Activation of adenylate cyclase in bovine corpus-luteum membranes by human choriogonadotropin, guanine nucleotides and NaF

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1. Preincubation of luteal membranes with human choriogonadotropin results in the formation of an activated state of adenylate cyclase which is not reversed by washing and which is limited only by the absence of guanine nucleotides, whereas preincubation with GTP yields only a partially activated adenylate cyclase which requires the presence of both GTP and human choriogonadotropin during assay to demonstrate maximal activity. 2. Preincubation of luteal membranes with GTP and human choriogonadotropin does not lead to a synergistic increase in wash-resistant activity. 3. Luteal membranes that had been preincubated with GTP and hormone exhibited a decreasing rate of cyclic AMP synthesis during the adenylate cyclase assay incubation; addition of GTP during the assay incubation reversed the decrease. 4. Membranes that had been preincubated in the absence of guanine nucleotide and hormone showed a 'burst' phase of cyclic AMP synthesis when GTP was present in the assay incubation and a 'lag' phase with p[NH]ppG (guanosine 5'-[β,γ -imido]triphosphate) present in the assay. The presence of human choriogonadotropin with either nucleotide in the assay incubation eliminated the curvatures in plots observed with guanine nucleotides alone. 5. Luteal adenylate cyclase was persistently activated by preincubation with p[NH]ppG alone or in combination with human choriogonadotropin; the activation caused by p[NH]ppG alone was still increasing after 70 min of preincubation, whereas that caused by p[NH]ppG in the presence of hormone was essentially complete within 10 min of preincubation. 6. Luteal adenylate cyclase that had been partially preactivated by preincubation with p[NH]ppG was slightly increased in activity by the inclusion of further p[NH]ppG in the adenylate cyclase assay incubation, but more so with p[NH]ppG and hormone. Human choriogonadotropin alone caused no further increase in the activity of the partially stimulated preparation unless p[NH]ppG was also added to the assay incubation. 7. GTP decreased the activity of adenylate cyclase in membranes that had been partially preactivated in the presence of p[NH]ppG; the decrease in activity was greater when GTP and hormone were present simultaneously in the assay. 8. The results indicate that stable activation states of adenylate cyclase can be induced by preincubation of luteal membranes *in vitro* with human choriogonadotropin or p[NH]ppG, and that in the presence of p[NH]ppG the hormone may accelerate events subsequent to guanine nucleotide binding. Stable activation of luteal adenylate cyclase by prior exposure to GTP is not achieved. The involvement of GTPase activity and of hormone-promoted guanine nucleotide exchange in the modulation of luteal adenylate cyclase activity is discussed.

The adenylate cyclase complex is now generally accepted to contain at least three interacting subunits (Rodbell, 1980); these include a catalytic

subunit, a hormone-receptor subunit and one or more guanine nucleotide-receptor subunits (Rodbell, 1980; Levitzki, 1980b; Iyengar *et al.*, 1979; Iyengar & Birnbaumer, 1979; Levitzki & Helmreich, 1979). There is, however, no general agreement on the molecular interactions involved in the activation of

Abbreviation used: p[NH]ppG, guanosine 5'-[β,γ -imido]triphosphate.

the catalytic subunit subsequent to the binding of hormone and guanine nucleotide to their respective receptors. Indeed, different models for the control of liver adenylate cyclase have been derived from data obtained by using the same experimental approach, namely target-size analysis by radiation inactivation (Rodbell, 1980; Martin *et al.*, 1979, 1980). Many other models have been proposed, but it is difficult at this time to distinguish properties that are generally characteristic of all adenylate cyclase systems from those that are unique variations arising from particular tissue specializations. Many more tissues need to have the properties of their adenylate cyclases examined before a widely applicable model can be developed.

We have studied the adenylate cyclase activity of bovine corpus luteum and have previously concluded that the activity *in vitro* reflects the state of activation of the enzyme achieved *in vivo* (Young & Stansfield, 1978a). This suggested that stable activation states could be produced *in vitro* by preincubation with the physiological effectors human choriogonadotropin and GTP. We have previously demonstrated that luteal adenylate cyclase in a preparation that was unresponsive to hormone *in vitro* could be stably activated by exposure to p[NH]ppG (Young & Stansfield, 1978a,b,c), a guanine nucleotide analogue which is resistant to hydrolysis by GTPase (Salomon & Rodbell, 1975) and which has been shown in other tissues to cause an activation of adenylate cyclase that is only slowly reversible (Levitzki & Helmreich, 1979; Birnbaumer *et al.*, 1980a).

We now report the results of studies on a hormone-responsive preparation of bovine luteal adenylate cyclase (Lydon *et al.*, 1979) in which we have sought: (a) to achieve stable activation states of the enzyme by preincubation with human choriogonadotropin and guanine nucleotides; (b) to examine the effect of hormone on the production of the p[NH]ppG-activated state, and (c) to determine some of the responses of the preactivated states to subsequent exposure to hormone, guanine nucleotides and NaF.

A preliminary account of some of these results has been presented (Lydon *et al.*, 1980).

Experimental

Materials

ATP (disodium salt, type A2383), phosphocreatine, creatine kinase (from rabbit muscle) and deoxyribonuclease 1 (from bovine pancreas) were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Cyclic AMP, GTP and p[NH]ppG (tetralithium salt) were purchased from Boehringer Corp. (London) Ltd., Lewes, E. Sussex, U.K. Cyclic AMP-

binding protein (bovine adrenal gland) and all other chemicals (analytical grade where available) were obtained from BDH, Poole, Dorset, U.K. Bovine ovaries were obtained from the local abattoir and transported on ice to the laboratory, where they were used immediately. Human choriogonadotropin (Pregnyl) was obtained from Organon Laboratories, Morden, Surrey, U.K. Cyclic [8-³H]AMP was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of partially purified plasma membranes

The method was based on that of Mintz *et al.* (1978) for the preparation of rat ovary plasma membranes. Corpora lutea of approx. 12 days development were removed from the ovaries, minced with scissors and the pooled tissue was suspended at a concentration of 1 g wet wt. of tissue per 5 ml of ice-cold bicarbonate medium (1 mM-NaHCO₃/1 mM-dithiothreitol) containing 0.2 mM-MgCl₂. Deoxyribonuclease 1 (1600 units/g wet wt. of tissue) was added and the mixture was incubated on ice for 10 min. The tissue suspension was then homogenized at 0–4°C with a motor-driven glass/Teflon homogenizer, and the homogenate was filtered through two layers of muslin. Solid sucrose was added to the filtrate to give a final concentration of 40% (w/v) as indicated by Abbé refractometer reading. Portions (15 ml) of the suspension were introduced into 50 ml polycarbonate centrifuge tubes and then overlaid with 10 ml of 36% (w/v) sucrose solution and 5 ml of 30% (w/v) sucrose solution; both sucrose solutions were made up in bicarbonate medium. The tubes were centrifuged in an angle rotor at 60000g ($r_{av.} = 7.72$ cm) for 120 min at 4°C. Material at the interface between the 30%- and the 36%-sucrose layers and in the 36%-sucrose layer, representing the partially purified plasma-membrane fraction, was harvested, diluted 5-fold to lower the sucrose concentration, sedimented by centrifugation at 100000g ($r_{av.} = 7.72$ cm) for 30 min at 4°C and resuspended in fresh bicarbonate medium.

Preincubation conditions

Luteal plasma membranes (1–3 mg of membrane protein/ml of medium) were preincubated at 32.5°C for various time periods (see text) in the presence of 25 mM-Tris/acetic acid buffer (pH 7.5), 5.5 mM-magnesium acetate, 1 mM-ATP, 1 mM-3-isobutyl-1-methylxanthine, 1.5 mM-dithiothreitol, 0.5 mM-NaHCO₃, 5 mM-phosphocreatine and 50000 units/ml of creatine kinase. Preincubations were initiated by the addition of membranes to medium which had been prewarmed to 32.5°C. Where stated (see the text), human choriogonadotropin was added to the preincubation medium at a concentration of 100 i.u./ml and GTP and p[NH]ppG were used at a concentration of 0.1 mM. The preincubation tem-

perature of 32.5°C is close to the optimum for retaining adenylate cyclase activity; the activity of preincubated adenylate cyclase decreases at preincubation temperatures above 35°C (results not shown). Preincubations were terminated by dilution with ice-cold bicarbonate medium, centrifugation and washing of the membranes in bicarbonate medium.

Washing of the preincubated membranes

Membranes were sedimented by centrifugation at 15000g ($r_{av.} = 7.72$ cm) for 15min at 4°C. The supernatant was discarded and the sediment was resuspended in bicarbonate medium by using a vortex mixer. The washing procedure was performed three times. It was shown that this procedure removed all detectable cyclic AMP present at the end of the preincubation period and that it caused a 30–40% loss of membrane protein (results not shown), without selective removal of components of the adenylate cyclase complex (Table 1).

Adenylate cyclase assay incubation conditions

Adenylate cyclase activity was assayed at 32.5°C for 15min or as indicated in the text in a medium identical with that used for the preincubation and in a total volume of 1ml. Preincubated membranes were suspended in half the volume used for their preincubation and were mixed with an equal volume of assay incubation medium. When included in the assay, human choriogonadotropin, GTP and p[NH]ppG were at the concentrations used in the preincubation and NaF was at 10mM. Incubations were terminated by immersion of assay tubes in boiling water for 3min. Insoluble material was removed by centrifugation at 8000g ($r_{av.} = 4.6$ cm). Cyclic AMP was determined in the boiled supernatant by a competitive protein-binding assay (Young & Stansfield, 1977). All assays were

performed in duplicate, and results shown are the mean values with the range of duplicates indicated.

Protein assay

The method of Bradford (1976) was followed, by using Coomassie Brilliant Blue G-250 and with bovine serum albumin as standard.

Results and discussion

Table 1 shows that the adenylate cyclase of luteal membranes which had been preincubated without additions was activated during assay by human choriogonadotropin (1.9-fold), GTP (3.2-fold), human choriogonadotropin plus GTP (9.3-fold) and NaF (32.5-fold). Table 1 also indicates the effects of preincubation in the presence of hormone or GTP on the adenylate cyclase activity observed after thorough washing of the preincubated membranes. The choriogonadotropin-preactivated state was not further increased in activity by the addition of human choriogonadotropin to the subsequent assay incubation, but it was further activated by the addition of GTP alone or in the presence of hormone to the assay incubation. The presence of GTP in the assay incubation of hormone-preactivated adenylate cyclase produced activity equal to that seen when non-stimulated membranes were assayed in the presence of hormone and GTP. Preincubation of membranes with GTP caused only a small increase in the adenylate cyclase activity observed after washing of the membranes, and maximal hormone stimulation of these membranes could only be achieved if additional GTP was present in the assay incubation. Preincubation of the membranes in the presence of both human choriogonadotropin and GTP did not give a synergized persistent activation of adenylate cyclase. Indeed, the addition of hormone alone to the subsequent assay incubation appeared to give somewhat less activity than did the addition of hormone to membranes that had been

Table 1. *Effect of preincubation with human choriogonadotropin and/or GTP on adenylate cyclase activity*

Preincubation, washing of membranes and assay were done as described in the Experimental section. Each preincubation contained 1.65mg of plasma-membrane protein in 1ml of medium. Results are expressed as the mean $\pm$ range of duplicate values.

Additions to preincubation medium	Additions to assay incubation ...	Adenylate cyclase activity (pmol of cyclic AMP/15 min)				
		None	Choriogonadotropin	GTP	Choriogonadotropin + GTP	NaF
—		24.5 $\pm$ 3.5	46.3 $\pm$ 0	78 $\pm$ 0	227 $\pm$ 22	795 $\pm$ 0
Choriogonadotropin		49 $\pm$ 0	47.5 $\pm$ 4.5	210 $\pm$ 0	212.5 $\pm$ 27	892 $\pm$ 7.5
GTP		32.5 $\pm$ 3.5	81.5 $\pm$ 13	79.5 $\pm$ 11	222 $\pm$ 36	780 $\pm$ 0
Choriogonadotropin + GTP		53 $\pm$ 3	62 $\pm$ 6	235 $\pm$ 15	210 $\pm$ 10	877 $\pm$ 7.5

preincubated in the presence of GTP alone, and the addition of GTP to the assay incubation was again necessary for full activation to be seen. Preincubation with GTP was without effect on the activity of luteal adenylate cyclase in the presence of NaF; preincubation with human choriogonadotropin alone or in the presence of GTP caused only minor change (+12%) in the NaF-stimulated activity (Table 1).

The results of Table 1 imply that preincubation of luteal membranes with human choriogonadotropin yields an activated state of adenylate cyclase which resists reversal by washing. We have shown that radioiodinated human choriogonadotropin remains bound to luteal membranes through extensive washing (results not shown), confirming previous observations by Azhar & Menon (1976). Hormonal activation of adenylate cyclase in the absence of added guanine nucleotide is consistent with the properties of the two-state activation model proposed by Iyengar *et al.* (1980). The persistently activated state induced by pre-exposure of luteal membranes to human choriogonadotropin is only prevented from expressing its potential activity by the absence of GTP from the assay incubation. On the other hand, Table 1 indicates that preincubation of luteal membranes with GTP does not lead to a significant wash-resistant activation of adenylate cyclase. Instances of persistent hormone activation of adenylate cyclase have been reported in adrenal cortex (Kelly & Koritz, 1971) and kidney (Goltzman *et al.*, 1976). There have also been reports of the reversal of GTP-preactivation of adenylate cyclase by washing of membranes from adrenal cortex (Glynn *et al.*, 1979), chick kidney (Michaelangeli *et al.*, 1977), rat ovary (Ezra & Salomon, 1980) and liver (Birnbaumer *et al.*, 1980a). However, the variable properties of adenylate cyclase complexes in different tissues are demonstrated by the reports that hormonal preactivation of adenylate cyclase failed to survive washing of membranes prepared from liver (Birnbaumer, 1973; Okamura & Terayama, 1978) and from astrocytoma cells (Clark, 1978), and that activation of adenylate cyclase by preincubation with GTP survived washing of membranes from heart (St. Louis & Sulakhe, 1976) and pigeon erythrocytes (Pfeuffer & Helmreich, 1975). Membranes that had been preincubated with GTP, hormone or GTP plus hormone were clearly not desensitized, since they allowed maximal adenylate cyclase activity to be observed when both hormone and GTP were present during assay (Table 1). Dithiothreitol, reported by Hunzicker-Dunn *et al.* (1979) to reverse the desensitization of adenylate cyclase by lutropin in membranes from pig ovaries, was included in all preincubations and assay incubations reported in Table 1. The role of dithiothreitol in preventing desensitization is,

however, not clear, since Ezra & Salomon (1980) have reported extensive desensitization of rat ovarian adenylate cyclase by lutropin and GTP during 40 min preincubations containing dithiothreitol. In preincubations of duration similar to those reported in Table 1, Ezra & Salomon (1980) observed desensitization to the extent of only 5%. Table 1 does not suggest that guanine nucleotides decrease the affinity of the luteal receptor for human choriogonadotropin, in contrast with findings in other tissues (Rodbell *et al.*, 1971a,b; Lefkowitz *et al.*, 1976; Lin *et al.*, 1977; Maguire *et al.*, 1977). This agrees with our observations that guanine nucleotides do not affect the binding of radioiodinated human choriogonadotropin to luteal membranes (results not shown) and with those of Labarbera *et al.* (1980), working with rat luteal tissue.

Fig. 1 shows that membranes that had been preincubated with hormone and GTP were able to synthesize cyclic AMP at an initially rapid rate, which decreased progressively and which was increased to about 2½ times the initial rate by the addition of GTP during the 10th min of assay. We conclude, therefore, that some GTP can remain bound to its receptor through washing, but that this is lost during the assay incubation, perhaps because of the GTPase activity, which is believed to be associated with guanine nucleotide receptors (Cassel & Selinger, 1977a, 1978a; Lambert *et al.*, 1979; Pfeuffer, 1977, 1979). Inclusion of a nucleoside triphosphate-regenerating system in both the preincubation and the assay incubation failed to increase the wash-resistant activation resulting from preincubation with GTP (results not shown). This is in accord with the results of Salomon & Rodbell (1975), who found that a regenerating system was without effect on the amount of membrane-bound GTP, although it enabled free GTP concentrations to be maintained. Thus, if any rephosphorylation of GDP to GTP occurs at the guanine nucleotide binding site it is more likely to be due to the activity of endogenous membrane-bound enzymes (Salomon & Rodbell, 1975; Kimura & Nagata, 1979).

Table 2 shows that the adenylate cyclase of luteal membranes which had been preincubated without additions was stimulated by p[NH]ppG during assay, that the activation was enhanced by hormone and that p[NH]ppG was a more potent agonist than GTP. When luteal membranes were assayed for adenylate cyclase activity in the presence of GTP or p[NH]ppG alone or in combination with human choriogonadotropin, the time-courses shown in Fig. 2 were obtained. They show that p[NH]ppG alone activated with a 1½–3 min 'lag' phase, whereas GTP activated with a 'burst' phase of similar duration. Kimura & Nagata (1979) observed similar effects with liver membranes during the first ½ min of

Fig. 1. Time course of cyclic AMP production by luteal membranes after preincubation with GTP and human choriogonadotropin

Luteal membranes (0.85 mg/ml) were preincubated, washed and subsequently assayed for adenylate cyclase activity as described in the Experimental section. The preincubation time was 10 min. Samples were removed at intervals from the subsequent adenylate cyclase assay incubation for measurement of cyclic AMP. The arrow at 10 min indicates the addition of GTP (0.1 mM) to the assay incubation. A sample was not taken at 10 min; the broken line indicates the assumed time-course between 9 and 11 min.

incubation whereas Birnbaumer *et al.* (1980a) have reported that liver membranes were activated by p[NH]ppG with a 5–10 min 'lag'. Fig. 2 also shows that human choriogonadotropin eliminates both the 'lag' and 'burst' phases; this has also been noted for hormonal activation in other tissues (Kimura & Nagata, 1979; Birnbaumer *et al.*, 1980a). The basal production of cyclic AMP was not measured in this experiment, because in similar experiments the amount of cyclic AMP was too low to be reliably

Table 2. Effect of GTP, human choriogonadotropin and p[NH]ppG on the activity of adenylate cyclase in luteal membranes after preincubation for 15 min in the absence of additions and washing before assay

See the Experimental section for details. The incubations each contained 1.1 mg of plasma-membrane protein in 1 ml of medium. Results are expressed as mean $\pm$ range of duplicate values.

Additions to assay incubation	Cyclic AMP (pmol/incubation)
None	13.6 $\pm$ 1.1
Human choriogonadotropin	30.0 $\pm$ 1.0
GTP	60.0 $\pm$ 4.0
Human choriogonadotropin + GTP	234.0 $\pm$ 30.0
p[NH]ppG	533.0 $\pm$ 27.0
p[NH]ppG + human choriogonadotropin	840.0 $\pm$ 30.0

Fig. 2. Time course of cyclic AMP production by luteal membranes in the presence of GTP, GTP and human choriogonadotropin, p[NH]ppG or p[NH]ppG and human choriogonadotropin

Luteal membranes (1.0 mg/ml) were assayed for adenylate cyclase activity in the presence of GTP (0.1 mM) alone (■) or in combination with human choriogonadotropin (100 i.u./ml) (□), or with p[NH]ppG (0.1 mM) alone (●) or in combination with human choriogonadotropin (100 i.u./ml) (○). Samples of the incubation were withdrawn at the indicated time intervals for assay of cyclic AMP as described in the Experimental section.

estimated during the first 5–10 min of the incubation. The 'lag' phase in the activation of adenylate cyclase by p[NH]ppG has been interpreted by some authors (Cassel & Selinger, 1978b; Swillens *et al.*, 1979) as being due to the need to expel GDP from the guanine-nucleotide-binding site before p[NH]ppG

Fig. 3. Time course of preactivation of luteal adenylate cyclase by p[NH]ppG alone or in combination with human choriogonadotropin

Luteal membranes (1.3 mg of protein/ml) were preincubated in a volume of 20 ml as described in the Experimental section in the presence of p[NH]ppG (0.1 mM) alone (●) or in combination with human choriogonadotropin (100 i.u./ml) (■). Portions (2 ml) of the membrane suspension were withdrawn at the indicated time intervals and were washed and assayed for adenylate cyclase activity (0.6 mg of membrane protein/ml) in a 15 min incubation as described in the Experimental section.

can enter the site. However, this view has been subsequently challenged (Birnbaumer *et al.*, 1980a,b; Iyengar *et al.*, 1980; Levitzki, 1980a).

Preincubation of luteal membranes with p[NH]ppG or p[NH]ppG plus hormone gave the preactivation time-courses shown in Fig. 3. The progressive wash-resistant activation caused by p[NH]ppG was still increasing at 70 min of preincubation, whereas the enzyme was maximally activated by p[NH]ppG and hormone within 10 min. Other experiments (results not shown) have indicated that p[NH]ppG in the absence of hormone can preactivate the enzyme to the value achieved by p[NH]ppG and hormone together, provided that the preincubation time is at least 90 min. The maximally preactivated state induced by p[NH]ppG and hormone in preincubation is not further increased in activity by the addition of p[NH]ppG to the

Table 3. Effect of p[NH]ppG on the adenylate cyclase activity of luteal membranes that had been preincubated in the presence of human choriogonadotropin and p[NH]ppG for 15 min and washed before assay

See the Experimental section for details. Each incubation contained 2.0 mg of plasma-membrane protein in 1 ml of medium. Results are expressed as the mean $\pm$ range of duplicate values.

Additions to preincubation...	Cyclic AMP (pmol/incubation)	
	None	Choriogonadotropin + p[NH]ppG
Additions to assay incubation		
None	63 $\pm$ 4	1069 $\pm$ 79
p[NH]ppG	557 $\pm$ 43	1036 $\pm$ 112

Table 4. Effect of GTP, human choriogonadotropin and p[NH]ppG on the adenylate cyclase activity of luteal membranes that had been preincubated in the presence of p[NH]ppG for 15 min and washed before assay

See the Experimental section for details. Each incubation contained 1.0 mg of plasma-membrane protein in 1 ml of medium. Results are expressed as the mean $\pm$ range of duplicate values.

Additions to preincubation...	Cyclic AMP (pmol/incubation)	
	None	p[NH]ppG
Additions to assay incubation		
None	47 $\pm$ 3	577 $\pm$ 22
Human choriogonadotropin	—	555 $\pm$ 45
GTP	—	465 $\pm$ 30
GTP + human choriogonadotropin	—	315 $\pm$ 4.5
p[NH]ppG	—	697 $\pm$ 52.5
p[NH]ppG + human choriogonadotropin	—	1020 $\pm$ 30

subsequent assay incubation (Table 3), indicating that there is no apparent removal of p[NH]ppG during washing of such maximally preactivated enzyme complexes. Thus p[NH]ppG can achieve an apparently maximal preactivation of luteal adenylate cyclase in the absence of hormone, in agreement with the predictions of the model proposed by Iyengar *et al.* (1980). The data of Figs. 2 and 3 are not directly comparable, but they are compatible; transformation of the data of Fig. 3 into an approximation of the situation described in Fig. 2 yields a time-course (not shown) with a 2 min lag phase in the activation by p[NH]ppG alone which is virtually eliminated by hormone.

Table 4 shows that partly preactivated luteal adenylate cyclase, produced by preincubation with p[NH]ppG alone for 15 min, does not respond to the presence of hormone in the subsequent assay. Thus the hormone cannot further activate adenylate cyclase complexes which have retained p[NH]ppG during washing. The partly preactivated state could, however, be enhanced in activity (approx. 20%) if p[NH]ppG was present during assay, indicating slow activation by p[NH]ppG via unoccupied guanine nucleotide receptors. This latter process was subject to synergistic increase by human chorionadotropin, the activity increasing by 79%. Similar observations have been made with liver (Iyengar *et al.*, 1979) and adrenal-cortex (Glynn *et al.*, 1978) adenylate cyclases.

Iyengar *et al.* (1980) have proposed that hormones activate adenylate cyclase by accelerating conformational changes in the occupied nucleotide receptors and associated catalytic subunits. Simpson & Pfeuffer (1980) have proposed that p[NH]ppG is retained through washing only by those guanine-nucleotide-binding subunits that are in association with catalytic subunits. On this basis it is thus to be expected that the activated adenylate cyclase complexes of the partly activated membranes described in Table 4 will already have undergone the conformational changes that cause them to retain their p[NH]ppG through washing. It is also to be expected, therefore, that such membranes will not respond to the presence of hormone, but that hormone will accelerate subsequent activation by free p[NH]ppG.

However, in contrast with the findings of Iyengar & Birnbaumer (1979) using liver membranes, Table 4 shows that the presence of GTP in the adenylate cyclase assay of partly preactivated luteal membranes was unable to support additional activation by hormone. Indeed, our data show that GTP decreased the measured adenylate cyclase activity and that hormone accentuated this loss. We believe that this indicates the presence in luteal membranes of guanine-nucleotide-exchange reactions which are activated by hormone. The loss of activity is caused by the substitution of the less effective ligand GTP for p[NH]ppG at the guanine-nucleotide-binding sites. The deactivation that we observed must be less than that actually caused by the exchange of GTP for p[NH]ppG, since it is to be expected that occupation by GTP of the unfilled guanine nucleotide receptors in the partly preactivated membranes would have led to some degree of activation of additional catalytic subunits. Guanine nucleotide exchange (Nielsen *et al.*, 1980; Birnbaumer *et al.*, 1980a) and the hormonal activation of such exchanges (Lad *et al.*, 1980; Svoboda *et al.*, 1978; Cassel & Selinger, 1977b) have been observed in other tissues.

The reason why the small amount of GTP that remains bound to luteal membranes during washing can support a limited synergism with subsequently added hormone (Table 1), whereas the residual p[NH]ppG cannot (Table 4), is not known. Perhaps p[NH]ppG is not strictly analogous to GTP in all of its effects on adenylate cyclase (Martin *et al.*, 1977).

In conclusion, the results in the present paper show: (a) that bovine luteal adenylate cyclase can be persistently activated by human chorionadotropin *in vitro*, supporting our suggestion (Young & Stansfield, 1978a) that the variable activity shown by this enzyme *in vitro* may be due to the retention, during processing of this cyclical tissue *in vitro*, of gonadotropin acquired *in vivo*; (b) that guanine nucleotides may resist removal by washing of the adenylate cyclase complex, with p[NH]ppG being more effectively retained than GTP; (c) that there is indirect evidence for the presence in bovine luteal membranes of GTPase activity and of hormone-stimulated guanine nucleotide exchange reactions that are associated with the adenylate cyclase complex; and (d) that human chorionadotropin may activate bovine luteal adenylate cyclase by accelerating events which occur as a consequence of guanine nucleotide binding rather than by facilitating the binding of guanine nucleotides.

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