THE ROLE OF GTP IN THE ACTIVATION OF ADENYLATE CYCLASE

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SUMMARY

An attempt is made to integrate the knowledge on the role of hormones and guanyl nucleotides in regulating adenylate cyclase into a single molecular model. It is suggested that the hormone catalyzes the activation of the enzyme adenylate cyclase by facilitating the conversion of the enzyme from its inactive state to its active form. The hormone is also responsible for the termination of the signal namely the deactivation of the enzyme by inducing the hydrolysis of GTP at its regulatory site. The relative rates of these two processes determine the steady state concentration of the active form of the enzyme. The model also explains the difference in behaviour between GTP and its non-hydrolyzable analogs GppNHp and GTPγS.

GTP was found to stimulate the activation of a variety of hormone-dependent adenylate cyclases (1-4). It is generally agreed that GTP functions as an intracellular allosteric activator which interacts with a specific regulatory site on the receptor-cyclase system and activates the enzyme with the hormone in a synergistic manner. It was also found to be generally true that the GTP analogs GppNHp (3 and references therein) and GTPγS activate the hormone-dependent adenylate cyclase in a quasi-irreversible fashion and in the presence of hormone induce the formation of a highly active and an extremely stable adenylate cyclase. More recently it was discovered that turkey erythrocytes possess a specific β-receptor dependent GTPase incapable of hydrolyzing GppNHp and which is blocked irreversibly by GTPγS (5). Avian erythrocytes which possess β-receptor dependent adenylate cyclase seem to offer a useful general model system to study the interrelationships between the hormone-receptor interaction and the guanyl nucleotide regulatory sites.

Abbreviations: GTP, guanosine triphosphate; GppNHp, guanylyl imidodiphosphate; GTPγS, guanosine-5'-O(3-thiotriphosphate).

This statement is justified on the basis of the following findings: (a) The adenylate cyclase activity in avian erythrocyte membranes possesses negligible basal activity and can be activated by β-agonists or fluoride ions; (b) the β-receptors on these cells are well characterized using direct binding studies (6-10); (c) direct evidence for the existence of a specific GTP regulatory subunit has been provided (11-12); (d) a detailed kinetic analysis on the interrelationship between hormone and GppNHp has been performed (13-14) and (e) the existence of a specific β-receptor dependent GTPase has been demonstrated (5). Although some of the features outlined above are recognized in a number of hormone-dependent adenylate cyclases only in turkey and pigeon erythrocytes are all the properties outlined above recognized. Therefore an attempt will be made to integrate the known facts within the frame of a single molecular model.

RESULTS AND DISCUSSION

Basic Postulates of the Model

(1) Binding of the hormone to the receptor is independent of the presence of GTP or GTP analogs.

This postulate is justified on the basis of the observation that ^{3}H-propanolol binding (9) and ^{125}I-hydroxybenzylpindolol binding (15) as well as agonist binding to the β-receptor are identical whether the system has been previously activated by GppNHp agonist or not.

(2) The binding of GppNHp is hormone independent. This postulate is supported by the finding that the adenylate cyclase can be activated to its super-active state when the turkey red cell membranes are first exposed to GppNHp, then thoroughly washed and only subsequently exposed to the β-agonist (14). Only in the last step the enzyme becomes activated.

(3) The β-receptor which is coupled to adenylate cyclase is also coupled to a specific GTPase. This GTPase is incapable of hydrolyzing GppNHp or GTPγS. Evidence for these statements has been recently presented (5).

The Model

The activation of adenylate cyclase by hormone and guanyl nucleotides and the termination of the hormone signal can be represented as follows:

$$(1)\quad R\cdot E\cdot GTP \underset{k_2}{\overset{H,k_1}{\rightleftarrows}} H\cdot R\cdot E\cdot GTP \xrightarrow{k_3} H\cdot R\cdot E'\cdot GTP \xrightarrow{k_4} H\cdot R\cdot E + GDP + P;$$

where R is the hormone receptor, E the enzyme and E' the activated form of the enzyme. In the course of enzyme activation the nature of the receptor R remains unchanged; the process of enzyme activation is characterized by the rate constant k_3 whereas the conversion of the active enzyme E' to its inactive form E is due to the hydrolysis of the bound GTP molecule. The species HRE is now available for another cycle of activation. If[E_o]denotes the total enzyme concentration it can be shown that the concentration of the active species H·R·E'·GTP is given by:

$$(2)\quad (H\cdot R\cdot E'\cdot GTP) = \frac{[E_o][H]}{\frac{k_2k_4}{k_1k_3} + \frac{k_4}{k_1} + (1 + \frac{k_4}{k_3})\,[H]}\ .$$

More details are given in the Appendix. At very high hormone concentrations the maximal concentration of HR·E'·GTP attainable is given by

$$(3)\quad [H\cdot R\cdot E'\cdot GTP]_{max} = \frac{[E_o]}{1 + \frac{k_4}{k_3}}\ .$$

The values of k_4 and k_3 have been measured independently. k_3 can be measured by substituting GTP with GppNHp, thus eliminating the hydrolytic step characterized by the rate constant k_4. Under these conditions one follows the rate of accumulation of H·R·E'·GppNHp and $k_4 = 0$. Therefore, under these conditions:

$$(4)\quad [H\cdot R\cdot E'\cdot GppNHp] = [E_o]$$

namely, all the enzyme is converted to its highly active form E'. The value found for k_3 is 0.7 min^{-1} for adenylate cyclase in turkey erythrocyte

membranes (14). The value of k_4 was measured independently by Cassel and Selinger for turkey erythrocyte membranes and was found to be k_3 = 4.0 to 6.0 min^{-1} (15). Inserting the values of k_3 and K_4 into (3) one can conclude that the maximal steady state concentration of the super-active enzyme is

$$(5) \qquad [H \cdot R \cdot E' \cdot GTP] = \frac{E_o}{6.5} \text{ to } \frac{E_o}{8.5}.$$

It follows therefore that the concentration of the highly active state of the enzyme is present only to the extent of 12% to 15% of the total enzyme concentration when the natural effector GTP is present. It is therefore clear that in the presence of GppNHp or GTPγS and hormone the enzyme is 7 to 10 more active than in its absence. It was also recently shown that the permanently active state of the enzyme in the presence of GppNHp can be reverted to the inactive state using GTP (or ATP) in the presence of hormone (14). This phenomenon can also be explained according to this model. GTP replaces GppNHp and is then hydrolyzed at the regulatory site thus converting E' to E. The fact that ATP is effective in the presence of hormone in reverting the enzyme to its inactive form may be due to the fact that ATP is contaminated with enough GTP to produce the reversal effect. Since ATP is used at concentrations of 1 x 10^{-4} M to produce the effect and since the affinity of the GTP site to the guanyl nucleotide is in the 0.1 μM (16) range a contamination of 0.1% GTP in the ATP is sufficient to produce the effect.

The model presented has been analyzed in a quantitative fashion for the turkey erythrocyte adenylate cyclase but may be of general significance, since the effects of TTP and GppNHp described are common to most if not all hormone activated adenylate cyclases.

APPENDIX

Using the scheme of equation (1) of the text one can write:

(1) $[E_o] = [R \cdot E \cdot GTP] + [H \cdot R \cdot E \cdot GTP] + [H \cdot R \cdot E' \cdot GTP]$

but

(2) $[H\cdot R\cdot E\cdot GTP](k_2+k_3) = k_1[R\cdot E\cdot GTP][H]$

namely

(3) $[H\cdot R\cdot E\cdot GTP] = \frac{k_1}{k_2+k_3}\ [R\cdot E\cdot GTP][H]$.

Also

(4) $k_3[H\cdot R\cdot E\cdot GTP] = k_4[H\cdot R\cdot E'\cdot GTP]$

namely

(5) $[H\cdot R\cdot E'\cdot GTP] = \frac{k_3}{k_4}[H\cdot R\cdot E\cdot GTP]$;

introducing (3) into (5) one obtains:

(6) $[H\cdot R\cdot E'\cdot GTP] = \frac{k_3}{k_4} \times \frac{k_1}{k_2+k_3}\ [R\cdot E\cdot GTP][H]$.

Introducing (3) and (6) into (1) one obtains:

(7) $[E_o] = [R\cdot E\cdot GTP] = \frac{k_1}{k_2+k_3}[R\cdot E\cdot GTP][H] + \frac{k_3}{k_4} \times \frac{k_1}{k_2+k_3}[R\cdot E\cdot GTP][H]$.

(8) $$[R\cdot E\cdot GTP] = \frac{[E_o]}{1 + \frac{k_1[H]}{k_2+k_3} + \frac{k_3}{k_4}\times\frac{k_1[H]}{k_2+k_3}} = \frac{[E_o]}{1 + \frac{k_1}{k_2+k_3}[H]\ (1 + \frac{k_3}{k_4})}\ .$$

Introducing (8) into (6) one obtains:

(9) $$[H\cdot R\cdot E'\cdot GTP] = \frac{\frac{k_3}{k_4} \times \frac{k_1}{k_2+k_3}\ [E_o][H]}{1+\frac{k_1}{k_2+k_3}[H](1 + \frac{k_3}{k_4})}$$

namely:

(10) $$[H\cdot R\cdot E'\cdot GTP] = \frac{[E_o][H]}{\frac{k_2+k_3}{k_1} \times \frac{k_4}{k_3} + \frac{k_4}{k_3}\ (1 + \frac{k_3}{k_4})[H]}$$

(11) $$[H\cdot R\cdot E'\cdot GTP] = \frac{[E_o][H]}{\frac{k_2k_4}{k_1k_3} + \frac{k_4}{k_1} + (1 + \frac{k_4}{k_3})[H]}\ .$$

At saturating hormone concentrations equation (11) is transformed into

$$(12)\quad [H\cdot R\cdot E'\cdot GTP] = \frac{[E_o]}{1 + \frac{k_4}{k_3}} \quad .$$

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