Inhibition of *Xenopus* Oocyte Adenylate Cyclase by Progesterone and 2',5'-Dideoxyadenosine Is Associated with Slowing of Guanine Nucleotide Exchange*

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Adenylate cyclase activity in *Xenopus* oocyte membranes measured in the presence of guanyl-5'-yl imidodiphosphate and 1.5 mM Mn²⁺ was maximally inhibited to 57% of control by progesterone and to 89% by the P site agonists, 2',5'-dideoxyadenosine and 9-β-D-arabinofuranosyladenine. Inhibition by saturating concentrations of 2',5'-dideoxyadenosine and progesterone was not additive, suggesting that inhibition of oocyte adenylate cyclase by progesterone may share a common mechanism with P site inhibition. Kinetic analysis of the effect of progesterone and 2',5'-dideoxyadenosine on the hysteretic activation of adenylate cyclase by guanyl-5'-yl imidodiphosphate indicates that both hormones exert their effects, at least in part, by lengthening the lag in cAMP formation, and this hysteretic effect is inversely proportional to the concentration of guanine nucleotide in the incubation mixture. Direct measurement of [³H]guanine nucleotide release from oocyte membranes preloaded with [³H]GTP demonstrated that treatment with either progesterone or 2',5'-dideoxyadenosine slows the rate of nucleotide exchange. Inhibition of oocyte adenylate cyclase by 2',5'-dideoxyadenosine was potentiated by millimolar concentrations of Mn²⁺, but inhibition by progesterone was abolished. The results indicate that inhibition of *Xenopus* oocyte adenylate cyclase by progesterone has features in common with both P site and receptor-mediated inhibitory mechanisms.

Ripe *Xenopus laevis* oocytes as isolated from the ovary are physiologically arrested in first meiotic prophase. Release from this arrested state occurs in response to administration of progesterone, leading to completion of the first meiotic division and arrest at second meiotic metaphase as an unfertilized egg. This process is termed oocyte maturation. A variety of experimental evidence indicates that steroids interact initially with the oocyte surface (see Ref. 1 and 2 for review) rather than with conventional cytosolic receptors. Studies in this and other laboratories (3-5) have described the ability of progesterone to inhibit adenylate cyclase in the oocyte plasma membrane, and this inhibition has been shown to be physiologically significant, since microinjection of cAMP-dependent protein kinase subunits into the oocyte demonstrated that the decrease in cAMP following administration of progesterone to the oocyte is a necessary and sufficient trigger for oocyte maturation (6). Inhibition of adenylate cyclase by progesterone appears to involve G/F, since basal activity as well as activity stimulated by cholera toxin, fluoride, or Gpp(NH)p can be inhibited, while activity stimulated by 10 mM Mn²⁺ is unaffected (3). Recently, the presence of a steroid receptor on the oocyte plasma membrane was demonstrated by photoaffinity labeling, and it was shown that occupancy of the receptor was correlated with both the induction of oocyte maturation and inhibition of adenylate cyclase activity (7). Thus, inhibition of adenylate cyclase by steroids in the oocyte appears to be receptor-mediated.

Several hormones and neurotransmitters have been shown to inhibit adenylate cyclase in various other cell types (see Refs. 8 and 9 for review), with adenosine being one of the most extensively characterized. In general, GTP is required for inhibition, and the nonhydrolyzable analog, Gpp(NH)p, will not substitute for GTP, indicating that GTP hydrolysis is required. Furthermore, receptor-mediated inhibition can be reversed by Mn²⁺.

Two distinct classes of adenosine action on adenylate cyclase have been described on the basis of functional and structural criteria for agonists (9-14). One class, the "R site", binds adenosine analogs that contain unmodified 2'- and 3'-hydroxyl groups on the ribose moiety of adenosine (13), while permitting various substitutions on the purine ring. R site effects require GTP hydrolysis, are antagonized by methylxanthines, and can be divided into subtypes based on their stimulatory or inhibitory actions on adenylate cyclase and the rank order of potency of agonists (14). The receptors for these subtypes bind adenosine and various of its analogs in nanomolar to low micromolar concentrations (14).

The second type of adenosine action has been designated the "P site" action (13), since effective analogs require an intact purine ring but allow modifications in the ribose ring of the nucleoside. Two of the most potent P site analogs are 2',5'-DDA and 9-β-D-AFA. P site agonists are thought to interact with the cytoplasmic side of the plasmalemma (9, 10), and adenosine effectively interacts with the P site in micromolar to millimolar concentrations (9, 13). In contrast to R site inhibition, P site inhibition is not affected by methylxanthines but is potentiated by divalent cations, especially Mn²⁺ (9, 11, 13). In contrast to receptor-mediated systems, the inhibition of adenylate cyclase by P site agonists does not require GTP hydrolysis, since Gpp(NH)p-stimulated

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1 The abbreviations used: G/F, guanine nucleotide regulatory subunit of adenylate cyclase; Gpp(NH)p, guanyl-5'-yl imidodiphosphate; 2',5'-DDA, 2',5'-dideoxyadenosine; 9-β-D-AFA, 9-β-D-arabinofuranosyladenine; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N' -tetraacetic acid; IC₅₀, concentration of inhibitory drug that causes 50% inhibition.
adenylate cyclase can be inhibited (11). However, inhibition by P site agonists does require the presence of G/F since no inhibition is seen in the soluble testicular enzyme (15) or in membranes from cyp variants of S49 lymphoma cells (9) that lack functional G/F protein (16, 17). Moreover, cyp membranes acquire the capacity for P site inhibition upon reconstitution with exogenous G/F (9). There is some controversy at present about whether solubilized preparations of adenylate cyclase are sensitive to P site inhibition (15, 18). Different results may be obtained depending on whether the solubilization is carried out in the presence of guanine nucleotides. It has been suggested that P site inhibition is deficient in cyclase solubilized in the absence of guanine nucleotide, leading to “uncoupling” of G/F and the catalytic subunit of cyclase (15). Consistent with this idea is the observation that P site inhibition in solubilized preparations of bovine brain extracted without guanine nucleotides is increased upon addition of G/F, particularly when G/F contains bound nucleotide (18).

It seems clear that inhibition of adenylate cyclase by progesterone in the oocyte plasma membrane and P site agonist inhibition of adenylate cyclase in other systems share a number of common features, especially with respect to a common involvement of the G/F protein and the ability to inhibit Gpp(NH)p-stimulated activity. In this paper, inhibition of adenylate cyclase by P site agonists is examined in oocyte plasma membranes and compared with inhibition by progesterone. The results suggest that the mechanism by which progesterone inhibits the oocyte adenylate cyclase is a hybrid of P site inhibition defined for adenosine agonists and receptor-mediated inhibition.

**EXPERIMENTAL PROCEDURES**

**Plasma Membrane Preparation**—Sexually mature *X. laevis* were primed, oocytes were isolated, and membranes were prepared as previously described (3). Each figure represents experimental results using oocytes from a separate animal, and each sample routinely contained plasma membranes from 10 oocytes, corresponding to 50 µg of total protein, as measured by the method of Lowry et al. (19), using bovine serum albumin as a standard.

**Adenylate Cyclase Assay**—Membrane-associated adenylate cyclase activity was determined by measuring the enzymatic conversion of [3H]ATP to [3H]cAMP, as previously described (3). As indicated in the figure legends, various concentrations of 2',5'-DDA (ICN Chemical and Radiosotope Division, Irvine, CA), 9-β-D-AFA (P-L Biochemicals), or progesterone (Sigma) were included in the assay mixture. Product recovery was measured by column chromatography of a known amount of [3H]cAMP, and all values shown have been corrected for recovery. The statistical significance of experimental data was determined using the Student's t test.

**Guanine Nucleotide Exchange**—Guanine nucleotide exchange was measured by the method of Pike and Lefkowitz (20). For each experiment of this type, the membrane sample was prepared by manual isolation of 320 oocyte membranes as previously described (3) and homogenized in 10 mM NaCl, 10 mM Hepes, pH 7.4, with 20 manual strokes of a glass/glass homogenizer. The sample was then centrifuged (15,000 × g) in a Fisher microcentrifuge for 1 min and resuspended in a 1-mL volume of 0.2 M adenyl-5'-yl imidodiphosphate, 0.1 mM ATP, 3 mM creatine phosphate, 18.8 units/mL of creatine phosphokinase, 2 mM β-mercaptoethanol, 0.1 mM EGTA, 1 mM MgCl₂, 30 mM Tris-Cl, pH 7.4, and warmed for 5 min at 37 °C. A 0.25-mL volume of [3H]GTP (New England Nuclear; 37 Ci/mmol; final concentration, 1 × 10⁻³ M) was added to the membrane suspension, and the mixture was incubated at 37 °C for 20 min to allow binding equilibrium, as determined by previous time course experiments (data not shown). The loaded membrane sample was then microcentrifuged for 1 min, and the resulting membrane pellet was washed three times with ice-cold buffer containing 25 mM MgCl₂, 0.1 mM ATP, 0.75 mM MgCl₂, 30 mM Tris-Cl, pH 7.4, and resuspended in 750 µL of the wash buffer without GTP. A few strokes of homogenization were used to get a uniform suspension of membrane.

Release of guanine nucleotide was measured by addition of 20 µL of the final membrane suspension (1.1 and 0.8 mg of protein/mL in Fig. 6, A and B, respectively) to 350 µL of release buffer containing 25 mM guanosine 5'-β-thiotriphosphate (Boehringer Mannheim), 2 mM β-mercaptoethanol, 0.1 mM EGTA, 12.5 mM MgCl₂, 37.5 mM Tris-HCl, pH 7.4. After various times of incubation at room temperature, the radioactivity released from triplicate samples was measured by liquid scintillation spectroscopy after rapid centrifugation of each sample and removal of a 100-µL sample of the supernatant. All results have been normalized by subtraction of the amount of radioactivity nonspecifically released at zero time, as measured by rapid centrifugation and sampling of membrane preparations in ice-cold release buffer. The background levels of nonspecifically released radioactivity were constant with increasing times of incubation on ice and were approximately 125,000 and 200,000 cpm/mg of protein for Fig. 6, A and B, respectively.

**RESULTS**

The abilities of the P site agonists, 2',5'-DDA and 9-β-D-AFA, to inhibit Gpp(NH)p-stimulated adenylate cyclase in the oocyte plasma membrane are demonstrated in Fig. 1. In the presence of 1.5 mM Mn²⁺, both analogs maximally inhibit 70-80% of the adenylate cyclase activity, and 2',5'-DDA is approximately 10-fold more potent than 9-β-D-AFA, with IC₅₀ values of approximately 5 and 50 µM, respectively (Fig. 1). This order of potencies is similar to that reported for the liver adenylate cyclase system (13). The more potent P site agonist, 2',5'-DDA, was used in subsequent experiments to characterize P site inhibition in the oocyte membrane.

Of the diverse mechanisms that may account for differences in the action of adenine in mammalian systems, the lack of antagonism by benzopyrinethiones and its potentiation by divalent cations, especially Mn²⁺ (9, 12). The methylxanthine did slightly elevate maximum enzyme activity, but it had no effect upon the dose-response curve for the inhibition of oocyte adenylate cyclase by 2',5'-DDA (Fig. 2A). In the experiment illustrated, enzyme activity was maximally inhibited by 70%, and the apparent IC₅₀ for inhibition by 2',5'-DDA was approximately 10 µM, irrespective of the presence or absence of methylxanthine. Fig. 2B shows potentiation of P site inhibition by Mn²⁺. In the absence of Mn²⁺, 2',5'-DDA was capable of inhibiting 30% of control activity, while in the presence of 1.5 mM Mn²⁺, there was a 3-fold increase in the level of control activity, and 2',5'-DDA was capable of inhibiting 70% of the activity with no apparent change in IC₅₀. These results indicate that P site action in the *Xenopus oocyte* membrane adenylate cyclase is similar to that described in mammalian systems.

The level to which the inhibition of oocyte adenylate cyclase by 2',5'-DDA is potentiated by Mn²⁺ is proportional to the concentration of Mn²⁺ in the assay mixture, as shown in Fig. 3. In the absence of Mn²⁺, 2',5'-DDA inhibited 30% of control activity.
activity; at 1.5 mM Mn++, the level of inhibition increased to 75% and enzyme activity was inhibited to 90% of control in the presence of 10 mM Mn++. In contrast, increasing concentrations of Mn++ diminished the level of inhibition in response to progesterone. Without Mn++, progesterone inhibited 60% of control activity; while in the presence of 10 mM Mn++, progesterone inhibition was abolished (Fig. 3). The ability of manganese to prevent progesterone inhibition is consistent with the evidence that a steroid receptor is coupled to adenylate cyclase on the oocyte membrane (7). This effect is consistent with the ability of Mn++ to abolish adenylate cyclase inhibition in other receptor-mediated systems (21, 22).

Since progesterone and the P site analogs are the only known hormones that inhibit adenylate cyclase activity stimulated by Gpp(NH)p, it was important to determine if progesterone inhibition might be acting through the same mechanism as P site inhibition. As one approach to this question, additivity experiments were performed, and representative results are shown in Table 1. The additivity of progesterone inhibition with that of the P site agonist, 2',5'-DDA, was examined in both the presence and absence of 1.5 mM Mn++. Using drug concentrations that each elicit maximum levels of inhibition of adenylate cyclase activity in the presence of 1.5 mM Mn++, 2',5'-DDA caused 78% inhibition and 9-β-D-AFA caused 51% inhibition of enzyme activity, while progesterone caused 38% inhibition (Table 1). In order to determine that the P site was indeed saturated under these conditions, 2',5'-DDA (0.32 mM) and 9-β-D-AFA (3.2 mM) were added together, and this combined treatment resulted in no further inhibition of enzyme activity. In a similar manner, the level of inhibition seen with 2',5'-DDA plus progesterone was not significantly different than the inhibition seen with the more powerful agonist, 2',5'-DDA, alone (Table 1). When Mn++ was absent from the assay mixture, the level of inhibition measured in the presence of the P site agonists was less than that measured in the presence of added Mn++, although in this experiment the cation effect was not as great as that seen in Figs. 2 and 3. In contrast, the level of inhibition seen with progesterone was greater in the absence of Mn++ (Table 1 and Fig. 3). Although Mn++ has opposing effects upon the inhibition by the two hormones, the presence or absence of Mn++ in the assay mixture did not affect the lack of additivity of the P site agonist and the steroid, since the level of inhibition measured in the presence of 2',5'-DDA and progesterone was not significantly different than the level of inhibition seen with 2',5'-DDA alone (Table 1). There are several possible explanations for the nonadditivity of the maximum inhibitory effects of 2',5'-DDA and progesterone. One explanation could be that these two agents share a common inhibitory mechanism. Alternatively, the inhibitory mechanisms of progesterone and 2',5'-DDA could be mutually exclusive, such that the enzyme system is maximally inhibited by 2',5'-DDA alone. Because additivity experiments are difficult to interpret and only suggest possible mechanisms of action, other aspects of the oocyte adenylate cyclase system were investigated.

![Inhibition of Xenopus Oocyte Adenylate Cyclase](http://www.jbc.org/)

**TABLE 1**

<table>
<thead>
<tr>
<th>Additions</th>
<th>MnSO4</th>
<th>Enzyme activity</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>4.4 ± 0.4</td>
<td>%</td>
</tr>
<tr>
<td>2',5'-DDA (0.32 mM)</td>
<td>-</td>
<td>1.5 ± 0.3°</td>
<td>65</td>
</tr>
<tr>
<td>9-β-D-AFA (3.2 mM)</td>
<td>-</td>
<td>1.2 ± 0.3°</td>
<td>72</td>
</tr>
<tr>
<td>Progesterone (30 μM)</td>
<td>-</td>
<td>2.3 ± 0.3°</td>
<td>48</td>
</tr>
<tr>
<td>2',5'-DDA/9-β-D-AFA</td>
<td>-</td>
<td>1.8 ± 0.1</td>
<td>71</td>
</tr>
<tr>
<td>2',5'-DDA/progesterone</td>
<td>-</td>
<td>1.7 ± 0.1</td>
<td>62</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>7.2 ± 0.1</td>
<td>%</td>
</tr>
<tr>
<td>2',5'-DDA (0.32 mM)</td>
<td>+</td>
<td>1.6 ± 0.1°</td>
<td>78</td>
</tr>
<tr>
<td>9-β-D-AFA (3.2 mM)</td>
<td>+</td>
<td>1.3 ± 0.2°</td>
<td>81</td>
</tr>
<tr>
<td>Progesterone (30 μM)</td>
<td>+</td>
<td>4.4 ± 0.3°</td>
<td>38</td>
</tr>
<tr>
<td>2',5'-DDA/9-β-D-AFA</td>
<td>+</td>
<td>1.8 ± 0.2</td>
<td>78</td>
</tr>
<tr>
<td>2',5'-DDA/progesterone</td>
<td>+</td>
<td>1.6 ± 0.2</td>
<td>74</td>
</tr>
</tbody>
</table>

* Expressed as mean ± S.E. (n = 3).

° Significantly less than control (p < 0.05).

°° Significantly less than control (p < 0.005).

Fig. 2. Effect of methylxanthine and Mn++ on inhibition of adenylate cyclase by 2',5'-dideoxyadenosine. A. effect of 3-isobutyl-1-methylxanthine on the inhibition of adenylate cyclase by 2',5'-DDA. Enzyme activity was measured in the presence of 0.15 mM Gpp(NH)p, 1.5 mM Mg(OOCCH3)2, 1.5 mM MnSO4, and the indicated concentrations of 2',5'-DDA, with (●) and without (○) 0.75 mM methylxanthine. Maximum activities were 5.4 ± 0.6 and 4.4 ± 0.2 pmol/mg/min in the presence and absence of methylxanthine, respectively. Each data point represents the average of duplicate measurements ± half-range. B. Potentiation of 2',5'-DDA inhibition of adenylate cyclase by Mn++. Enzyme activity was measured in the presence of 0.15 mM Gpp(NH)p, 1.5 mM Mg(OOCCH3)2, 0.75 mM 3-isobutyl-1-methylxanthine with (●) and without (○) 1.5 mM MnSO4. Maximum activities were 5.4 ± 0.6 and 1.9 ± 0.05 pmol/mg/min in the presence and absence of Mn++, respectively. Each data point represents the average of duplicate measurements ± half-range.

![Inhibition effect of Mn++ on inhibitory action of progesterone and 2',5'-dideoxyadenosine](http://www.jbc.org/)

**FIG. 3.** Differential effect of Mn++ on inhibitory action of progesterone and 2',5'-dideoxyadenosine. Membrane samples were prepared and assayed for adenylate cyclase activity in the presence of 1.5 mM Mg(OOCCH3)2, the indicated amounts of MnSO4, and 0.15 mM Gpp(NH)p. The values for percent inhibition were calculated from the mean of triplicate determinations for each assay addition. The percent inhibition relative to control is shown for 10 μM progesterone (□) and 30 μM 2',5'-DDA (●). Control activities (pmol/mg/min) were 2.1 ± 0.07 in the absence of Mn++, 5.8 ± 0.4 with 1.5 mM Mn++, and 6.2 ± 0.4 with 10 mM Mn++.

*Significantly less than control (p < 0.05).*

**Role of Guanine Nucleotide Exchange**—A widely accepted model for the role of guanine nucleotides in the regulation of adenylate cyclase (see Refs. 28–29 for review) involves an
active cycle in which enzyme activity is maximal when GTP or an appropriate analog of GTP is bound to the guanine nucleotide regulatory protein. Recycling of the enzyme occurs when enzyme activity is turned off by hydrolysis of bound GTP to GDP, followed by dissociation of GDP from the regulatory site. The exchange of GTP for GDP has been proposed to be the rate-limiting step in the cyclic activation process in avian systems. This model is largely derived from studies by Cassel and Selinger (25–28) of turkey erythrocyte membranes which demonstrate increased GTPase activity (24) and an increased rate of guanine nucleotide exchange (27, 28) upon hormonal stimulation of enzyme activity.

When adenylate cyclase activity in various cell systems is stimulated by nonhydrolyzable GTP analogs, such as Gpp(NH)p, slow transitions in enzyme activity occur that appear as lag periods in AMP accumulation (29–31). The lag is thought to be due to either the time required for displacement of bound GDP by the nonhydrolyzable analog (28) or the transition from an inactive to an active enzyme conformation (32). In order to further investigate the mechanisms by which progesterone and 2',5'-DDA inhibit adenylate cyclase in the oocyte membrane, the effects of these two agents on the activation of enzyme activity by Gpp(NH)p were investigated. Fig. 4 shows a representative time course of activation by 50 μM Gpp(NH)p. Kinetic data such as that shown in Fig. 4 were analyzed using graphical techniques described elsewhere (see Ref. 33 for review), and the calculated t1/2 values quantitatively define the half-life for the transition of enzyme activity from initial to steady state activated levels. In Fig. 4, the t1/2 in the absence of added drug was 13.9 min; progesterone and 2',5'-DDA treatments of the membrane samples increased t1/2 to 16.5 and 17.3 min, respectively. Thus, at least part of the mechanism by which progesterone and 2',5'-DDA inhibit oocyte plasma membrane adenylate cyclase involves a decreased rate of activation by guanine nucleotide.

As shown in Fig. 5, the magnitude of the increase in t1/2 was inversely proportional to the concentration of Gpp(NH)p in the incubation mixture in the presence of either hormone. In this series of experiments, there were significant variations in the absolute lag times measured in membrane samples from oocytes taken from different animals, with the control values for t1/2 (measured in the absence of added hormone) ranging from 4.0 to 13.9 min. Similarly, considerable variations in the actual length of lag for activation of adenylate cyclase activity in S49 wild type membranes have been reported for different cell batches and cholate extracts (31, 34). In spite of this variability in control values for t1/2, progesterone and 2',5'-DDA generally caused similar increases in the calculated values for t1/2, as shown in Fig. 4, and the magnitude of t1/2 was inversely proportional to the concentration of Gpp(NH)p in the release buffer. The fact that the hysteretic effects of both agents were diminished by high concentrations of Gpp(NH)p suggested that both progesterone and 2',5'-DDA induce a slowing of the rate of guanine nucleotide release from oocyte membranes.

Fig. 4. Effect of progesterone and 2',5'-dideoxyadenosine on the kinetics of stimulation of oocyte membrane adenylate cyclase by 50 μM Gpp(NH)p. Membrane samples were prepared as described under “Experimental Procedures” (25 μg of protein/tube) and assayed at 30 °C in the presence of 1.5 mM Mg(OOCCH3)2 and 59 μM Gpp(NH)p in the absence (●) or presence of either 30 μM progesterone (○) or 0.32 mM 2',5'-DDA (▲). The reaction was initiated by addition of [α-32P]ATP and Gpp(NH)p at time zero, reactions were stopped at the indicated times, and total [32P]cAMP formed was determined (n = 2, ± half-range).

Fig. 5. Effect of Gpp(NH)p upon the hysteretic effects of progesterone and 2',5'-dideoxyadenosine. Membrane samples were prepared as described under “Experimental Procedures,” and the time course of activation of cAMP formation by various concentrations of Gpp(NH)p was measured in the presence of 1.5 mM MgCl2 after preincubation of the membrane samples with either 30 μM progesterone (A) or 0.32 mM 2',5'-DDA (B). Kinetic data similar to that in Fig. 4 were analyzed to determine t1/2 values, calculated as t1/2 (+drug) − t1/2 (−drug). The correlation coefficients were determined by least squares analysis of the means, and the means were compared by general one-factor analysis of variance (n = 3; ± S.E.).
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exchange at the regulatory site. In the intact cell, this would result in a decreased rate of GDP-GTP exchange and contribute to an overall inhibition of adenylate cyclase activity.

In order to directly evaluate this hypothesis, the effects of progesterone and 2',5'-DDA upon guanine nucleotide release were measured in a typical release assay illustrated while the 64, 2',5'-DDA (100 μM) decreased the rate of [H]guanine nucleotide release from membrane samples that had been previously loaded with [H]GTP. In a similar manner, progesterone (30 μM) also slowed the rate of guanine nucleotide exchange (Fig. 6B). Similar results were obtained when guanosine 5'- (3-thio)triphosphate in the release buffer was replaced with Gpp(NH)p. These findings provide direct evidence that these inhibitory hormones decrease the "turn-on" reaction of adenylate cyclase as monitored by guanine nucleotide exchange.

DISCUSSION

The experimental evidence presented in this paper suggests that two seemingly unrelated compounds, progesterone and 2',5'-DDA, share a common mechanism of action for inhibiting adenylate cyclase that involves a decreased rate of guanine nucleotide exchange. The primary impetus for these comparative studies was the observation that both agents were capable of inhibiting oocyte adenylate cyclase activity measured in the presence of Gpp(NH)p (3) (Fig. 6). The action of other inhibitory hormones generally requires GTP hydrolysis (11, 32). The additivity experiments (Table 1) suggested that the effects of the steroid and adenosine analog were either attributed to a common mechanism or were mutually exclusive. The kinetic studies indicated that both progesterone and 2',5'-DDA slowed the rate of enzyme activation (Fig. 4), and this hysteretic effect was inversely proportional to the concentration of Gpp(NH)p in the activation mixture (Fig. 5). These results combined with a common inhibitory action on guanine nucleotide exchange (Fig. 6) suggested that these agents somehow affected the guanine nucleotide binding site.

Guanine nucleotide exchange has been shown to be rate-limiting in avian systems (25-28) and may contribute to the hysteretic lag seen with Gpp(NH)p stimulation in other systems (33). Furthermore, agents that stimulate adenylate cyclase activity have been shown to stimulate the rate of guanine nucleotide exchange. For example, isoproterenol has been shown to stimulate the release of [H]GDP from turkey erythrocyte membranes, and the rate of release was correlated with the level of activation of adenylate cyclase activity (28). More recently, it has been shown that treatment of turkey erythrocyte membranes with the presence of NAD resulted in an increase in the rate of release of GDP from sites on these membranes (36) and that the mechanism for activation of turkey erythrocyte adenylate cyclase involves, at least in part, an increased rate of release of guanine nucleotide from G/F (37); thus, cholera stimulates the turn-on reaction for adenylate cyclase.

By direct analogy, one might expect inhibitory hormones to have the opposite effect, i.e. to slow the rate of guanine nucleotide exchange at the G/F regulatory protein, thereby slowing the turn-on reaction. As reported here, this effect does occur with progesterone and 2',5'-DDA. However, since the effects of most other inhibitory hormones require GTP hydrolysis, as evidenced by the fact that inhibition is not measured in the presence of Gpp(NH)p, mechanistic analysis of hormonal inhibition by these hormones has focused on their effects upon GTPase activity. Koski and Klee (38) demonstrated that inhibitory hormones, such as opiates, α-adrenergic receptor agonists, and muscarinic receptor agonists, stimulated low K_m GTPase activity in neuroblastoma X glioma cell membranes in a GTP-dependent manner. Conversely, stimulatory agents such as prostaglandin E_1, 2-Cl-adenosine, and secretin had little or no effect on GTPase activity. Thus, the authors proposed a model for the dualistic control of adenylate cyclase activity in which stimulatory hormones interact with stimulatory receptors, and this complex facilitates the exchange of GTP for GDP at the regulatory site. On the other hand, inhibitory hormones bind to inhibitory receptors, and the inhibitory hormone-inhibitory receptor complex stimulates the hydrolysis of GTP bound to the regulatory site, thus limiting the half-life of the active species. This role for GTP hydrolysis has been previously proposed (39, 40), and suggests that many inhibitory hormones act, at least in part, by stimulating the turn-off reaction for adenylate cyclase. The results reported in this paper establish that certain inhibitory hormones can also decrease the turn-on reaction for adenylate cyclase. It remains to be determined whether progesterone also stimulates GTPase activity in the oocyte membrane or whether other inhibitory hormones also decrease the rate of guanine nucleotide exchange in mammalian systems. In any case, while guanine nucleotide exchange has been shown to be rate-limiting in the turkey erythrocyte, it is not clearly rate-limiting in the oocyte since exchange can occur in the absence of hormone (Fig. 6).

The results presented in this paper are consistent with the concept that inhibition of adenylate cyclase by progesterone occurs by a novel mechanism that has many properties in common with the P site previously described for adenosine agonists. Both progesterone and 2',5'-DDA inhibit Gpp(NH)p-stimulated activity, require a guanine nucleotide regulatory protein, and decrease the rate of guanine nucleotide exchange. This result was somewhat surprising because the P site has been thought to be neither receptor-mediated nor physiologically significant. Since impermeable adenosine analogs inhibit adenylate cyclase in membrane preparations by interacting at the P site but do not inhibit enzyme activity in whole cells (10, 35), the P site action is apparently not mediated by a conventional extracellularly exposed hormone-receptor interaction. In addition, P site inhibition can be observed in solubilized preparations of cyclase if extraction is performed in the presence of guanine nucleotides (15), and hormone-receptor binding is generally not thought to be present under these conditions. The physiological significance of the P site has been questioned because certain P site agonists only acts at this site in high micromolar or millimolar concentrations, well above the normal physiological concentration of adenosine found in cells (11, 13, 35). However, it has been observed recently (41, 42) that potent adenosine compounds are present in cells at concentrations sufficient to act at the P site. It is of interest that one of these compounds, 2'-deoxyadenosine 3'-monophosphate, is present in amphibian cells in much higher concentrations than in mammalian cells (41). Further experimentation is required to evaluate the possible role of endogenous P site agonists in regulating the amphibian oocyte adenylate cyclase.

The available evidence supports the view that inhibition of adenylate cyclase by progesterone is receptor-mediated. In photoaffinity labeling studies with membranes using the synthetic progestin, R5020, a single oocyte membrane protein was photolabeled (7). The apparent affinity of the photolabeled protein for R5020 was equal to the IC50 for cyclase inhibition, and the degree of inhibition was proportional to the amount of steroid covalently bound to the receptor (7). Furthermore, while Mn²⁺ potentiates P site action, it abolishes progesterone inhibition, as one would expect on the basis of observed cation effects in other receptor-mediated
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systems (21, 22). Further evidence for surface receptor mediation of progesterone inhibition comes from studies in which steroids bound to polymers are active in stimulating oocyte maturation (43, 44), while microinjection of steroid is without effect (45, 46). The polymer experiments are not necessarily inconsistent with the action of a progesterone receptor near the P site, since the lipophilic nature of steroids might permit action inside the membrane even when steroid coupled to a polymer is applied extracellularly. In this connection, it should be remembered that the photoaffinity labeling of a steroid receptor on oocyte membranes does not establish on which side of the membrane the receptor is located.

The original report by Sadler and Maller (3) of adenylate cyclase inhibition by steroids implicated a requirement for the G/F protein in the process. This conclusion was based largely on the finding that only activity stimulated by agents known to activate via G/F was subject to inhibition by progesterone. The results in this paper demonstrating an effect of progesterone on guanine nucleotide exchange provide further support for this hypothesis. Other experiments have shown that cholera toxin ADP-ribosylates oocyte membrane proteins of Mr = 45,000 and 52,000,2 indicating oocyte G/F is structurally similar to mammalian G/F (47). The evidence that G/F is required for P site inhibition includes the absence of inhibition of cyclase by adenosine in cyc- membranes (9), which lack a functional G/F (16, 17), and the absence of inhibition of the soluble testicular enzyme and solubilized (uncoupled) hepatic cyclase extracted in the absence of guanine nucleotide (15). With both hormones, the results imply that the requirement for G/F reflects a property other than GTP hydrolysis, since Gpp(NH)p-stimulated activity is inhibited. The idea that G/F has multiple properties has been supported by other experiments. Other experiments have demonstrated that progesterone in the amphibian oocyte leads to inhibition of adenylate cyclase by modulating this type of hormone-receptor interaction may be regulated intracellularly by the level of adenosine compounds such as 2'-deoxyadenosine 3'-monophosphate (41). Further work is needed to evaluate whether progesterone in the amphibian oocyte leads to inhibition of adenylate cyclase by modulating this type of inhibitory response.

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REFERENCES


2 S. E. Sadler and J. L. Maller, unpublished data.
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Inhibition of Xenopus oocyte adenylate cyclase by progesterone and 2',5'-dideoxyadenosine is associated with slowing of guanine nucleotide exchange.

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