Mechanism of Desensitization of Adenylate Cyclase by Lutropin

IMPARED INTRODUCTION OF GTP INTO THE REGULATORY SITE*

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Activity of adenylate cyclase (EC 4.6.1.1) ATP pyrophosphate lyase (cyclizing) in purified rat ovarian plasma membranes undergoes partial desensitization (30–60%) upon exposure to saturating lutropin (LH, 0.1 µg) and GTP (1 µg). Decline in hormone responsiveness of the enzyme is dose-dependent with regard to LH (Km = 1.7 µg ± 1) and GTP (Km = 0.19 µg ± 0.02), indicating that the action of these ligands is probably mediated by the hormone receptor and guanine nucleotide regulatory sites, respectively. The action of GTP in this process is highly specific as demonstrated by the inability of ITP, CTP, or ATP and other nucleotides to substitute for GTP.

The desensitizing reaction induced by LH proceeds well in the complete absence of ATP or adenosine 5'-(β,γ-imido)triphosphate, both substrates for adenylate cyclase. Furthermore, GTP is shown to accelerate desensitization in the presence of adenosine 5'-(α,β-methylene)triphosphate or CTP, which do not serve as substrates for adenylate cyclase. These findings indicate that continuous catalytic activity of adenylate cyclase is probably not a prerequisite for desensitization.

The addition of guanosine 5'O-(2-thiodiphosphate) (a competitive inhibitor of GTP) results in the cessation of adenylate cyclase activity within seconds. The rate with which this inhibition is achieved is enhanced (3- to 4-fold) by LH. In the desensitized state the ability of LH to accelerate this process is reduced along with a similar reduction in the ability of the hormone to stimulate adenylate cyclase. It is suggested that this effect of the hormone provides a new measure for the interaction of the hormone receptor with the guanine nucleotide regulatory site.

These results support the view that the function of the hormone is to accelerate the interaction of guanine nucleotides with the regulatory site, thus enhancing the influx of GTP into the GTP-regulatory cycle and, consequently, stimulating adenylate cyclase activity. In the desensitized state this capacity of the hormone is partially lost, resulting in the uncoupling of the enzyme system.

Desensitization of cells to hormones and neurotransmitters

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is one of the mechanisms for termination of primary external stimuli (1–3). Rapid decay of the response, in some cases within seconds or minutes, may well reflect processes which prevent an overshoot of the primary response.

Attempts to evaluate in detail the molecular processes which may be responsible for desensitization of hormonesensitive adenylate cyclase have recently been made in cell-free systems, utilizing membrane preparations of several tissues in which adenylate cyclase is sensitive to LH (2, 4–7), vasopressin (8), or isoproterenol (9). Many of these studies as well as some that described desensitization in whole cells or tissues indicate that loss of responsiveness results from impaired coupling of the hormone receptor. It was therefore of interest to identify the site of the lesion involved in desensitization by approaching the coupling step itself.

According to the model presented by Cassel and Selinger (10) and others (11–13), hormone-dependent interaction of GTP with the GTP regulator (G) initiates several molecular rearrangements that result in adenylate cyclase activation. Hydrolysis of the bond GTP by the "turnoff" GTPase (14) deactivates the enzyme and permits initiation of an additional cycle of activation. This "GTP-regulatory cycle" which includes all the events that take place between successive interactions of 2 GTP molecules with the GTP-binding protein presents the current chemical basis for receptor cyclase coupling. In this study we used a technique which allows the determination of the rate at which GDPβS (a competitive inhibitor of GTP (22)) interacts with the GTP regulatory site and causes cessation of adenylate cyclase activity. The effect of LH on this rate in the normal and desensitized membranes has been studied.

Some of the basic requirements of the desensitizing process and the activation process of adenylate cyclase in the cell-free system were compared. These include the requirement for ATP and p[NH]ppA, a question that is in dispute with respect to the desensitizing process (2, 5, 9). It will be shown that the process that leads to loss of hormonal sensitivity occurs also under conditions which do not permit catalytic expression of adenylate cyclase. Furthermore, the specificity of the GTP effect as compared to other nucleotides, as well as the dose dependency of the desensitizing process of GTP and LH, are described.

MATERIALS AND METHODS

p[NH]ppA, adenosine 5'-(α,β-methylene)triphosphate, and CTP were from Sigma. GDPβS was kindly made available by Dr. P.

† The abbreviations used are: GDPβS, guanosine 5'-(2-thiodiphosphate); LH, lutropin, luteinizing hormone; pp(CH2)ppA, adenosine 5'-(α,β-methylene)triphosphate; p[NH]ppA, adenosine 5'-(β,γ-imido)triphosphate; p[NH]ppG, guanosine 5'-(β,γ-imido)triphosphate; G, GTP-binding protein or G-protein; GTP, GTP-occupied protein; GDPβS, GDPβS-occupied G-protein; G, degree of desensitization (%).

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Desensitization of LH-sensitive Adenylate Cyclase

Eckstein of the Max-Planck Institute for Experimental Medicine, Göttingen, W. Germany. All other reagents, rats, procedures for preparation of purified ovarian plasma membranes were as previously described (2, 16). Luteinizing hormone ovine (oLH-NIH-S18) was kindly supplied by the United States National Institutes of Health. For calculation of molar concentrations the LH preparation was assumed 100% pure and molecular weight was taken as 30,000 as previously described (16). Protein was determined according to Lowry et al. (17).

Adenylate Cyclase Assay—Adenylate cyclase was assayed by measuring the formation of [32P]cAMP from [γ-32P]ATP as previously described (2), and cyclic [32P]AMP was isolated according to Salomon (18). All determinations were performed in triplicate. One unit of adenylate cyclase was defined as the amount of enzyme that catalyzes the formation of 1 pmol of cAMP/min at 30 °C.

Pretreatment of Ovarian Plasma Membranes—Desensitization of rat ovarian LH-sensitive adenylate cyclase was achieved by preincubating ovarian plasma membranes in adenylate cyclase assay mixture for 40 min at 30 °C in the presence of LH + GTP as previously described (2), except that control membranes which are fully responsive were preincubated for only 2 min instead of 10 min. The concentration of GTP in the preincubation step was 1 μM instead of 10 μM. Membranes incubated for 2 min or 40 min were designated E0 and E6 respectively. The additions made during the preincubation step are indicated in subscripts within parentheses, e.g. membranes incubated for 2 min with LH + GTP are designated E(LH) + GTP.

The nucleotide composition and the concentration of LH during the preincubation step deviated in some experiments from that used in the standard adenylate cyclase assay mixture as indicated in the individual experiments.

Termination of the preincubation step and preparation of membranes for adenylate cyclase assay was as described earlier (2). The degree of desensitization (D) was calculated as previously described (2) as per cent activity of adenylate cyclase assayed in the presence of 0.1 μM LH + 10 μM GTP in E6 relative to that of E(LH) + GTP. Untreated enzyme is designated E0.

RESULTS

Desensitization of Rat Ovarian Plasma Membranes by LH + GTP in the Presence of p(NH)ppA or ATP—We have previously shown (2) that desensitization induced by LH in a cell-free system containing rat ovarian plasma membranes does not occur in the presence of 0.5 mM ATP as the sole nucleotide. Induction of the desensitization state was only observed when GTP along with LH were present throughout the preincubation period. As ATP was always present one could not eliminate the possibility that this nucleotide is in some way required.

We therefore tested whether the effect of GTP can be seen in the absence of ATP. In the experiment (Table I, Experiment 1) 0.5 mM p(NH)ppA substituted for 0.5 mM ATP in the preincubation step. Adenylate cyclase activity of E(LH) + GTP (following 2 min incubation) was 192 ± 16 units/mg. Following 40 min of incubation, the activity of E(LH)+GTP was desensitized to the extent of 42%, declining to an absolute value of 112 ± 5 units/mg protein. A similar observation (37% desensitization) was made in a control experiment in which standard incubation conditions, i.e. 0.5 mM ATP, were used. In another experiment (Table I, Experiment 2) we carried out the incubation in the presence of 0.5 mM p(NH)ppA the absolute requirement of the desensitizing process on GTP was tested by preincubating ovarian plasma membranes with or without 1 μM GTP in the presence of LH. E(LH) and E(LH)GTP differed by only 14%, whereas the activity of E(LH) + GTP, declined by 66% relative to E(LH) + GTP (from 145 ± 5 units/mg of protein to 55 ± 3 units/mg of protein). Thus, ATP is not essential and desensitization also proceeds well in the presence of p(NH)ppA, provided GTP is present.

Desensitization of Adenylate Cyclase in the Absence of Productive Substrate—The possible dependence of the desensitizing process on continuous catalysis (i.e. formation of cAMP by adenylate cyclase) was tested. Preincubation was carried out in the presence of either pp(CH2)pA (0.5 mM) or CTP (0.5 mM) instead of 0.5 mM p(NH)ppA. Both pp(CH2)pA and CTP do not serve as substrates for adenylate cyclase and therefore no catalytic activity is expected to take place. However, these nucleotides do afford sufficient protection to the low concentration of GTP (1 μM) (required to promote desensitization) against nonspecific triphosphonucleotide hydrolase activity. E(LH) + GTP, prepared in the presence of p(NH)ppA had an absolute activity of 225 units/mg (Fig. 1). The activity of all E(LH)p preparations was not different (<3%) from the respective E(LH) preparations incubated with 0.5 mM p(NH)ppA, pp(CH2)pA, or CTP, i.e. D was not significantly different from zero (Fig. 1). In contrast, preincubation of ovarian plasma membranes with LH + GTP in the presence of either p(NH)ppA, pp(CH2)pA, or CTP for 40 min yielded substantial desensitization (at least 10-fold higher) D values in the range of 36-40%.

The results indicate that the effect of GTP and LH are not dependent on the presence of ATP or even on continuous production of cAMP by adenylate cyclase.

Comparison of the Effect of GTP and Other Nucleotides on the Desensitizing Process—To demonstrate that GTP is indeed selective in promoting LH-dependent desensitization as it is in the case of cyclase activation, we substituted this nucleotide in the preincubation step by 1 μM ATP, CTP, or ITP. In this experiment (Fig. 2) we used 0.5 mM p(NH)ppA throughout the preincubation step. The activity of E(LH) + GTP was 218 units/mg of protein and that of E(LH) + GTP declined by 30% to an absolute value of 152 units/mg of protein. If 1 μM ATP, CTP, or ITP substituted for GTP, the extent of desensitization was minimal (2-5%) and did not differ significantly from zero. When tested under these conditions the same nucleotides were also unable to substitute for GTP in activation of adenylate cyclase. Thus, GTP is so far the only nucleotide clearly required for desensitization in the cell-free system.

The Dependence of the Desensitization Process on the Concentration of GTP and LH—To test the dose dependence of the desensitization reaction for the two essential ligands, LH and GTP, we kept the concentration of one constant and varied the concentration of the other throughout the preincubation step. Subsequently, E6 preparations were assayed for adenylate cyclase in the presence of LH + GTP under standard conditions. In the experiment shown in Fig. 3A, LH

Table I

<table>
<thead>
<tr>
<th>Preincubation condition</th>
<th>Adenylate cyclase activity</th>
<th>D units/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E1</td>
<td>E2</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p(NH)ppA, 0.5 mM (LH + GTP)</td>
<td>192 ± 16</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>ATP, 0.5 mM (LH + GTP)</td>
<td>176 ± 12</td>
<td>111 ± 4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p(NH)ppA, 0.5 mM (LH)</td>
<td>117 ± 8</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>p(NH)ppA, 0.5 mM (LH + GTP)</td>
<td>145 ± 5</td>
<td>55 ± 3</td>
</tr>
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</table>
Variation in the range of units/mg of protein, respectively. GTP increased in the preincubation step with 0.5 mM p(NH)ppA replacing ATP. The medium also contained GTP. Adenylate cyclase activity in the washed membranes and calculation "Materials and Methods." Preincubation: ATP CTP ITP GTP

Calculation of the extent of desensitization and all other details were as described under "Materials and Methods." Preincubation: p(NH)ppA pp(CH2)pA CTP

When GTP concentration was kept constant throughout the preincubation step (Fig. 3B), the activity of E(LH + GTP) and E(GTP), were almost the same (243 ± 12 and 220 ± 7 units/mg). Upon increasing concentrations of LH from 0.5 to 50 nM, D increased and reached plateau levels of about (45%) at a concentration of 75 nM LH. Half-maximal concentration required in this experiment as 3.6 ± 1 nM LH, and the average of three experiments was \( K_a = 1.7 \text{ nM} ± 1 \text{ S.E.} \). This value is about 4 times higher than \( K_a = 0.4 \text{ nM} \) obtained for activation of this enzyme by LH (18).

The Effect of LH on the Rate of Inhibition of Adenylate Cyclase by GDPpS—Other than some suggestions of loss of hormone receptors that is the primary measure of desensitization in this and other laboratories is the reduction in responsiveness of the adenylate cyclase to hormonal stimulation. We looked for other parameters that could serve to locate the lesion responsible for desensitization. It occurred to us that an additional measure of the interaction of the receptor with the GDPpS regulatory site might be reflected in the rate of inhibition of activity by GDPpS (15).

We found that GDPpS competitively inhibits LH-sensitive adenylate cyclase in the rat ovarian system (\( K_i = 0.7 \text{ nM} \)). We then determined the rate at which GDPpS inhibited adenylate cyclase activity. This rate is expressed as the reciprocal of the time (min)\(^{-1}\) designated \( K_{on} \) required for the nucleotide analogue to inhibit activity according to Cassel et al. (15). In these experiments we used a GTP concentration of 0.5 nM. The selected GDPpS concentration was 0.35 nM which afforded an inhibition in excess of 85%. A typical experiment in which adenylate cyclase activity was stimulated by LH + GTP in the presence and absence of GDPpS is shown in Fig. 4 and yielded a value \( K_{on} \) = 11.2 min\(^{-1}\). The figure also illustrates the extrapolation procedure proposed by Cassel et al. (15) for calculation of the rate constant \( K_{on} \).

Previous determinations of the \( K_{on} \) in several tissues (15) were all performed in the presence of the respective stimulating hormones or neurotransmitters. In no system has there been a direct comparison of the \( K_{on} \) in the basal and hormone-stimulated state. As GTP alone is sufficient to generate appreciable activity of adenylate cyclase in this membrane preparation, it was possible to assess the effect of the hormone on the rate constant \( K_{on} \) by determination of activity in the presence and absence of LH. As shown in Table II, the apparent \( K_{on} \) was 3-fold higher in the presence of LH (\( K_{on} = 13.5 \text{ min}^{-1} ± 2 \text{ S.E.} \) than in its absence (\( K_{on} = 4.0 \text{ min}^{-1} ± 0.75 \text{ S.E.} \)). Consequently we concluded that the hormone significantly stimulates \((p < 0.01)\) the rate at which GDPpS inhibits the activity of the enzyme. Thus, the change in \( K_{on} \) provides an additional measure for hormone-receptor interaction at the G site.

The Rate of Inhibition of Adenylate Cyclase by GDPpS in Desensitized Membrane Preparations—To evaluate further the mechanism of LH- and GTP-dependent desensitization and the possible site that is affected within the hormone-sensitive machinery, we compared the rate of inhibition of adenylate cyclase in desensitized E(LH + GTP) and control E(LH + GTP) membrane preparations. The average adenylate cyclase activity of responsive membranes of E(LH + GTP) was 247 units/mg ± 20 S.E. (\( n = 5 \); Table II), and the activity of desensitized membranes E(LH + GTP) was 95 units/mg ± 10 S.E. (\( n = 5 \)), i.e. an average D = 62%.

The apparent \( K_{on} \) in control membranes E(LH + GTP) (9.8 min\(^{-1} ± 1.1 \text{ S.E.} \); \( n = 5 \)) was not significantly different \((p <
FIG. 3. Desensitization of rat ovarian plasma membranes by LH + GTP. A, GTP concentration dependence. Ovarian plasma membranes (100 ng/ml) were preincubated for 2 or 40 min at 30 °C in the presence of 0.5 mM p(NH)pA and subsequently washed as described under “Materials and Methods.” LH concentration in the preincubation step was kept constant (100 nM) and GTP concentration was varied over the indicated range. The washed membranes were assayed for adenylate cyclase activity under standard assay conditions in the presence of 100 nM LH + 10 μM GTP. D was calculated as described under “Materials and Methods” and plotted against GTP concentration in the preincubation step. K, was calculated and fitted according to Wilkinson (20). B, LH concentration dependence. Ovarian plasma membranes were preincubated as described in the legend to Fig. 1, except that GTP concentration (1 μM) in the preincubation step was kept constant and LH concentration was varied from 0.5-10 nM as indicated. D is plotted against LH concentration in the preincubation step and calculated as in A.

FIG. 4. Inhibition of LH-sensitive adenylate cyclase by GDPβS. Ovarian plasma membranes (60 μg) were incubated at 30 °C for 2 min in adenylate cyclase assay mixture containing LH and GTP but devoid of [α-32P]ATP. At time zero the reaction was initiated by the addition of 100 μl of [α-32P]ATP (5.4 × 10^4 cpm) with ( ) or without ( ) 0.14 μmol of GDPβS. Final concentrations of the assay components were as described under “Materials and Methods” except that GTP concentration was 0.5 μM and GDPβS concentration was 0.35 μM. At the indicated times 50-μl samples were transferred to test tubes containing stopping solution. All other details were as described under “Materials and Methods.”

TABLE II
Rate of inhibition of adenylate cyclase by GDPβS in untreated and desensitized membrane preparations

Determinations of K, were carried out with untreated Eo, control Eo+LH + GTP, or desensitized Eo+LH + GTP membrane preparations. The experimental details were as in Fig. 4, except that in the first set of experiments LH was omitted from the assay mixture. Preparation of Eo+LH + GTP and Eo+LH + GTP were as described under “Materials and Methods.” Plots of activity for each experiment were fitted by least square analysis of the last five points (+) GDPβS and for all points obtained (−)GDPβS. K, was obtained by calculation of the cross-over point of both lines. Values are mean ± S.E. n, number of experiments.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>n</th>
<th>Adenylate Cy-class assay with</th>
<th>Adenylate Cy-class</th>
<th>K,</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>[α-32P]ATP</td>
<td>units/mg protein</td>
<td>min⁻¹</td>
<td></td>
</tr>
<tr>
<td>Eo untreated</td>
<td>6</td>
<td>GTP</td>
<td>28 ± 5</td>
<td>4 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eo untreated</td>
<td>5</td>
<td>LH + GTP</td>
<td>262 ± 25</td>
<td>13.6 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eo+LH + GTP, control</td>
<td>5</td>
<td>LH + GTP</td>
<td>247 ± 20</td>
<td>9.8 ± 1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eo+LH + GTP, desensitized</td>
<td>5</td>
<td>LH + GTP</td>
<td>95 ± 10</td>
<td>5.2 ± 0.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td>62%</td>
<td>47%</td>
</tr>
</tbody>
</table>

0.05) from that determined in untreated plasma membranes Eo, (K, = 13.6 ± 2 S.E.; Table II). In parallel to the desensitization of adenylate cyclase we found that the apparent K, in Eo+LH + GTP also decreased (47%) to K, = 5.2 min⁻¹ ± 0.65 S.E. (n = 5), a value which is significantly lower (p < 0.01) than that obtained in control membranes. This rate is similar to the basal rate (4.0 min⁻¹ ± 0.75 S.E.) determined in the complete absence of LH. It was therefore possible to conclude that desensitization leads to a similar reduction in the ability of LH to stimulate adenylate cyclase and to stimulate the rate of inhibition by GDPβS.

DISCUSSION

It was previously suggested by us (2) that a GTP-dependent, hormone-stimulated process leads to the formation of a low
activity intermediate of adenylate cyclase which is refractory to further hormonal stimulation. It was also demonstrated that loss of hormone responsiveness in the ovarian system results from uncoupling of the enzyme system rather than from a decrease in the number of hormone binding sites or from inactivation of the catalytic subunit.

In this study we addressed ourselves to two major questions: (i) are the kinetic and other requirements of adenylate cyclase activation and desensitization similar or not? and (ii) what step in the coupling mechanism is affected in the desensitized state.

A diversity exists with respect to whether cell-free desensitization requires the presence of ATP. Bockaert et al. (5) demonstrated in pig ovarian membranes that ATP is an essential nucleotide and cannot be replaced by p(NH)ppA. Using membranes from normal rat kidney cells Anderson and Jaworski (9) showed that GTP is required but that ATP cannot be omitted from the incubation medium. In our system ATP alone did not have any effect. However, since GTP-dependent desensitization was always determined in the presence of ATP, the participation of this nucleotide could not be excluded.

The results (Table I, Figs. 1 and 2) clearly demonstrated that ATP is not at all required for cell-free desensitization in rat ovarian membranes. When ATP or other nucleotides, CTP, IPT, or pp(CH3)ppA were added, they could not replace GTP. It seems therefore that in our system GTP is so far the only required nucleotide. Should a phosphorylation step be involved in cell-free desensitization as previously proposed (2, 5), then in the rat ovarian system GTP would be the preferred phosphate donor. It should be noted, however, that so far no direct evidence exists that may link desensitization with phosphorylation. The results (Fig. 1) also suggest that continuous catalytic expression of adenylate cyclase is not a prerequisite for desensitization. The degree of desensitization was the same in the absence or presence of substrates for adenylate cyclase.

Desensitization is dose-dependent with respect to GTP and LH concentrations (Fig. 3, A and B). The $K_a$ for GTP (0.19 nM) is close to the $K_a = 0.4$ nM observed previously for activation of this enzyme by GTP (16). The $K_a$ for LH (1.7 nM) is about 4-fold higher than that observed for the activation process $K_a = 0.4$ nM (18). The requirements therefore of adenylate cyclase activation and desensitization seem to be rather similar with respect to specificity and the dependency of both processes on LH and GTP concentration. Also indicated by these results is the fact that these ligands may be acting through identical sites in promoting both reactions. Desensitization, however, may require a slightly higher degree of receptor occupancy as also noted by others (5). In contrast, the requirement for ATP in both reactions seems to differ in that desensitization is independent of ATP while adenylate cyclase activation obviously requires that substrate be present. Another differences already reported earlier (2) is that p(NH)ppG, which successfully substitutes for GTP in the stimulation of adenylate cyclase, fails to participate in the desensitizing process.

We have previously demonstrated that GDP is a competitive inhibitor of GTP in the hepatic glucagon-sensitive adenylate cyclase (19). Similar observations have recently been made with GDPβS (a GDP analogue) in several other tissues (15, 21, 22) and in this study also in the rat ovary.

Inhibition of adenylate cyclase activity by these diphospho nucleotides may be explained by the hypothesis (10) that G<sub>GDPS</sub> or G<sub>GDPβS</sub>, in contrast to G<sub>GTP</sub>, are unable to activate adenylate cyclase. The preferential association of GDPβS with the G-protein at high GDPβS/GTP ratio leads to rapid cessation of adenylate cyclase activity. The experimental approach proposed by Cassel et al. (15) permits the measurement of the rate of inhibition of adenylate cyclase expressed as a first order decay constant $K_{off}$.

Using this method we obtained a value for (LH + GTP)-stimulated rat ovarian adenylate cyclase, $K_{off} = 13.6 \pm 2 \text{ min}^{-1}$ at 30°C (Table II), which is in the range of values obtained by Cassel et al. (15) for catecholamine-sensitive adenylate cyclase of turkey erythrocytes (10 ± 2 min<sup>-1</sup>), of rat parotid (7.5 min<sup>-1</sup>) and of rat hepatic glucagon-sensitive adenylate cyclase (6.2 min<sup>-1</sup>). With the same methodology we were also able to show that LH increases over 3-fold the apparent $K_{off}$ from 4 to 13.6 min<sup>-1</sup> (Table II). This determination was possible since the basal adenylate cyclase activity in the ovarian preparation is sufficiently high to be measured even in the absence of hormone. In the turkey system (15), the basal rate is too low to be detected.

The mechanism by which LH enhances the apparent $K_{off}$ deserves some attention as this observation is seemingly paradoxical. It is suggested that in this system hormone-dependence augmentation of the apparent $K_{off}$ results from enhancement of the interaction of GDPβS with the G-protein. This hormone-stimulated process accelerates the formation of G<sub>GDPβS</sub> and thus promotes the deactivation of adenylate cyclase. Under physiological conditions, in the absence of GDPβS, the same mechanism would lead to activation of adenylate cyclase, as free GTP associates with the G-protein.

This suggestion is consistent with other reports which predicted that the hormone facilitates the interaction of GTP with the G site (10, 13, 23, 24). The direct demonstration of this hormone-stimulated reaction in the ovarian system certainly represents an early step in the sequence of events that lead to hormonal activation of adenylate cyclase. This step must even precede hydrolysis of GTP by a putative reaction analogous to the "turnoff GTPase" demonstrated recently in turkey (25) and frog erythrocytes (26). Based on the assumption that the same principles apply for hormonal control of adenylate cyclase in different cell types, one is tempted to speculate that the hormone receptor does probably not enhance GTP hydrolysis directly. It seems more likely that apparent stimulation of GTPase activity by the hormone in those systems reflects, in a secondary fashion, the enhanced uptake of GTP by G-protein.

It was of interest to find that desensitization of ovarian adenylate cyclase by LH + GTP (Table II) was accompanied by a similar decrease in $K_{off}$. In the light of the above interpretations we suggest that desensitization results from a decrease in the ability of the LH-receptor complex to facilitate the uptake of GTP by G-protein and consequently to activate adenylate cyclase. Impairment of the coupling mechanism at a step that controls the influx of GTP into the GTP-regulatory cycle seems therefore to be the basis of the observations described. The lesion responsible for desensitization may well be associated with a modification of the hormone receptor, the G-protein or yet another unidentified component.

In a recent report by Joy Pike and Lefkowitz (26), the authors arrived at somewhat similar conclusions with respect to the activation and desensitization of agonist-coupled GTPase in turkey and frog erythrocytes. We would like to emphasize that while their studies were conducted in whole cells ours were derived from observations in broken cell preparations. It is therefore likely that a similar lesion may be responsible for hormone-dependent desensitization in broken cell preparations and in intact cells. Future experiments will be aimed at defining the exact site of this lesion and the nature of the modification incurred.

<sup>1</sup>The report appeared while this study was under review.
Desensitization of LH-sensitive Adenylate Cyclase

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