Programmable messengers: a new theory of hormone action

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Many hormone receptors are linked to GTP-regulatory proteins in membranes. When these proteins are activated by hormones and GTP, the α-subunits are released from the membrane as soluble proteins. It is proposed that these α-subunits are modified by kinases, proteases and other protein-modifying enzymes to give new forms with differing functions. This provides a way of explaining the multiple actions of a hormone on its target cell, and the released α-subunits of GTP-regulatory proteins can be called 'programmable messengers'.

Two ideas have dominated the field of signal transduction over the past 25 years. One is that hormone/neurotransmitter receptors interact with various effector enzymes in the plasma membrane to generate signals in the form of small molecules. The classical example is the receptor-controlled adenylate cyclase system in eukaryotic cells. The other is that receptors exist either in membranes or in the cytosol as 'mobile' elements which, when combined with the activating hormone, induce the receptor to collide with or move to the site(s) of the effector systems. Examples of theories that have evolved from the mobile-receptor theory are the 'collision-coupling'[1] and 'two-step'[2] theories proposed for the coupling of β-adrenergic receptors to the adenylate cyclase system. Another example is the estrogen receptor; it has been thought that the receptor first reacts with the steroid in a cytosolic compartment, and that the activated receptor then enters the nucleus where it regulates gene expression.

There is ample evidence that cyclic AMP and other small molecules (cyclic GMP and inositol trisphosphates are recent examples) mediate some of the effects of hormones. The question is whether the pleiotypical responses induced by a hormone are due solely to any of these molecules. If not, what type of molecule might be more closely linked to receptors that could serve as primary messengers of hormone action? As for the concept of receptor mobility, there is evidence that membrane receptors can be induced by agonists to move about in the plane of the membrane. However, there is no compelling evidence that mobility is necessary or causal for signal transduction to take place. Indeed, there is a report that increasing the fluid environment to enhance receptor mobility in membranes is detrimental to hormone action[3]. For the estrogen receptor, recent studies indicate that most of the receptors are bound to the nuclear matrix prior to their occupation by hormone; receptor release into the cytosolic compartment is an artifact of the methods used for isolating the nucleus[4].

This article proposes an alternative view of the function of membrane receptors and develops a logical framework for a theory that the primary messengers of hormones acting on membrane receptors are proteins that bind and degrade GTP. These are the so-called GTP-regulatory proteins (G) that are linked to numerous receptor types in eukaryotic cells. The fundamental aspects were presented five years ago in a theory called 'Disaggregation Theory of Hormone Action'[5]. This theory is now extended and modified in the light of information acquired recently.

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The disaggregation theory

Briefly, this theory suggests that various classes of receptors are complexed with a family of oligomeric GTP-regulatory proteins. When the receptors are occupied by agonists and the G units by GTP, the oligomers dissociate into monomers. In the process, the receptors are transformed from a high affinity state when they can bind physiological concentrations of hormones, into a low affinity state in which they are no longer active. At the same time, the G units are transformed to a 'monomeric' structure that reacts specifically with an effector unit (E) such as adenylate cyclase. The theory is thermodynamically sound[6]; it explains the apparent paradox of receptors undergoing transitions from high to low affinity states during concerted activation of G by hormone and GTP; it explains the findings of target analysis that the ground-state structure of receptors coupled to G exhibits a much higher molecular weight than the activated adenylate cyclase. This theory predicts that the putative monomeric form of G is the primary messenger of hormone action, whereas the product of the effector unit(s) is a secondary signal.

G units are oligomeric proteins

In recent years, G units have been purified and structurally analysed[7]. It is now clear that G units coupled to rhodopsin (termed transducin) and those coupled to receptors (R) that stimulate or inhibit adenylate cyclase (termed G_s and G_i, respectively), and a newly discovered G unit of unknown action (termed G_o) are composed of three distinct protein subunits, only one of which, the α-unit, binds GTP. The type of α-subunit coupled depends on the type of G unit (and associated R) to which it is attached. The other two sub-

	HE* + cyc⁻		HE + cyc⁻	HE	cyc⁻
Adenylate cyclase (pmol/min)	92		0.9	0.1	0.8
Cholera toxin + ^{32}p NAD	+	–	+	+	+
Pertussis toxin + ^{32}p NAD	+	+	–	–	–

Fig. 1. Effects of pretreatment of human erythrocyte ghosts (HE) with pertussis toxin + NAD (HE) on levels of adenylate cyclase activity and levels of α_s subunit transferred to S49 lymphoma cyc– membranes. HE and cyc– membranes were co-incubated for 15 min at 30°C in presence of 0.1 mM Gpp(NH)p + 10 mM $MgCl_2$. The mixtures were layered over 33% sucrose and centrifuged for 20 min at 30000 × g. The upper layer containing only cyc– membranes was assayed for adenylate cyclase activity (with 10 μM Gpp(NH)p, 5 mM $MgCl_2$, 50 μM ATP). Cyc– membranes were also treated with either cholera toxin or pertussis toxin, or both in presence of [^{32}P] NAD. Membranes were extracted and extracts electrophoresed (PAGE) for separation of α_s (43 kDa) and α_i (39 kDa) subunits, followed by autoradiography.*

units, designated β and γ, are highly conserved proteins – they are found in many cell types and species and have similar if not identical structures irrespective of the type of attached α-subunit.

Coupling to receptors

In reconstitution studies with purified components, G units interact with receptors when incorporated into lipid vesicles. The complexes formed exhibit the properties of R–G complexes in native membranes, i.e. hormones induce binding and degradation of GTP; R can take different affinity states, the higher affinity presumably linked to G; and GTP decreases the affinity of R for agonists[8,9]. Kinetically, the process of activation of G by agonists does not require hormone-induced associations between R and G, suggesting that the pre-formed complexes are the active species. Thus, there is no need to invoke the theories suggesting that hormones act by promoting such associations.

Reconstitution studies with rhodopsin and β-adrenergic receptors indicate that all three subunits of G are required for coupling between receptors and G. It follows that factors that disrupt the G unit must functionally uncouple R from this unit.

Disaggregation of G oligomers

In their purified, detergent-soluble form, G units dissociate when incubated with non-hydrolysable analogs of GTP (e.g. Gpp(NH)p or GTP-γ-S) or with aluminum fluoride in the presence of high concentrations of Mg^{2+} (Ref. 10). GTP is probably ineffective because GTP is hydrolysed to GDP as soon as the α unit dissociates, and the subunits re-aggregate to form the holoprotein. This cyclical behaviour of the trimer may explain why GTP is relatively ineffective in the receptor-coupled systems within native membranes in the absence of hormones.

The observation most relevant to the 'disaggregation' theory is that G units are oligomers which, in the absence of activating ligands, cannot dissociate to release the 'active' GTP binding α-subunit. In this sense, the postulated monomer of G is equivalent to the activated α-subunit(s). Theoretically, activation of the R–G complex by concerted actions of hormone and GTP should lead to two interrelated phenomena: release of activated free α-subunits and conversion of receptors to a lower affinity, inactive form of R. Until α re-associates with the β/γ subunits, R is de-sensitized, even if it is still linked to the β/γ subunits.

α-Subunits are released from membranes

Proof that α-subunits are released from R–G complexes in membranes by actions of hormones and GTP has been lacking. A possible means of testing release from native membranes arose from an apparently peculiar finding: co-incubation of membranes containing G_s units (rat liver, RL, and human erythrocyte ghosts, HE), with membranes lacking this unit (isolated from a variant termed cyc– of S49 mouse lymphoma cells) rendered the cyc– membrane able to be activated by Gpp(NH)p or fluoride[11]. For this activation to occur, the cyc– membranes must be co-incubated with HE membranes which lack R units, or with RL membranes in the presence of glucagon plus GTP, or with donor membranes pretreated with cholera toxin and NAD (a procedure that ADP-ribosylates the α-subunit and which renders the G_s unit susceptible to activation by GTP).

Recently, we succeeded in separating donor and recipient cyc– membranes after co-incubation under various activating conditions[12]. Separation was achieved because the cyc– membranes have a lower density than either HE or RL membranes; layering the mixture of membranes over a sucrose gradient followed by centrifugation resulted in a layer of cyc– membranes free of donor membranes, as indicated by assays of various enzymes present in donor but not in cyc– membranes. When isolated after co-incubation with donor membranes under appropriate activating conditions, cyc– membranes acquired an active α_s subunit (α of G_s) donated by HE or RL membranes. This was indicated by (1) the levels of Gpp(NH)p-stimulatable adenylate cyclase activity induced in cyc– membranes and (2) by the quantity of α_s transferred to cyc–. The latter was monitored by labelling α_s with [^{32}P] ADP-ribose catalysed by cholera toxin. A typical example of the relationship between transfer of α_s and the degree of activation of cyclase is illustrated in Fig. 1 using HE membranes co-incubated with cyc–.

This experiment also revealed, indirectly, that when G_i in HE membranes is activated by Gpp(NH)p and Mg^{2+} there is simultaneous activation of G_s. Activation of cyclase and transfer of α_s to cyc– membranes in HE membranes was slight unless the donor membranes were pre-treated with pertussin toxin plus NAD. This toxin ADP-ribosylates α_i and renders G_i inactive[13]. As shown in Fig. 1, toxin-treatment of HE causes cyc– membranes to acquire high levels of Gpp(NH)p-stimulatable cyclase

actitivy with concomitant transfer of α-$_s$ (labelled with cholera toxin and ^{32}-P NAD on re-isolated cyc–).

We interpret these findings as evidence that α-$_i$ released from G_i in the donor membranes influences the ability of α-$_s$ to interact with cyc– adenylate cyclase. We are investigating whether this is due to competition between released α-$_s$ and α-$_i$ for sites on adenylate cyclase or to some other process, such as the 'scavenging' of released α-$_s$ by exposed β/γ subunits of G_i (Ref. 10). Irrespective of the mechanism, the thrust of these findings is that simultaneous activation of G_i and G_s, with consequent release of their respective α-subunits from the membrane, can dramatically affect the amount of α-$_s$ transferred to cyc–. Similar results are seen with liver membranes using combinations of hormones and GTP to induce activation.

Obviously, results obtained with a test system of two isolated membranes do not necessarily simulate what happens in an intact cell. Nonetheless, it is reasonable to speculate that release of α-subunits is the primary step leading to the pleiotropic effects of hormones on their target cells. If it is true that these proteins are primary messengers of hormone action, the present concepts of hormone action will have to be altered radically.

Programmable messengers

Perhaps the most significant difference between proteins and small molecules such as cyclic AMP is that a protein messenger is pluripotent in its capacity to react as a regulatory signal. Proteins can be phosphorylated, methylated, sulfated, oxidized, appended to other proteins via disulfide groups, and degraded to smaller forms by proteases, to name a few well known covalent modifications. Such modifications yield different structures with different functions. If α-subunits are modified after their release into the cytosolic compartments of the cell, and if some of the modifications lead to a different regulatory structure, then the α-subunit, the initial primary messenger, can be considered programmable. This concept of 'programmable messengers' is illustrated in Fig. 2.

In this scheme, each type of α-subunit released from the plasma membrane as a consequence of actions by hormone and GTP becomes exposed to different modifiers (M) that alter the structure and function of that unit. Each new form of α reacts selectively with an effector (E) which emits a signal (S) that can bring forth one or more responses. Possible examples of M include protein kinase C, insulin-receptor tyrosine kinase and calcium-activated protease. Examples of E are adenylate cyclase, guanylate cyclase, calcium transporters, phospholipases and glucose transporters. The central point of this thesis is that a single primary signal can give rise to an array of new signals which, in wave-like fashion, can propagate vast changes in the structure and metabolism of target cells. Specificity of response will depend on the types of receptor and G (or α-) units, and on the modifiers and effector of the cell phenotype. Given that there are various classes of receptors linked to G units and many potential signal-generating effector systems, a variety of cell responses can be envisaged.

The idea of programmable α-subunits as messengers provides an explanation for the frequently cited lack of correlation between hormone-stimulated AMP levels, for example, and other responses given by a hormone. Levels of activated cAMP-dependent protein kinase are also not correlated in all responses; a recent example is the discordance in the actions of β-adrenergic agonists on lipolysis and glucose transport in rat adipocytes[14]. There are examples of cer-

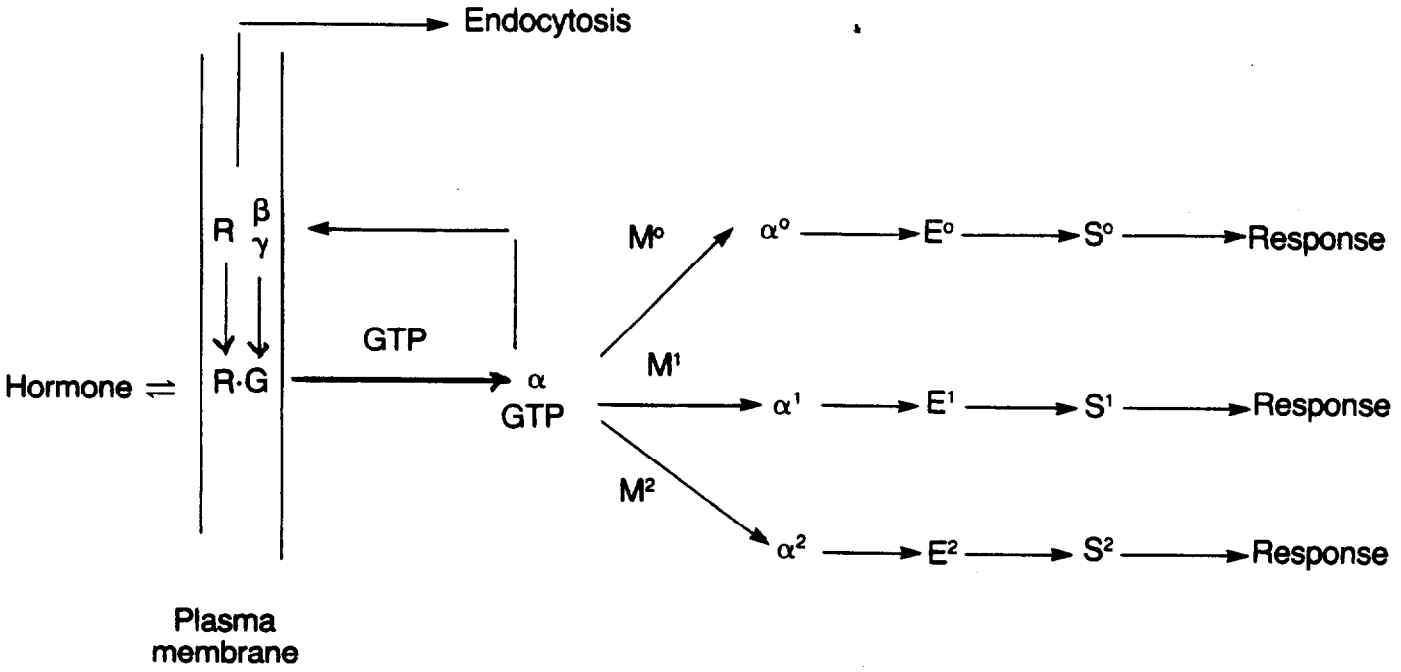

Fig. 2. Theory of 'Programmable Messengers'. Hormones interact with receptor/GTP-regulatory complexes (R.G) causing, in presence of GTP, release of the α subunit of G from the interior face of the plasma membrane. R and β/γ subunits of G remain in the membrane and can either re-bind α (-GTP) to re-form R.G or they are taken into cell by endocytosis. The released α subunit is exposed to modifying enzymes (M) that transform α into new structures having affinities for different effector (E) units which, activated, yield signals (S) the combination of which give the pleiotypical responses of the target cell.

tain hormones inducing simultaneous rises in adenylate cyclase and cAMP-phosphodiesterases by apparently independent processes. Hormones that induce activation of G_i, while inhibiting the production of cAMP induced by a hormone operating through G_s, exert effects which are clearly unrelated to regulation of cAMP production[15].

Receptor desensitization

The programmable messenger theory can explain why receptor desensitization in intact cells can be reversed rapidly with low pulses of hormone and short times of exposure, but not with high concentrations and longer exposure to hormone. In the theory, only a fraction of the α-subunit would be released in a short pulse-type experiment with submaximal concentrations of hormone; during this brief interval, there may not be sufficient modification of α by modifiers to alter the equilibrium between bound and free α-subunit (Fig. 2) when the hormone is withdrawn. By the same reasoning, with higher concentrations of hormones and longer exposure times, more α-subunit is discharged from its union with β/γ subunits and there is greater opportunity for modifiers to prevent α from re-associating with these β/γ. If this is so, reconstitution of functional receptors coupled to G units may require internalization of receptors (with or without attached β/γ), resynthesis of new α, and recycling of the units back to the plasma membrane.

Synergy

Perhaps the most interesting possible consequence of the programmable messenger theory is an explanation for the long-known synergism with which two hormones, operating through completely different mechanisms, exert effects on cells. A good example is the synergistic effect of insulin and adenosine on the metabolism of rat adipocytes[16]. Neither insulin nor adenosine alone have much effect at physiological concentrations. Combined at such concentrations, the hormones exert large effects on such metabolic processes as lipolysis and glucose transport. Since insulin activates a tyrosine kinase associated with the receptor[17], it is possible that the activated kinase phosphorylates a liberated α-$_i$ subunit (adenosine operates through a receptor linked to G_i in these cells) converting it from a weak or inactive regulatory signal into one that is very active. There are many examples of synergism between two hormones or neurotransmitters operating on the same cell through different primary mechanisms. The point to be stressed is that the search for the usual small molecule messengers, such as cAMP, as the primary agents of synergism has not yet been successful. Hopefully, the ideas put forth here will stimulate investigations along different, more productive lines of research.

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