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GTP-RECEPTOR INTERRELATIONSHIPS IN ADENYLATE CYCLASE SYSTEMS

THEORETICAL CONSIDERATIONS

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Summary

Theoretical considerations concerning the effect of guanyl nucleotides on hormone-dependent adenylate cyclases show that the phenomena observed can be accounted for by postulating only one type of guanyl nucleotide regulatory site. The binding of GTP or of its non-hydrolizable analogues to the guanyl nucleotide regulatory site induces cyclase activation and may induce a decrease in agonist affinity towards the receptor. From basic principles it is shown that the potency ratio of guanyl nucleotides in inducing the decreased agonist affinity does not necessarily reflect their order of affinities to the GTP regulatory site.

Introduction

GTP is now recognized as an essential regulatory ligand for the activation of adenylate cyclase by hormones and neutrotransmitters [1—4]. In all cases investigated (for recent reviews, see Refs. 3 and 4) it is also established that when non-hydrolizable GTP analogues such as GppNHp, GTPγS and $GppCH_2p$ are used instead of GTP the adenylate cyclase is activated to a permanently active state. This finding led to the hypothesis [4,5] and to the discovery [5] that GTP is hydrolyzed at the guanyl nucleotide regulatory site concomitantly with the deactivation of the enzyme. Thus, reactivation of the enzyme to a cyclic AMP-producing state requires the binding of a new molecule of GTP to the regulatory site, a step which requires the presence of the agonist on the receptor. Thus it is clear that if a non-hydrolyzable GTP analogue occupies the

regulatory site, the enzyme remains permanently active. Furthermore, cholera toxin which is known to activate adenylate cyclase in the presence of NAD^+ [6,7] has been shown to inhibit the GTPase [8] step due to ADP ribosylation [9] of the guanyl nucleotide regulatory subunit. This subunit has been separated from the cyclase [10] and was shown to induce the activation of the enzyme in the presence of GppNHp.

In summary, the receptor-dependent adenylate cyclase is composed of three basic units: the receptor, the GTP regulatory unit and the catalytic moiety which is responsible for the conversion of ATP to cyclic AMP. It appears that the simultaneous occupancy of the receptor by the agonist and the GTP regulatory site by GTP is required to induce the activation of the cyclase from its inactive state to its activated form. There are, however, some observations on hormones and neurotransmitter-dependent adenylate cyclases which have not yet been accounted for by the general model outlined above:

(a) Several reports have shown that in many systems GTP induces the decrease in the affinity of the receptor towards the hormone [11—14]. Interestingly, in cases where an antagonist [13,14] is available it was shown that GTP does not induce any change in the affinity of the receptor towards the antagonists.

(b) Rodbell and his associates have suggested [15,16] that there are two types of guanyl nucleotide regulatory sites, one linked to the receptor, N_1, and one linked to the enzyme, N_2. The authors have also suggested that the nucleotide specificity of the two sites is different.

The purpose of this communication is to suggest: (a) a simple explanation for the GTP-induced decrease in agonist affinity without a change in antagonist affinity, and (b) explain why one type of guanyl nucleotide regulatory site is in fact sufficient to explain both the GTP effect on cyclase and the GTP effect on the receptor.

Theory and Discussion

(a) GTP-induced decrease in receptor affinity towards agonists

For simplicity we shall assume that the receptor cyclase system binds the hormone and the antagonist in a non-cooperative (Michaelian) fashion. This is true in most cases. Let us assume that the receptor cyclase complex binds GTP and hormone as follows:

$$\begin{array}{ccccc} R \cdot E & \underset{K_G,\,GTP}{\overset{GTP}{\rightleftharpoons}} & R \cdot E \cdot GTP & \rightleftharpoons & R'_T \cdot E' \cdot GTP \\ H \Big\| K_T & & H \Big\| K_T & & H \Big\| K_R \\ H \cdot\cdot R \cdot E. & & H \cdot R \cdot E \cdot GTP & & H \cdot R' \cdot E' \cdot GTP \end{array} \qquad (1)$$

where R is the inactive conformation of the receptor, E the inactive cyclase, R′ a new conformational isomer of the receptor stabilized by the bound GTP and E′ the activated state of the enzyme. K_T and K_R are the hormone-receptor dissociation constants characterizing ligand binding to the inactive complex and to the activated complex, respectively. L is the equilibrium constant characterizing the transition between the two states. K_G is the dissociation constant of the guanyl nucleotide to its regulatory site. The scheme shown in Eqn. 1 is in

fact the simple two state model of Monod-Wyman-Changeux [17] for a case with no cooperativity. Let us consider, for simplicity, the equation which describes hormone binding in the presence of saturating GTP.

If we define

$$C = \frac{K_T}{K_R} \tag{2}$$

and

$$L = \frac{[R \cdot E \cdot GTP]}{[R' \cdot E' \cdot GTP]} \tag{3}$$

As in the original derivation of Monod et al. [17] one can derive quite easily the equation describing hormone binding to the receptor, in terms of the parameters defined.

The measured hormone-receptor dissociation constant is given by

$$K_{diss} = \frac{[\text{Receptor}]_{free}[H]}{[\text{Receptor}]_{bound}} \tag{4}$$

but since, in the presence of saturating GTP

$$[\text{Receptor}]_{free} = [R \cdot E \cdot GTP] + [R' \cdot E' \cdot GTP] \tag{5}$$

and

$$[\text{Receptor}]_{bound} = [H \cdot R \cdot E \cdot GTP] + [H \cdot R' \cdot E' \cdot GTP] \tag{6}$$

we can write, using Eqns. 2 and 3:

$$K_{diss} = \frac{1 + L}{c + L} \cdot K_T \tag{7}$$

From Eqn. 7 it is clear that when $c < 1$, $K_{diss} > K_T$, namely when R′ binds H with less affinity than R, the observed hormone-receptor dissociation constant in the presence of GTP is higher than in its absence. It is therefore clear that within the frame of the simple non-exclusive concerted model [17] one can account for the GTP-induced decreased receptor affinity towards the hormone without assuming an additional type of GTP binding site [15,16]. If an antagonist binds to R and R′ with the same affinity ($c = 1$) no change is expected in the affinity of the receptor towards the latter when GTP is added to the system. Such a situation is predicted by the simple concerted model and is well recognized in regulatory systems where it is often found that certain ligands bind with different affinities to the R and T states whereas others bind with equal affinity.

The conversion of R to R′ and E to E′ do not have to occur simultaneously as depicted in Eqn. 1. One can therefore consider a more general scheme and write:

$$R \cdot E \underset{K_G}{\overset{GTP}{\rightleftharpoons}} R \cdot E \cdot GTP \begin{array}{l} \overset{L_1}{\rightleftharpoons} R \cdot E' \cdot GTP \overset{L_2}{\rightleftharpoons} \\ \underset{L_2}{\rightleftharpoons} R' \cdot E \cdot GTP \underset{L_1}{\rightleftharpoons} \end{array} R' \cdot E' \cdot GTP \tag{8}$$

where the isomerization of E to E′ is characterized by L_1 ($L_1 = [E]/[E']$) and that of R to R′ by L_2 ($L_2 = [R]/[R']$). The intrinsic affinity of GTP or its analogues to the cyclase system is governed by K_G and the values of L_1 and L_2.

The extent of enzyme activation by GTP or its analogues depends on the value of L_1. The fraction of the receptor in its low-affinity form, R′, depends on L_2 and thus the extent of nucleotide-induced hormone release depends strongly on L_2. Namely, the nucleotide may bind with high affinity to its regulatory site and induce very little hormone release if L_2 is very high. Similarly, if L_1 is very low the nucleotide will be found very effective in inducing enzyme activation. There is no a priori reason to assume that L_1 and L_2 must depend in an identical fashion on the chemistry of the guanyl nucleotide. Thus a guanyl nucleotide which induces enzyme activation is not necessarily an efficient effector of the receptor and vice versa. In extreme cases the nucleotide may induce only one of the effects. Thus for example, guanyl nucleotides induce the activation of turkey erythrocyte β-adrenergic receptor-dependent adenylate cyclase with a small effect on the affinity of the receptor towards β-agonists in some cases (Lefkowitz, R.J., personal communication and Harmatz, D. and Levitzki, A., unpublished data) and no effect in others [18]. Similarly it is in principle possible that certain guanyl nucleotides will affect the R to R′ transition without inducing the E to E′ transition.

(b) The linkage between the GTP site, the cyclase and the receptor

The simple schemes described in Eqns. 1 and 8 show how the binding of GTP to its regulatory subunit could affect both the cyclase and the receptor. The effect of GTP binding on the cyclase is manifested by the activation of the latter and the effect on the receptor is reflected in its diminished affinity towards the agonist as shown above. Indeed, recently Limbird et al. [19] have shown that in the presence of Mn^{2+} the affinity of pigeon erythrocyte β-receptor towards β-agonists is reduced in the presence of GppNHp whereas the latter is no more capable of activating the cyclase moiety. They suggest that Mn^{2+} uncouples the enzyme from the receptor but not the guanyl nucleotide binding protein from the receptor.

Different GTP analogues are expected to yield different quantitative relationships between the measured dissociation constant K_{diss} and K_T since L is likely to depend on the nature of the nucleotide bound to the cyclase system.

Therefore, if one examines the decrease in receptor affinity towards the hormone H as a function of nucleotide concentration one expects a certain potency ratio where the more efficient nucleotide will be the one for which L is smaller. Thus following the release of H as a function of nucleotide concentration will yield a dose-response curve which can be characterized by an apparent affinity of the nucleotide to its site. The extent of hormone release is a function of both the intrinsic affinity of the guanyl nucleotide to its regulatory site and of L_2 (Eqn. 8) which determines the fraction of the low-affinity form of the receptor induced by the nucleotide.

On the other hand, when one follows the formation of the activated state of the adenylate cyclase (E′), namely the activity of the enzyme as a function of the nucleotide concentration, the apparent affinity of the cyclase system towards the nucleotide is determined by the intrinsic affinity of the latter to the guanyl nucleotide regulatory unit, and by L_1.

(c) High and low-affinity GTP sites

The guanyl nucleotide-induced shift in the affinity of β-receptors towards

β-agonists is found only when the guanyl nucleotide is continuously present during the binding experiment. Therefore when membranes are pretreated with GppNHp and β-agonist to induce permanent cyclase activation and then the excess free nucleotide is removed, the enzyme remains in its highly active form whereas the β-receptor does not reveal the typical low affinity towards β-agonists in spite of the tightly bound GppNHp [20—22]. This observation is usually taken to mean that the GppNHp site involved in cyclase activation is different from the receptor-linked guanyl nucleotide site. It must, however, be recalled [19,22] that the high-activity form of the enzyme is stable in the absence of free GppNHp only in the absence of β-agonist [21]. In the presence of a β-agonist and the absence of free GppNHp, the latter dissociates from the regulatory site [23] concomitantly with the decrease in enzyme activity [22,24]. Namely, the permanently active form of the enzyme is stable as such either in the absence of β-agonist altogether or in the presence of both β-agonist and excess GppNHp. Excess of free nucleotide is probably required to offset the decrease in the affinity of the cyclase complex towards GppNHp in the presence of the β-agonist. These observations imply that the affinity of the GTP regulatory site towards guanyl nucleotides is reduced in the presence of β-agonists. This is to be expected if the opposite effect, namely a guanyl nucleotide-induced decrease in receptor affinity towards the agonist, takes place. It was indeed demonstrated that GppNHp can be released effectively from its regulatory site only in the presence of an agonist [22,24] in the turkey erythrocyte cyclase system. Similarly, the release of GDP from the regulatory site was also shown to be enhanced by β-agonists in the turkey erythrocyte system [25] (and Harmatz, D., and levitzki, A., unpublished results). Obviously these guanyl nucleotide agonist interrelationships are not unique to the β-adrenergic system and are probably responsible also for the effects of guanyl nucleotides on glucagon binding [15].

An alternative possibility is that the guanyl nucleotide binding unit is a dimer composed of identical subunits exhibiting either negative cooperativity towards the guanyl nucleotide [21] or which possess 'tight' (high-affinity) and 'loose' (low-affinity) sites for GTP and GppNHp. In this model one has to assume that the occupancy of the high-affinity sites is sufficient to induce cyclase activation, whereas the occupancy of both the high-affinity and the low-affinity site is necessary to express reduced β-agonist affinity. This latter possibility has some appeal from the physiological point of view, since the level of cyclase activity is determined by the simultaneous occupancy of the β-receptor and the GTP site(s). It is feasible that once the level of GTP is elevated the affinity of the β-receptor is reduced in order to buffer the output of cyclic AMP by the cyclase system.

Conclusion

In summary, a simple model such as the non-exclusive concerted model depicted in Eqn. 1 assuming one class of guanyl nucleotide regulatory sites, accounts for (1) the guanyl nucleotide-induced decrease in agonist affinity to the receptor; (2) the independence of antagonist affinity on the presence of guanyl nucleotides, and (3) the absence of a correlation between the potency ratio of different nucleotides in inducing a decreased agonist affinity and the

order of their affinities to the guanyl nucleotide regulatory site.

It must be added at this point that the hypothesis that a single class of guanyl nucleotide sites exists in the cyclase system can be tested experimentally. This will be possible when a complete separation between the receptor and the enzyme is achieved. In the single case where the effect of GTP was examined on the release of hormone from its solubilized receptor such a separation was not achieved [16]. Therefore these latter experiments cannot be used as an argument for the existence of a receptor-linked GTP site distinct from the enzyme-linked GTP site.

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References

1 Rodbell, M., Birnhaumer, L., Pohl, S.L. and Krans, H.M.J. (1971) J. Biol. Chem. 246, 1877—1882
2 Rodbell, M., Lin, M.C. and Salomon, Y. (1974) J. Biol. Chem. 249, 59—65
3 Helmreich, E.J.M., Zenner, H.P., Pfeuffer, T. and Cori, C.F. (1976) in Current Topics in Cellular Regulation (Horecker, B.L. and Stadman, E.R., eds.), pp. 41—87, Academic Press, New York
4 Levitzki, A. (1977) Biochem. Biophys. Res. Commun. 74, 1154—1159
5 Cassel, D. and Selinger, Z. (1976) Biochim. Biophys. Acta 452, 538—551
6 Finkelstein, R.A. (1973) CRC Crit. Rev. Microbiol. 2, 533—623
7 Gill, D.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 72, 2064—2068
8 Cassel, D. and Selinger, Z. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3307—3311
9 Cassel, D. and Pfeuffer, T. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2669—2673
10 Pfeuffer, T. (1977) J. Biol. Chem. 252, 7224—7234
11 Rodbell, M., Krans, H.M.J., Pohl, S.L. and Birnbaumer, L. (1971) J. Biol. Chem. 246, 1861—1871
12 Rodbell, M., Krans, H.M.J., Pohl, S.L. and Birnbaumer, L. (1971) J. Biol. Chem. 246, 1872—1876
13 Maguire, M.E., Van Arsdale, P.M. and Gilman, A.G. (1976) Mol. Pharmacol. 12, 335—339
14 Lefkowitz, R.J., Mulikin, D. and Caron, M.G. (1976) J. Biol. Chem. 251, 4686—4692
15 Lad, P.M., Welton, A.F. and Rodbell, M. (1977) J. Biol. Chem. 252, 5942—5946
16 Welton, A.F., Lad, P.M., Newby, A.C., Yamamura, H., Nicosia, S. and Rodbell, M. (1977) J. Biol. Chem. 252, 5947—5950
17 Monod, J., Wyman, J. and Changeux, J.P. (1965) J. Mol. Biol. 12, 88—118
18 Weiland, G.A., Minnemen, K.P. and Molinoff, P.B. (1979) Nature 281, 114—117
19 Limbird, L.E., Mickey, A.R. and Lefkowitz, R.J. (1979) J. Biol. Chem. 2677—2683
20 Ross, E.M., Maguire, M.E., Sturgill, T.W., Biltonen, R.L. and Gilman, A.G. (1977) J. Biol. Chem. 252, 5761—5775
21 Sevilla, N., Steer, M.L. and Levitzki, A. (1976) Biochemistry 15, 3433—3439
22 Tolkovsky, A.M. and Levitzki, A. (1979) Biochemistry 17, 3785—3810
23 Cassel, D. and Selinger, Z. (1977) J. Cyclic Nucl. Res. 3, 11—22
24 Sevilla, N. and Levitzki, A. (1977) FEBS Lett. 76, 129—134
25 Cassel, D. and Selinger, Z. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4155—4159