrations. Furthermore, negative co-operativity buffers adenylate cyclase against acute changes in circulating hormone concentrations. In these instances, the steady state concentration rather than rapid fluctuations in the amount of circulating hormone would limit the biological action. The steady state concentration determines the extent of loading of receptors ready for biological action. But so long as hormone–receptor

* To the contary, Cuatrecasas and Hollenberg [12] make self-aggregation responsible for non-linear Scatchard plots of binding data obtained from competition–displacement experiments.

interactions are not better defined molecularly, negative co-operativity can not be proved. Therefore at present negative co-operative interactions among hormone binding sites should only be considered as a plausible, albeit attractive, possibility.

4. Control of coupling

In cases where hormones – i.e.: growth hormone and neurohypophyseal hormones – bind to receptors in a non-cooperative (or even in a positive cooperative) manner but activate in a negative co-operative manner, one should look for regulatory control at a step or steps beyond the hormone–receptor interaction. Although the binding of hormone to receptor is essential for the activation of adenylate cyclase or for other biological actions, factors which control the coupling between liganded hormone receptor and adenylate cyclase could detemine how many of the hormone–receptor complexes are translated into biological function.

It has been suggested recently that the hormone–receptor complex is mobile and moves on or in the fluid membrane. Coupling would thus be a stochastic process depending on the probability of collision between the mobile hormone–receptor complex and a likewise mobile adenylate cyclase, cf [3]. The probability of encounter would be influenced by changes in the liquid–crystalline state of the membrane. Unspecific means unrelated to specific hormone effects can alter membrane fluidity and could conceivably affect the coupling reaction: temperature, pH, cations, changes in membrane lipids and cholesterol etc. Although such general alterations may occur they would not seem especially suited for specific rapid rate control of hormonally activated adenylate cyclase in homoiothermic membranes. Moreover, lipid halos surrounding the coupling proteins might shield them from fluctuations of membrane fluidity. Protein and lipid could form a structural and functional unit. Among specific factors guanylnucleotides have recently received much attention as possible modulators of the coupling reaction. Guanylnucleotides appear to play a rather universal role in amplification of hormonal activation of adenylate cyclases. They could act at a step removed from the hormone–receptor interaction [15]. This has recently been discussed in detail, cf [2,15]. Another effector is Ca^{2+} which may play an even more universal role as modulator. In some systems, Ca^{2+} appears to act as another second messenger which is independent of or interdependent with cAMP [16,17]. In other systems, for example in brain, Ca^{2+} bound to a specific protein may control adenylate cyclase activity itself [18]. Additional effectors and feed back modulators of adenylate cyclase activity can be anticipated [19–21].

5. Translation of hormone–receptor interactions

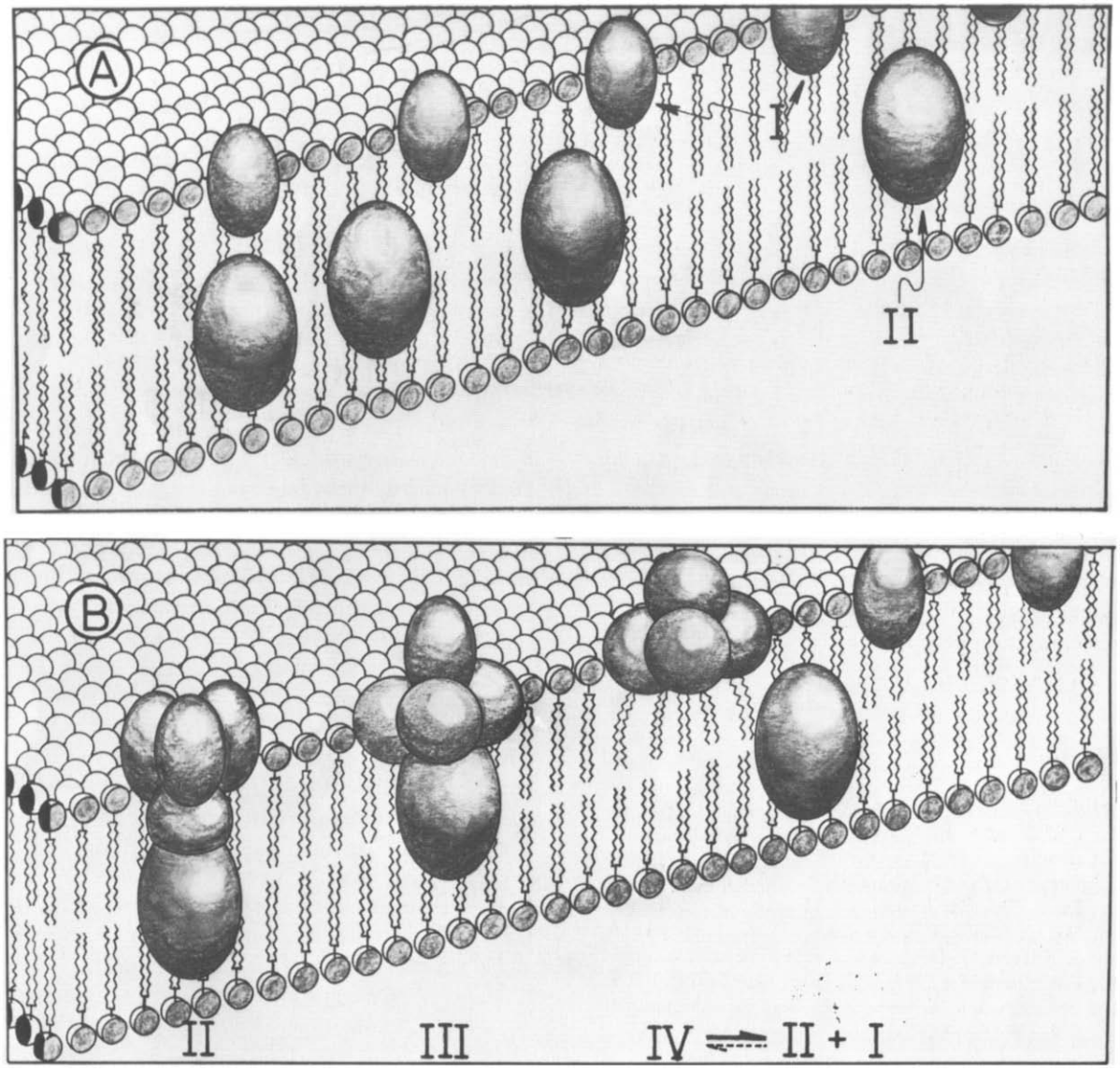
Let us now consider how hormone receptor interactions are translated into biological function:

Some (if not all hormones) have pleiotropic biological actions: binding to one type of specific receptor in the membrane may elicit several seemingly unrelated effects. In some but not all cases these effects might be mediated by $3',5'$-cAMP-dependent protein kinases with broad substrate specificities [22], although the relationships of protein phosphorylation to biological effects of hormones are still a matter of speculation [2].

Another possible explanation for different biological actions arising from one and the same hormone–receptor interaction would be functional links by means of multiple coupling reactions of the same liganded hormone receptor or the assumption that membrane binding sites which have the same specificity and affinity for a hormone are structurally linked to different proteins and enzymes. In both cases coupling would require a remarkable lack of specificity of the interacting proteins. This would make actin-like proteins, like the microfilaments in membranes attractive candidates for multiple coupling reactions. Actins are remarkably unselective in choosing their partner. They combine with themselves but also with troponins, heavy meromyosins, tropomyosin, DNAase I, fibrin, and perhaps with glycolytic enzymes.

Next let us consider cells which have receptors for more than one hormone: When hormone receptor and adenylate cyclase are coupled nonlinearly activation of adenylate cyclase with several hormones should not be additive and only limited by the number of available adenylate cyclase molecules in the membrane pool. This actually seems to be the case, cf [2]. Since additive activation would be anticipated when each hormone couples stoichiometrically with its own adenylate cyclase, nonadditive activation and nonlinear coupling also support the idea that receptor and adenylate cyclase are separate entities in association–dissociation equilibria which interact functionally.

Therefore, one of the questions which need to be answered is whether hormone–receptor interactions can be uncoupled from adenylate cyclase and perhaps other membrane systems. There are many ways to uncouple



Scheme 1. Hormone–receptor interactions cause formation of clusters of liganded hormone receptors. Cluster formation and coupling with adenylate cyclase is modulated by negative co-operativity.

A: I. Unliganded receptor.

II. Uncoupled, hormonally non-stimulated adenylate cyclase.

B: Liganded receptor.

II. and III. Partially liganded hormone–receptor adenylate cyclase complexes. IV. The fully liganded hormone–receptor adenylate cyclase complex loses affinity because of negatively co-operative interactions and partially dissociates:
IV ⇌ II + I

hormonal activation of adenylate cyclase [23] but there is no experiment of which I am aware, which would allow one to decide whether uncoupling involves dissociation of an intact hormone–receptor adenylate cyclase complex or whether it is a consequence of desensitization of adenylate cyclase against the hormone, a phenomenon quite common with allosterically regulated enzymes. The use of somatic cell hybrids deprived of one or the other component should help to answer that question [24,25].

The questions which I have raised focus on the translation of hormone–receptor interactions into biological function and specifically adenylate cyclase activation. Hypothetical control steps are summarized schematically. Perhaps, some of the suggestions I have made will stimulate new experiments on signal transmission from hormone receptors which is the key to the function of hormones.

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