

## Prostacyclin Activation of Adenylyl Cyclase in Rabbit Corpus Luteum Membranes: Comparison with 6-Keto Prostaglandin $F_{1\alpha}$ and Prostaglandin $E_1$ <sup>1</sup>

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### ABSTRACT

Rabbit corpus luteum membranes contain an adenylyl cyclase system that in addition to being stimulated by luteinizing hormone (LH), is stimulated by both prostacyclin ( $PGI_2$ ) and prostaglandin  $E_1$  ( $PGE_1$ ). Stimulation of rabbit corpus luteum adenylyl cyclase by  $PGI_2$  and  $PGE_1$  demonstrates an absolute requirement for guanyl nucleotides. In the presence of  $10 \mu M$  GTP,  $10$ – $20 \mu g/ml$   $PGI_2$  and  $PGE_1$  produce a 3.5–4-fold stimulation of adenylyl cyclase. Under identical conditions,  $100 \mu g/ml$  6-keto prostaglandin  $F_{1\alpha}$  (6-keto  $PGF_{1\alpha}$ ), the breakdown product of  $PGI_2$ , produce only a marginal 1.3-fold stimulation of adenylyl cyclase activity. The concentration at which  $PGI_2$  and  $PGE_1$  stimulate rabbit corpus luteum adenylyl cyclase half-maximally is dependent upon the concentration and type of guanyl nucleotide present at the time of assay. In the presence of low ( $0.2 \mu M$ ) GTP, the concentrations of  $PGI_2$  and  $PGE_1$  required for 50% activation were  $0.5$  and  $0.3 \mu g/ml$ , respectively. With high ( $10 \mu M$ ) GTP in the assay,  $1.5$  and  $2.2 \mu g/ml$   $PGI_2$  and  $PGE_1$ , respectively, were required. With  $10 \mu M$  guanylyl 5'-imidodiphosphate (GMP-P(NH)P, a nonhydrolyzable analog of GTP), on the other hand, concentrations required for half-maximal stimulation differed for each prostanoid, being  $0.2 \mu g/ml$  for  $PGI_2$  and  $0.7 \mu g/ml$  for  $PGE_1$ .

During a standard 0–10 min assay in the absence of prostanoid, GTP and GMP-P(NH)P half-maximally activate luteal adenylyl cyclase at  $0.36$  and  $0.55 \mu M$ , respectively. After a 30 min preincubation under adenylyl cyclase assay conditions, GTP and GMP-P(NH)P activated half-maximally at  $0.15$  and  $0.18 \mu M$ , respectively. These values remained unaltered upon addition of either  $PGI_2$  or  $PGE_1$ .

Adenylyl cyclase activities were dependent on Mg ion ( $MgCl_2$  added in excess of ATP plus EDTA). In the absence of guanyl nucleotide, half-maximal activities were obtained at  $12$  mM Mg ion. After addition of  $10 \mu M$  of either GTP or GMP-P(NH)P, this value decreased to  $3.6$  and  $1.1$  mM, respectively. We found that  $PGI_2$  and  $PGE_1$  had no effect on the Mg ion requirements of the system.

It appears that  $PGI_2$  and  $PGE_1$  are acting via the same receptors because their activities are not additive. Further 13–40-fold excess 6-keto  $PGF_{1\alpha}$  does not alter  $PGI_2$  or  $PGE_1$  stimulation of adenylyl cyclase.

### INTRODUCTION

Receptors for  $PGE_1$  (Rao, 1973, 1974) and a  $PGE_1$ -sensitive adenylyl cyclase system (Marsh, 1971; Birnbaumer et al., 1976) have been identified in the corpus luteum. With the availability of prostacyclin ( $PGI_2$ ) and its breakdown product 6-keto prostaglandin  $F_{1\alpha}$  (6-keto  $PGF_{1\alpha}$ ) (Johnson et al., 1976), we determined whether the rabbit corpus luteum

contains a  $PGI_2$ -sensitive adenylyl cyclase and compared the effects of  $PGI_2$ , 6-keto  $PGF_{1\alpha}$  and  $PGE_1$  on rabbit corpus luteum adenylyl cyclase. Further, as guanyl nucleotides play an essential role in the regulation of adenylyl cyclase (for review see Birnbaumer, 1977), we also examined the effects of GTP and its nonhydrolyzable analog, guanylyl 5'-imidodiphosphate [GMP-P(NH)P], on  $PGI_2$  and  $PGE_1$  stimulation of adenylyl cyclase in rabbit corpus luteum membranes.

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### MATERIALS AND METHODS

#### Animals

New Zealand White rabbits (3.5–4.5 kg) were used throughout. Pseudopregnancy was induced by inject-

ing 100 IU hCG (Ayerst) in saline, i.v. The rabbits were sacrificed by cervical dislocation on Day 7 of pseudopregnancy (the day of hCG injection was Day 0). The ovaries were removed and placed in ice cold Krebs-Ringer bicarbonate (KRB), pH 7.4, until dissection of the corpora lutea. The dissected corpora lutea were homogenized and membrane particles were prepared as previously described (Birnbaumer et al., 1976).

#### Adenylyl Cyclase Assay

Adenylyl cyclase activity was determined in a final volume of 50  $\mu$ l containing the indicated concentration of ATP (with  $3-8 \times 10^6$  cpm of [ $\alpha$ - $^{32}$ P]-ATP), 5.0 mM MgCl<sub>2</sub>, 1.0 mM EDTA, 1.0 mM cAMP (with  $\sim 10,000$  cpm [ $^3$ H]-cAMP), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.02 mg/ml myokinase and 25 mM Tris HCl buffer, pH 7.5. Incubations were carried out at 32.5°C for 10 min. The reaction was stopped with 0.1 ml of solution containing 40 mM ATP, 10 mM cAMP and 1% sodium dodecyl-sulfate, followed by immediate boiling for 3.5 min. The [ $^{32}$ P]-cAMP that formed and the [ $^3$ H]-cAMP that was added to monitor recovery were isolated according to the method of Salomon et al. (1974) using Dowex and alumina chromatography with minor modifications described elsewhere (Bockaert et al., 1976).

Protein was determined by the method of Lowry et al. (1951), using crystalline bovine serum albumin (fraction V) as standard.

All experiments were performed a minimum of 3 times and representative data have been presented.

#### Materials

The ATP, GTP, cAMP, Trizma base, EDTA and myokinase (1925 units/mg) were purchased from Sigma Chemical Co; GMP-P(NH)P was purchased from Boehringer Mannheim. Creatine phosphate and creatine phosphokinase (214 units/mg) were purchased from Calbiochem. The Core Laboratory on Cyclic Nucleotide Research, Center for Population Research and Studies on Reproductive Biology, Baylor College of Medicine supplied the [ $\alpha$ - $^{32}$ P]-ATP, synthesized as described by Birnbaumer et al. (1979). The PGI<sub>2</sub>, 6-keto PGF<sub>1 $\alpha$</sub>  and PGE<sub>1</sub> were gifts from Upjohn (Dr. J. E. Pike). The hCG used to induce pseudopregnancy was a gift from Ayerst (Dr. J. B. Jewell). Stock solutions of PGI<sub>2</sub>, 6-keto PGF<sub>1 $\alpha$</sub>  and PGE<sub>1</sub> were prepared in 10 mM Tris base at a concentration of 1 mg/ml and stored in small aliquots at -70°C until used. On the day of use, stock solutions were appropriately diluted immediately before assay.

## RESULTS

Due to the inherent instability of PGI<sub>2</sub>, we compared the time course of cAMP accumulation by rabbit corpus luteum membranes in the presence of PGI<sub>2</sub> and PGE<sub>1</sub> (Fig. 1). Basal adenylyl cyclase activity was linear over the 20 min time period examined. In the presence of 10  $\mu$ M GTP, cAMP accumulation was not

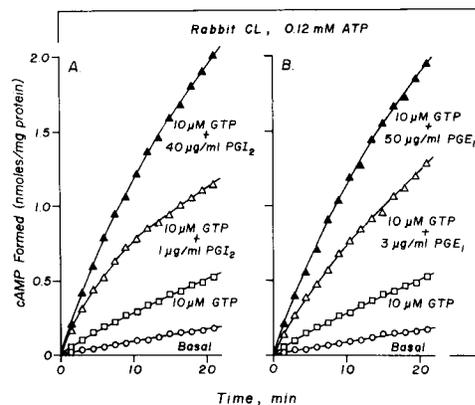


FIG. 1. Time course of cAMP accumulation by rabbit corpus luteum membranes in the presence or absence of A) PGI<sub>2</sub> and B) PGE<sub>1</sub>. Rabbit corpus luteum membranes were incubated as indicated under Materials and Methods in the absence of guanyl nucleotide and prostanoid (basal,  $\circ$ ), in the presence of 10  $\mu$ M GTP ( $\square$ ), 10  $\mu$ M GTP plus half-maximally stimulating concentrations of prostanoid ( $\Delta$ ) or 10  $\mu$ M GTP plus saturating concentrations of prostanoid ( $\blacktriangle$ ). Each curve was obtained from a single incubation from which 50  $\mu$ l aliquots were withdrawn at 1.5 min intervals and processed for cAMP isolation as indicated in Materials and Methods. ATP concentration was 0.12 mM and membrane protein was 60  $\mu$ g/ml.

linear. Addition of saturating (40 and 50  $\mu$ g/ml) or half-maximally stimulating concentrations (1 and 3  $\mu$ g/ml) of PGI<sub>2</sub> or PGE<sub>1</sub> to the assay resulted in a nonlinear accumulation of cAMP. Adenylyl cyclase activity decreased considerably in the presence of 10  $\mu$ M GTP plus 1  $\mu$ g/ml PGI<sub>2</sub> after 10 min of incubation (Fig. 1A); therefore, assays were run routinely for 10 min.

#### Effect of Guanyl Nucleotides

In the absence of any guanyl nucleotide, PGI<sub>2</sub> and PGE<sub>1</sub> marginally stimulated rabbit corpus luteum adenylyl cyclase activity (Table 1, Figs. 2, 3), while 6-keto PGF<sub>1 $\alpha$</sub>  did not stimulate adenylyl cyclase activity. In the presence of 10  $\mu$ M GTP, saturating concentrations (10–20  $\mu$ g/ml) of either PGI<sub>2</sub> or PGE<sub>1</sub> produced a 3.5–4-fold stimulation of cyclase activity, while in the presence of the non-hydrolyzable analog of GTP, GMP-P(NH)P, PGI<sub>2</sub> and PGE<sub>1</sub> produced only a 1.6–1.7-fold stimulation of adenylyl cyclase activity over that obtained with 10  $\mu$ M GMP-P(NH)P alone. In contrast, 100  $\mu$ g/ml 6-keto PGF<sub>1 $\alpha$</sub>  produced only a 1.3-fold stimulation of adenylyl cyclase

TABLE 1. Effect of PGI<sub>2</sub> and 6-keto PGF<sub>1α</sub> on adenylyl cyclase activity in rabbit corpus luteum membranes.

Additions to assays	Adenylyl cyclase activities <sup>a</sup> in the presence of		
	...	10 μM GTP	10 μM GMP-P(NH)P
		pmoles/min/mg (-fold)	
None	7.9 ± 0.02	20.7 ± 1.1	94.1 ± 3.7
PGI <sub>2</sub>			
1 μg/ml	12.7 ± 0.6 (1.61)	58.1 ± 1.0 (2.81)	157.2 ± 3.7 (1.67)
100 μg/ml	16.8 ± 0.5 (2.13)	89.1 ± 1.8 (4.30)	172.7 ± 7.1 (1.84)
6-keto PGF <sub>1α</sub>			
1 μg/ml	9.0 ± 0.5 (1.14)	21.2 ± 0.7 (1.02)	98.5 ± 3.7 (1.05)
100 μg/ml	10.6 ± 3.0 (1.34)	27.2 ± 0.8 (1.31)	92.0 ± 3.9 (0.98)

<sup>a</sup> Assays were performed for 10 min at 32.5°C as described under Materials and Methods. Prostanoids dissolved in 10 mM Tris base were added in 10 μl. ATP was 0.13 mM and membrane protein was 3.2 μg/assay. Values represent means ± SD of triplicate determinations. Fold-stimulations were calculated by dividing each experimental value by its appropriate control value.

activity in the presence of 10 μM GTP and did not significantly alter cyclase activity in the presence of 10 μM GMP-P(NH)P (Table 1).

The presence of GTP and GMP-P(NH)P

altered the apparent affinity for PGI<sub>2</sub> and PGE<sub>1</sub> to activate adenylyl cyclase activity (Figs. 2, 3). At 0.2 μM GTP, the effective concentrations of PGI<sub>2</sub> and PGE<sub>1</sub> required to

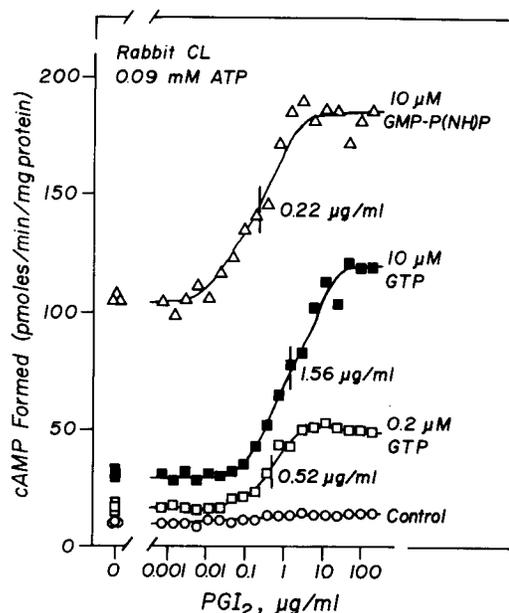


FIG. 2. Effect of guanyl nucleotides on concentration effect curves for PGI<sub>2</sub>. Rabbit corpus luteum membranes were incubated as indicated under Materials and Methods in the absence of any guanyl nucleotide (control, ○), in the presence of 0.2 μM GTP (□), 10 μM GTP (■) or 10 μM GMP-P(NH)P (△). ATP concentration was 0.09 mM and membrane protein was 3.2 μg/assay.

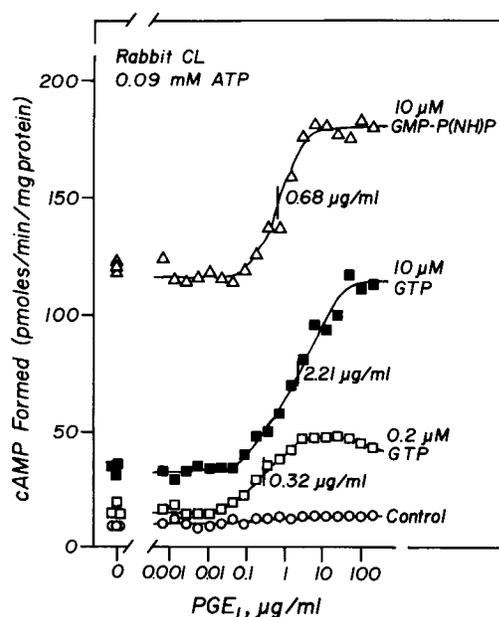


FIG. 3. Effect of guanyl nucleotides on concentration effect curves for PGE<sub>1</sub>. Rabbit corpus luteum membranes were incubated as indicated under Materials and Methods in the absence of any guanyl nucleotide (control, ○), in the presence of 0.2 μM GTP (□), 10 μM GTP (■) or 10 μM GMP-P(NH)P (△). ATP concentration was 0.09 mM and membrane protein was 3.2 μg/assay.

TABLE 2. Characteristics of guanyl nucleotide activation of adenylyl cyclase activity in rabbit corpus luteum membranes in the presence and absence of  $\text{PGI}_2$  and  $\text{PGE}_1$ .<sup>a</sup>

	Additions to assay	
	None	10 $\mu\text{g/ml}$ $\text{PGI}_2$
Adenylyl cyclase activities (pmoles/min/mg)		
Basal <sup>c</sup>	11.9 $\pm$ 0.9	14.0 $\pm$ 0.9
GTP (10–100 $\mu\text{M}$ ) <sup>d</sup>	37.1 $\pm$ 2.8	121.9 $\pm$ 8.0
GMP-P(NH)P (10–100 $\mu\text{M}$ ) <sup>d</sup>	139.1 $\pm$ 5.2	209.3 $\pm$ 7.1
EC-50 (95% confidence limits) $\mu\text{M}$ <sup>e</sup>	0.36 (0.27–0.48)	0.21 (0.16–0.28)
GTP	0.55 (0.46–0.70)	0.48 (0.39–0.58)
GMP-P(NH)P		0.19 (0.15–0.25)
		0.70 (0.52–1.00)
Adenylyl cyclase activities (pmoles/min/mg)		
Basal <sup>c</sup>	8.3 $\pm$ 0.4	11.0 $\pm$ 0.4
GTP (10–100 $\mu\text{M}$ ) <sup>d</sup>	15.3 $\pm$ 0.8	25.7 $\pm$ 1.4
GMP-P(NH)P (10–100 $\mu\text{M}$ ) <sup>d</sup>	144.7 $\pm$ 5.6	142.3 $\pm$ 7.6
EC-50 (95% confidence limits) $\mu\text{M}$ <sup>e</sup>	0.15 (0.10–0.29)	0.18 (0.14–0.24)
GTP	0.18 (0.14–0.22)	0.09 (0.07–0.13)
GMP-P(NH)P		0.11 (0.06–0.19)
		0.11 (0.08–0.15)

<sup>a</sup>Concentration effect curves were constructed from experiments in which the concentration of GTP or GMP-P(NH)P was varied from  $3.8 \times 10^{-9}$  to  $10^{-4}$  M at 2-fold intervals for a total of 19 concentrations in the absence or presence of the indicated concentration of  $\text{PGI}_2$  or  $\text{PGE}_1$ .

<sup>b</sup>Adenylyl cyclase was determined as described under Materials and Methods except that ATP was 0.10 mM and membrane protein was 3.2  $\mu\text{g/assay}$ .

<sup>c</sup>Activity determined in the absence of guanyl nucleotide. Values represent means  $\pm$  SD of sextuplet determinations.

<sup>d</sup>Activity determined in the presence of the indicated concentrations of guanyl nucleotide. Values represent means  $\pm$  SD of triplicate determinations.

<sup>e</sup>Estimates of the intercepts of Hill plots with the abscissa were obtained by calculating linear regressions via the least square method. Values represent the  $\mu\text{M}$  concentrations of guanyl nucleotide necessary to activate adenylyl cyclase to 50% of maximum.

<sup>f</sup>Membrane particles from rabbit corpora lutea were preincubated for 30 min at  $32.5^\circ\text{C}$  in 40  $\mu\text{l}$  of medium 1.25 times the concentration of ingredients indicated under Materials and Methods without the [ $\alpha$ - $^{32}\text{P}$ ]-ATP or [ $^3\text{H}$ ]-cAMP. At 30 min, 10  $\mu\text{l}$  of solution containing [ $\alpha$ - $^{32}\text{P}$ ]-ATP and [ $^3\text{H}$ ]-cAMP were added to the assay. The incubation was continued for another 10 min and terminated. The final ATP concentration was 0.10 mM and the membrane protein was 3.2  $\mu\text{g/ml}$ .

half-maximally stimulate adenylyl cyclase ( $EC_{50}$ 's) were 0.52 and 0.32  $\mu\text{g}/\text{ml}$ , respectively. Increasing the GTP concentration to 10  $\mu\text{M}$  increased the  $EC_{50}$ 's of  $\text{PGI}_2$  and  $\text{PGE}_1$  to 1.56 and 2.21  $\mu\text{g}/\text{ml}$ , respectively. In the presence of 10  $\mu\text{M}$  GMP-P(NH)P, half-maximal stimulation by  $\text{PGI}_2$  and  $\text{PGE}_1$  was at 0.22 and 0.68  $\mu\text{g}/\text{ml}$ , respectively.

When adenylyl cyclase activity was determined in a standard 0–10 min assay, 10  $\mu\text{g}/\text{ml}$  of  $\text{PGI}_2$  or  $\text{PGE}_1$  stimulated adenylyl cyclase activity in presence of GTP or GMP-P(NH)P without significantly altering the apparent affinity for either guanyl nucleotide, GTP stimulating half-maximally at 0.36  $\mu\text{M}$  and GMP-P(NH)P doing so at 0.56  $\mu\text{M}$  (Table 2). However, when adenylyl cyclase activity was determined after a 30 min preincubation under adenylyl cyclase assay conditions, GTP stimulated cyclase activity half-maximally at 0.15  $\mu\text{M}$  and GMP-P(NH)P did so at 0.18  $\mu\text{M}$  (Table 2). After a 30 min preincubation in the presence of 10  $\mu\text{M}$  GMP-P(NH)P (30–40 min assay), neither  $\text{PGI}_2$  nor  $\text{PGE}_1$  had any further effect than that obtained with the nucleotide analog alone. On the other hand, if the 30 min

preincubation was carried out in the presence of GTP instead of GMP-P(NH)P, activity due to GTP alone was much lower than that obtained with GMP-P(NH)P and further stimulation by  $\text{PGI}_2$  and  $\text{PGE}_1$  was obtained (Table 2).

#### Effect of Mg Ion

In the absence of any prostanoid, guanyl nucleotides increased the apparent affinity of the adenylyl cyclase system for Mg ion from 12 mM  $\text{MgCl}_2$  in excess of ATP and EDTA in the absence of guanyl nucleotide to 3.6 mM and 1.1 mM  $\text{MgCl}_2$  in the presence of GTP and GMP-P(NH)P, respectively (inset Fig. 4). Neither  $\text{PGI}_2$ ,  $\text{PGE}_1$  nor 6-keto  $\text{PGF}_{1\alpha}$  significantly altered the apparent affinity of the rabbit corpus luteum adenylyl cyclase system for Mg ion. In the presence of guanyl nucleotide,  $\text{PGI}_2$  and  $\text{PGE}_1$ , but not 6-keto  $\text{PGF}_{1\alpha}$ , stimulated adenylyl cyclase activity over the entire  $\text{MgCl}_2$  concentration range tested (0.05–100 mM in excess of ATP and EDTA). Maximal stimulation of adenylyl cyclase activity occurred at 6.2 mM  $\text{MgCl}_2$  (in excess of ATP and EDTA) in the presence of guanyl nucleotide and  $\text{PGI}_2$  or  $\text{PGE}_1$  (Fig. 4).

#### Other Characteristics of $\text{PGI}_2$ -Sensitive Adenylyl Cyclase

To determine whether  $\text{PGI}_2$  and  $\text{PGE}_1$  activate adenylyl cyclase through the same receptor, rabbit corpus luteum membranes were incubated as indicated in Table 3. When adenylyl cyclase activity was determined in the presence of half-maximally stimulating concentrations of  $\text{PGI}_2$  plus  $\text{PGE}_1$ , there was a slight increase in activity compared with the activity obtained in the presence of  $\text{PGI}_2$  or  $\text{PGE}_1$  alone (Table 3). This partial additive effect was not seen, however, when saturating concentrations of  $\text{PGI}_2$  plus  $\text{PGE}_1$  were used. Further, 6-keto  $\text{PGF}_{1\alpha}$ , the breakdown product of  $\text{PGI}_2$ , did not affect the ability of either  $\text{PGE}_1$  (Table 3) or  $\text{PGI}_2$  (Table 4) to stimulate corpus luteum adenylyl cyclase when present in a 13- or 40-fold excess, respectively.

#### DISCUSSION

The rabbit corpus luteum adenylyl cyclase system is stimulated by LH, epinephrine and  $\text{PGE}_1$  (Birnbaumer et al., 1976; Hunzicker-Dunn and Birnbaumer, 1976). The results reported here indicate that this adenylyl cyclase

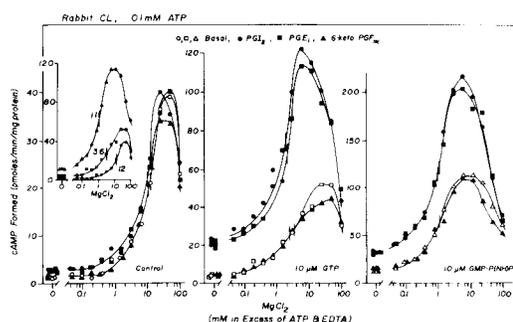


FIG. 4. Effect of guanyl nucleotides,  $\text{PGI}_2$ ,  $\text{PGE}_1$  and 6-keto  $\text{PGF}_{1\alpha}$  on concentration effect curves for Mg ion. Rabbit corpus luteum membranes were incubated as indicated under Materials and Methods except at the indicated  $\text{MgCl}_2$  concentration in excess of ATP and EDTA. Therefore, the "0"  $\text{MgCl}_2$  point of the abscissa contains 1.1 mM  $\text{MgCl}_2$ . Membrane particles were incubated in the absence or presence of the indicated guanyl nucleotide, without ( $\circ, \square, \triangle$ ) or with 10  $\mu\text{g}/\text{ml}$   $\text{PGI}_2$  ( $\bullet$ ), 10  $\mu\text{g}/\text{ml}$   $\text{PGE}_1$  ( $\blacksquare$ ) or 10  $\mu\text{g}/\text{ml}$  6-keto  $\text{PGF}_{1\alpha}$  ( $\blacktriangle$ ). Inset: Rabbit corpus luteum membranes incubated in the absence ( $\circ$ ) or presence of 10  $\mu\text{M}$  GTP ( $\square$ ) or 10  $\mu\text{M}$  GMP-P(NH)P ( $\triangle$ ). Vertical lines represent the  $EC_{50}$  (concentrations required for 50% of maximal effect) of Mg activation of adenylyl cyclase activity. The ATP concentration was 0.10 mM and the membrane protein concentration was 3.2  $\mu\text{g}/\text{assay}$ .

TABLE 3. Effect of PGI<sub>2</sub> and 6-keto PGF<sub>1α</sub> on the ability of PGE<sub>1</sub> to stimulate adenylyl cyclase in rabbit corpus luteum membranes.

Addition to assay	Adenylyl cyclase activities <sup>a</sup> in the presence of		
	...	3 μg/ml/PGE <sub>1</sub>	50 μg/ml PGE <sub>1</sub>
	pmoles/min/mg (-fold)		
None	18.5 ± 1.4	48.0 ± 1.4 (2.59)	68.4 ± 1.4 (3.70)
PGI <sub>2</sub>			
1 μg/ml	42.3 ± 2.3 (2.29)	52.9 ± 3.6 (2.86)	65.6 ± 4.9 (3.55)
40 μg/ml	69.8 ± 2.0 (3.77)	67.5 ± 3.7 (3.67)	64.9 ± 0.9 (3.51)
6-keto PGF <sub>1α</sub>			
1 μg/ml	20.2 ± 1.7 (1.09)	49.2 ± 6.0 (2.66)	70.6 ± 5.2 (3.82)
40 μg/ml	23.0 ± 1.9 (1.24)	50.5 ± 4.0 (2.73)	71.8 ± 1.6 (3.88)

<sup>a</sup>All assays were performed in the presence of 10 μM GTP. ATP was 0.14 mM and membrane protein was 4.0 μg/assay. For the rest of the conditions, see Materials and Methods. Values represent means ± SD of triplicate determinations. Fold-stimulations were determined by dividing each experimental value by the value in the absence of prostanoid.

system is also stimulated by the prostanoid, PGI<sub>2</sub> (Figs. 1, 2, Table 1). The effects of prostanoids on luteal adenylyl cyclase are dependent on both Mg ion and nucleotides.

The presence of an allosteric regulatory site for Mg on adenylyl cyclase was first suggested from studies on adipocyte adenylyl cyclase (Birnbaumer et al., 1969). The findings of the present study in which GTP and GMP-P(NH)P increased the apparent affinity for Mg ion to stimulate the rabbit corpus luteum adenylyl cyclase (Fig. 4) would support such a suggestion. Similar increases in the apparent affinity of the allosteric site for Mg ion in the presence of guanyl nucleotides have been reported for cardiac (Alvarez and Bruno, 1977) and hepatic (Londos and Preston, 1977) adenylyl cyclase.

Unlike the cardiac (Alvarez and Bruno, 1977) and hepatic adenylyl cyclase (Londos and Preston, 1977) in which the presence of hormone further increases the apparent affinity of the Mg ion allosteric site neither PGI<sub>2</sub> nor PGE<sub>1</sub> increased the apparent affinity of the Mg site in the rabbit corpus luteum adenylyl cyclase (Fig. 4). Although adenylyl cyclase appears to have an allosteric regulatory site for Mg, the exact role for Mg in the regulation of adenylyl cyclase remains to be determined.

Unlike LH and hCG stimulation of luteal adenylyl cyclase, which requires high (mM) concentrations of ATP (Birnbaumer and Yang, 1974), stimulation by PGI<sub>2</sub> and PGE<sub>1</sub> of corpus luteum adenylyl cyclase demonstrates an absolute requirement for guanyl nucleotides

TABLE 4. Effect of 6-keto PGF<sub>1α</sub> on the ability of PGI<sub>2</sub> to stimulate adenylyl cyclase in rabbit corpus luteum membranes.

Addition to assay	Adenylyl cyclase activities <sup>a</sup> in the presence of		
	...	1 μg/ml PGI <sub>2</sub>	40 μg/ml PGI <sub>2</sub>
	pmoles/min/mg (-fold)		
None	25.8 ± 1.2	67.7 ± 1.0 (2.47)	98.8 ± 1.5 (3.83)
6-keto PGF <sub>1α</sub>			
1 μg/ml	23.5 ± 1.8 (0.91)	61.4 ± 3.5 (2.38)	96.4 ± 4.6 (3.74)
40 μg/ml	29.7 ± 0.4 (1.15)	55.6 ± 10.4 (2.16)	91.2 ± 2.2 (3.53)

<sup>a</sup>All assays were performed in the presence of 10 μM GTP. ATP was 0.10 mM and membrane protein was 3.2 μg/assay. For the rest of the conditions see Materials and Methods. Values represent means ± SD of triplicate determinations. Fold-stimulations were determined by dividing each experimental value by the value in the absence of prostanoid.

(Table 1, Figs. 2, 3). Nucleotides play an essential role in the regulation of the adenylyl cyclase system and affect the system at 3 functional levels: 1) they regulate the catalytic moiety (Salomon et al., 1975; Birnbaumer and Swartz, 1977); 2) they promote coupling of the hormone-receptor complex to adenylyl cyclase (Birnbaumer and Yang, 1974; Iyengar et al., 1979); and 3) they affect the binding of stimulatory hormone to its receptor in all systems thus far tested (Rodbell et al., 1971; Ross et al., 1977; Lefkowitz et al., 1977). It appears that responsiveness to prostanoids in the luteal cyclase system is regulated solely by guanyl nucleotides, as both basal activities and the receptor-mediated stimulation of activity are fully dependent on addition of guanyl nucleotide (Table 1, Figs. 2, 3). Although the effects of GTP or GMP-P(NH)P on the interaction of the luteal receptor with prostanoids (PGI<sub>2</sub> or PGE<sub>1</sub>) have not yet been studied, the findings by Lefkowitz et al. (1977) that PGE<sub>1</sub> binding to frog erythrocyte membrane receptor is under regulation by guanyl nucleotides, suggest that luteal prostanoid receptor binding is probably also regulated by guanyl nucleotides. The "all G" regulation of prostanoid action on luteal cyclase is in distinct contrast to the nucleotide regulation of gonadotropin action on the same adenylyl cyclase. Thus, while the basic adenylyl cyclase (assessed in the absence of hormone) is still under regulation of GTP (or analogs of GTP), coupling of LH (and possibly also binding) depends on ATP (Birnbaumer and Yang, 1974; Birnbaumer et al., 1976). The observations presented here indicate that both maximal stimulations and the positions of the dose-response curves for PGI<sub>2</sub> and PGE<sub>1</sub> vary with the concentration and type of guanyl nucleotide [GTP or GMP-P(NH)P] added. This is undoubtedly the result of the various individual effects of guanyl nucleotides on the system. An understanding of the details of these effects will require further studies, especially on PGI<sub>2</sub> and PGE<sub>1</sub> binding, which would provide information on actual affinity changes of receptor for prostanoid due to guanyl nucleotide addition.

Based on the findings of the present study, it is clear that the corpus luteum contains an adenylyl cyclase system that responds to both PGI<sub>2</sub> and PGE<sub>1</sub>. However, even though the corpus luteum contains prostaglandin receptors (Rao, 1973, 1974) and a prostaglandin-sensitive adenylyl cyclase system, the exact physiological

significance of PGI<sub>2</sub> and PGE<sub>1</sub> in the regulation of corpus luteum function remains to be determined.

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