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Progesterone Inhibition of *Xenopus* Oocyte Adenylate Cyclase Is Not Mediated via the *Bordetella pertussis* Toxin Substrate

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SUMMARY

Preincubation of intact *Xenopus laevis* oocytes with islet-activating protein (IAP) stimulates plasma membrane adenylate cyclase activity measured in the presence of either GTP or 5′-guanylyl imidodiphosphate. The toxin appears to act by catalyzing the ADP-ribosylation of a 41,000-D protein in the oocyte plasma membrane that is distinct from the 45,000- and 52,000-D proteins labeled by cholera toxin. Preincubation of intact oocytes with IAP for 2.5 hr inhibits 80% of the ADP-ribosylation of oocyte membranes subsequently prepared and treated *in vitro* with IAP in the presence of [α-^{32}P]NAD. The abilities of progesterone and the P site agonist, 2′,5′-dideoxyadenosine, to inhibit plasma membrane adenylate cyclase are not inhibited by IAP treatment, but IAP pretreatment abolishes inhibition by acetylcholine. In addition, IAP pretreatment of oocytes slows the time course of germinal vesicle breakdown induced by suboptimal concentrations of progesterone. This effect of toxin upon oocyte maturation is apparently due to its effect upon basal cyclase activity, since IAP has no effect upon the time course of maturation induced by 10 μM progesterone. These results indicate that, even though the oocyte adenylate cyclase does contain a functional N_i subunit, inhibition of *Xenopus* oocyte adenylate cyclase by progesterone or 2′,5′-dideoxyadenosine is not mediated via the *Bordetella pertussis* toxin substrate.

INTRODUCTION

Islet-activating protein, *Bordetella pertussis* toxin, modifies the activity of hormone-mediated adenylate cyclase in a number of cell types. This toxin catalyzes the NAD-dependent ADP-ribosylation of a cell membrane protein of 41,000-D molecular mass (1–5), and the action of IAP[2] has been shown to prevent receptor-mediated or GTP-dependent inhibition of adenylate cyclase (3–6). The site of IAP action appears to be a component of the guanine nucleotide regulatory protein N_i, and the function of N_i is abolished by IAP-catalyzed ADP-ribosylation of the 41,000-D protein that appears to be an active subunit of N_i (4, 7, 8).

Our laboratory has previously shown that progesterone inhibits oocyte plasma membrane adenylate cyclase by a mechanism that involves a guanine nucleotide regulatory protein (9), and the inhibitory action of the steroid has been confirmed by other investigators (10–12). In keeping with the model that hormonal regulation of adenylate cyclase is usually receptor-mediated, the steroidal photoaffinity label [^{3}H]R5020 has been used to identify a steroid receptor in oocyte plasma membrane samples (13). The oocyte plasma membrane receptor has an apparent M_r = 110,000 by SDS-polyacrylamide gel electrophoresis (13) and 102,000 by sucrose density centrifugation (14). In addition, the amount of [^{3}H]R5020 covalently bound to the 110,000-D protein after photolysis correlates with both the level of inhibition of adenylate cyclase activity and the EC_{50} for germinal vesicle breakdown induced by R5020 (13).

Inhibition of amphibian oocyte adenylate cyclase is the first example of a direct effect of steroids upon a target cell enzyme system that is not mediated by altered transcription or translation, and it was of interest to investigate further the mechanism by which progesterone inhibits oocyte adenylate cyclase. Comparative studies showed that inhibition of oocyte plasma membrane adenylate cyclase by both progesterone and 2′,5′-DDA, a potent P site agonist (15), was correlated with slowing of guanine nucleotide exchange (16). The steroid inhibition shared certain other common characteristics with P

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[2] The abbreviations used are: IAP, islet-activating protein, *Bordetella pertussis* toxin; 2′,5′-DDA, 2′,5′-dideoxyadenosine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; GVBD, germinal vesicle breakdown; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; N_i, N_s, the guanine nucleotide regulatory proteins that are responsible for inhibition and stimulation of adenylate cyclase, respectively; R5020, 17,21-dimethyl-19-norpregn-4,9-diene-3,10-dione (New England Nuclear); Gpp(NH)p, 5′-guanylyl imidodiphosphate, SDS, sodium dodecyl sulfate.

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site action, including inhibition of Gpp(NH)p-stimulated enzyme activity and a slowing of the rate of Gpp(NH)p activation of the enzyme that was inversely proportional to the concentration of guanine nucleotide in the activation mixture (16). However, a major difference between the action of progesterone and the P site agonist was encountered in the effects of divalent cations. While 10 mM Mn^{2+} dramatically potentiated the ability of 2′,5′-dideoxyadenosine to inhibit oocyte adenylate cyclase (16) as would be predicted for P site action (15, 17, 18), Mn^{2+} abolished the inhibitory action of progesterone (16) as would be predicted for receptor-mediated GTP-regulatory protein-dependent action (19, 20). These results suggested that, while the mechanism by which progesterone inhibits oocyte adenylate cyclase is similar to P site action, progesterone inhibition is not identical to P site action.

In the present study, IAP was used to study the possible role of the N_i subunit of the adenylate cyclase system in the inhibition of oocyte plasma membrane adenylate cyclase activity and the induction of germinal vesicle breakdown by progesterone. The results indicate that while the IAP substrate is present and functional in oocyte membranes, the inhibitory effects of progesterone or 2′,5′-DDA on the amphibian adenylate cyclase are not mediated by the N_i protein.

EXPERIMENTAL PROCEDURES

IAP treatment and membrane isolation. Sexually mature *Xenopus laevis* were primed with pregnant mare's serum gonadotropin (Sigma), and oocytes were manually dissected from ovarian fragments, as previously described (9). Groups of 300 oocytes were incubated for 2 hr at room temperature in 0.5 ml of Buffer A (83 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM KCl, 10 mM Hepes, pH 7.9) containing 1 mg/ml bovine serum albumin in the presence or absence of 0.2–0.4 μg/ml IAP. The stock solution of IAP, a gift of Dr. Michio Ui (Hokkaido University) was stored at 4° at a concentration of 1 mg/ml in 0.1 M phosphate buffer, pH 7.0, containing 2 M urea. The oocytes were then washed in Buffer A, and membrane samples were prepared as previously described (9). Each figure represents experimental results using oocytes from a different animal, and each sample used for adenylate cyclase measurements routinely contained plasma membranes from 10 oocytes, corresponding to 50 μg of total protein as measured by the method of Lowry *et al.* (21) using bovine serum albumin as standard.

Adenylate cyclase assay. Plasma membrane-associated adenylate cyclase activity was determined by measuring the enzymatic conversion of [α-^{32}P]ATP (ICN, >1000 Ci/mmol) to [^{32}P]cyclic AMP, as previously described (9). The reaction was carried out in a final volume of 0.2 ml that contained membrane samples resuspended in 50 mM Tris-maleate, pH 7.5, 1.5 mM $MgCl_2$, 0.5 mM EGTA, 5 mM cyclic AMP, 0.75 mM 3-isobutyl-1-methylxanthine, 0.1 mg/ml creatine phosphokinase, 10 mM creatine phosphate, 0.15 mM guanine nucleotide, 0.25 mM ATP, and 10 μCi of [α-^{32}P]ATP. Assays were initiated by addition of ATP and terminated after 1 hr at 30° (9). When indicated in the figure legends, 150 μM Gpp(NH)p was substituted for GTP, and 2′,5′-dideoxyadenosine (ICN) or progesterone (Sigma) was included in the assay mixture. Product recovery was determined by co-chromatography of [^{3}H]cyclic AMP.

Toxin-catalyzed ADP-ribosylation of membrane samples. Cholera toxin-catalyzed ADP-ribosylation of oocyte membrane samples was performed using conditions modified from Schleifer *et al.* (22) and Kaslow *et al.* (23). The cholera toxin was preactivated for 20 min at 37° in a mixture containing 20 mM dithiothreitol, 1 mg/ml bovine serum albumin, 25 mM KH_2PO_4, pH 8.0, and 500 μg/ml cholera toxin (Schwarz/Mann). Cholera toxin-catalyzed ADP-ribosylation was performed by resuspending a membrane sample prepared from 100 oocytes (0.5 mg of total protein) in 100 μl of a solution containing 5 mM ATP, 15 mM thymidine, 1 mM $MgCl_2$, 0.5 mM EDTA, 250 mM KH_2PO_4, pH 6.8, 150 μM GTP, 50 μg/ml preactivated cholera toxin, and 10 μM [α-^{32}P]NAD (10 μCi, ICN). IAP (100 μg/ml) was preactivated for 20 min at 37° in 25 mM dithiothreitol, 12.5 mM Tris-HCl, pH 7.5, and IAP-catalyzed ADP-ribosylation of oocyte membranes was performed by the method of Burns *et al.* (24). A membrane sample prepared from 100 oocytes (0.5 mg of total protein) was resuspended in 100 μl of ADP-ribosylation mixture containing 25 mM glycine, 0.4 mM ATP, 0.4 mM GTP, 15 mM thymidine, 10 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 40 mM KH_2PO_4, pH 7.5, 5 μg/ml preactivated IAP, and 10 μM [α-^{32}P]NAD (10 μCi, ICN). After both ADP-ribosylation mixtures were incubated for 45 min at 30°, the membrane samples were washed by centrifugation (16,000 × *g* for 1 min, Fisher Microfuge) in 130 mM NaCl, 0.01% sodium azide, 100 μM phenylmethylsulfonyl fluoride, 10 mM Hepes, pH 7.9, and suspended in sample buffer (10% glycerol, 5% β-mercaptoethanol, 3% sodium dodecyl sulfate, 70 mM Tris, pH 6.8). After the membrane samples were warmed in a boiling water bath for 4 min, aliquots (100 μg of protein per lane) were subjected to SDS-polyacrylamide gradient slab gel electrophoresis (10–15% acrylamide) by the method of Laemmli (25). Autoradiography was performed by exposure of the dried gel to Kodak X-RP film.

Steroid-induced maturation (GVBD). After IAP treatment as described above, oocytes were washed and groups of 50 were treated with various concentrations of progesterone (as indicated in Fig. 6) at room temperature in 3 ml of Buffer A. At successive time points following addition of steroid, each group of oocytes was scored for GVBD as evidenced by the appearance of a white spot on the pigmented animal pole. All GVBD responses were verified by manual dissection of oocytes after fixation in 10% trichloroacetic acid.

RESULTS

Pretreatment of oocytes with IAP for 2 hr prior to membrane isolation and assay of adenylate cyclase activity elevated the level of enzyme activity measured in the presence of either 0.15 mM GTP or 0.15 mM Gpp(NH)p, as shown in Fig. 1. In several experiments of this type, the level of IAP-stimulated activity ranged from 1.5- to

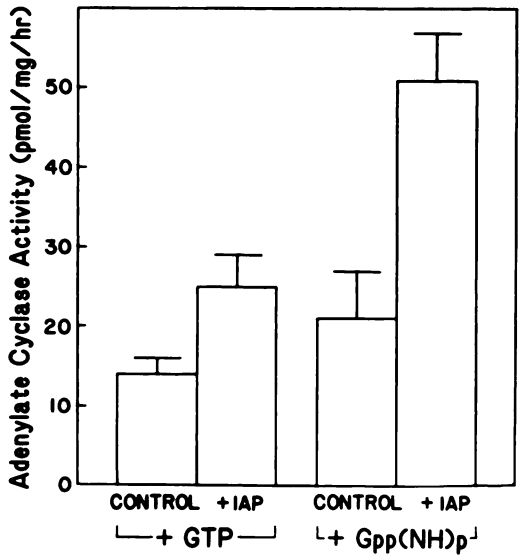

FIG. 1. *Effect of IAP preincubation on Xenopus oocyte adenylate cyclase*

Oocytes were preincubated for 2 hr in the presence or absence of 0.2 μg/ml IAP prior to membrane preparation and enzyme assay (as described under Experimental Procedures) in the presence of either 0.15 mM GTP or 0.15 mM Gpp(NH)p as indicated.

3-fold that of enzyme activity in membranes prepared from untreated cells (data not shown).

As described in numerous other cell systems (1–6, 24), IAP catalyzes the ADP-ribosylation of a 41,000-D membrane protein that alters the function of the inhibitory guanine nucleotide regulatory subunit (N_i) of the adenylate cyclase complex. As shown in Fig. 2, incubation of oocyte membranes with [α-^{32}P]NAD and IAP also resulted in ADP-ribosylation of a 41,000-D protein. This 41,000-D protein was the only IAP substrate detected in the oocyte membrane (Fig. 2) and was clearly different from the proteins ADP-ribosylated in a parallel sample of membranes treated with cholera toxin.

Figure 3 demonstrates the inability of IAP pretreatment of intact cells to prevent inhibition of Gpp(NH)p-stimulated adenylate cyclase by either progesterone or 2′,5′-dideoxyadenosine in membrane samples prepared subsequently. When membranes of untreated cells were assayed in the presence of 20 μM progesterone, there was a 27% inhibition of enzyme activity. In samples of membranes from IAP-treated cells, the level of enzyme activity in the absence of added drug was increased approximately 50% and the level of inhibition measured in the presence of 20 μM progesterone increased to 65%. In a similar fashion, the level of inhibition by 2′,5′-dideoxyadenosine was increased by IAP pretreatment. A previously established optimal concentration of 2′, 5′-dideoxyadenosine, 0.32 mM (16), caused 18% inhibition in untreated cells, and the level of inhibition was increased to 43% in membranes from IAP-treated cells. The effect of Mn^{2+} to potentiate P site action has previously been demonstrated for other cell systems (15, 17, 18), as well as oocyte adenylate cyclase (16). In the experiment shown in Fig. 3, the addition of 5 mM Mn^{2+} to the assay mixture increased the level of inhibition measured in the presence of 0.32 mM 2′,5′-dideoxyadenosine from 18% in the absence of added Mn^{2+} to 56% in the presence of Mn^{2+}. The most striking effect of IAP treatment was also measured in the presence of Mn^{2+}. IAP pretreatment increased the level of enzyme activity measured in the presence of 5 mM Mn^{2+} by more than 2-fold, and the inhibitory action of 2′,5′-dideoxyadenosine upon Mn^{2+}-stimulated activity increased from 56% in membranes prepared from untreated oocytes to 76% in membranes prepared from IAP-treated oocytes. The increase in the degree of P site inhibition in the presence of stimulatory agents has been seen in numerous other systems (26–31). Clearly, IAP treatment of intact cells does not prevent inhibition of the oocyte adenylate cyclase by either progesterone or 2′,5′-dideoxyadenosine.

An important question in these experiments relates to whether enough of the pertussis toxin substrate is modified under the experimental conditions to affect the

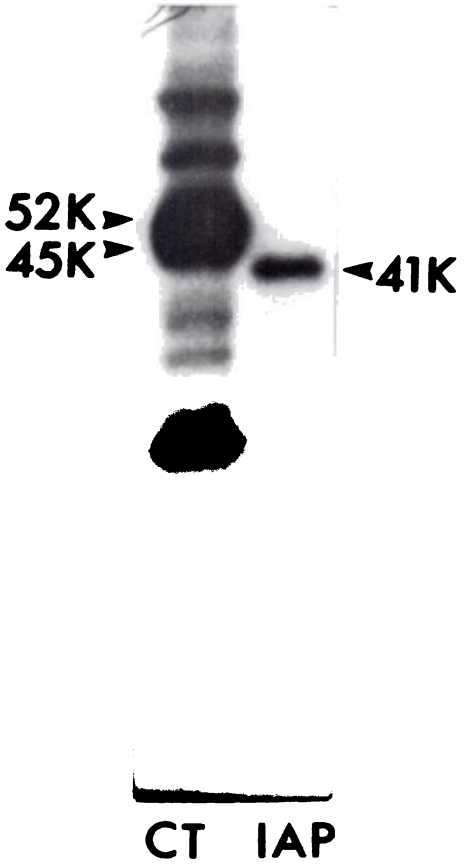

FIG. 2. *Polyacrylamide gel analysis of cholera toxin- and IAP-catalyzed ADP-ribosylation of oocyte membranes*

Membranes were treated with [α-^{32}P]NAD in the presence of cholera toxin (*CT*) or IAP followed by electrophoresis and autoradiography, as described under Experimental Procedures. The molecular weights indicated were determined by electrophoresis and staining of molecular weight standards (phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactalbumin).

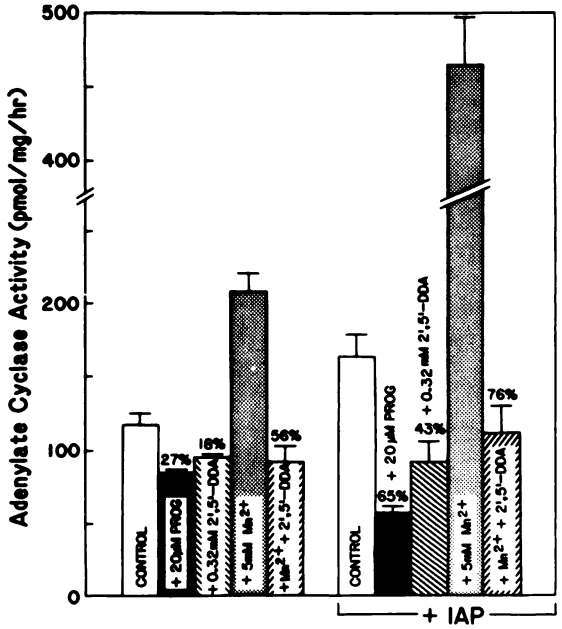

FIG. 3. *Effect of IAP on inhibition of Gpp(NH)p-stimulated oocyte adenylate cyclase by progesterone or 2′,5′-dideoxyadenosine*

Groups of oocytes were preincubated for 2 hr in the presence or absence of 0.4 μg/ml IAP as indicated. Membranes were isolated and assayed with no additions to the assay mixture (*CONTROL*), or in the presence of 20 μM progesterone (*PROG*), 0.32 mM 2′,5′-DDA, 5 mM Mn^{2+}, or 5 mM Mn^{2+} plus 0.32 mM 2′,5′-DDA. The numbers over the bars indicate the percentage inhibition caused by the various drug treatments relative to their respective controls.

action of a hormone known to require N_i for inhibition of adenylate cyclase. When intact oocytes were preincubated with IAP for 2.5 hr prior to membrane isolation and subsequent labeling with [α-^{32}P]NAD and IAP, 80% of the IAP-catalyzed ADP-ribosylation was inhibited (Fig. 4), indicating that most of the IAP-substrate is ADP-ribosylated in the intact oocyte during the preincubation period. Under these same conditions, IAP pretreatment of the oocytes did block the inhibitory action of acetylcholine (Fig. 5). In membrane samples prepared from oocytes following a control incubation in Buffer A without IAP, 36% of the adenylate cyclase activity measured in the presence of 0.15 mM GTP was inhibited by 5 μM acetylcholine (Fig. 5A) and 39% of Gpp(NH)p-stimulated enzyme activity was inhibited by 20 μM progesterone (Fig. 5B). In membrane samples prepared from oocytes that were preincubated for 2.5 hr in the presence of IAP, the inhibitory effect of acetylcholine was abolished (Fig. 5A), but the level of inhibition by progesterone was slightly increased due to the increased level of basal activity following IAP pretreatment. Thus, IAP pretreatment blocked the inhibitory action of acetylcholine but not the action of progesterone.

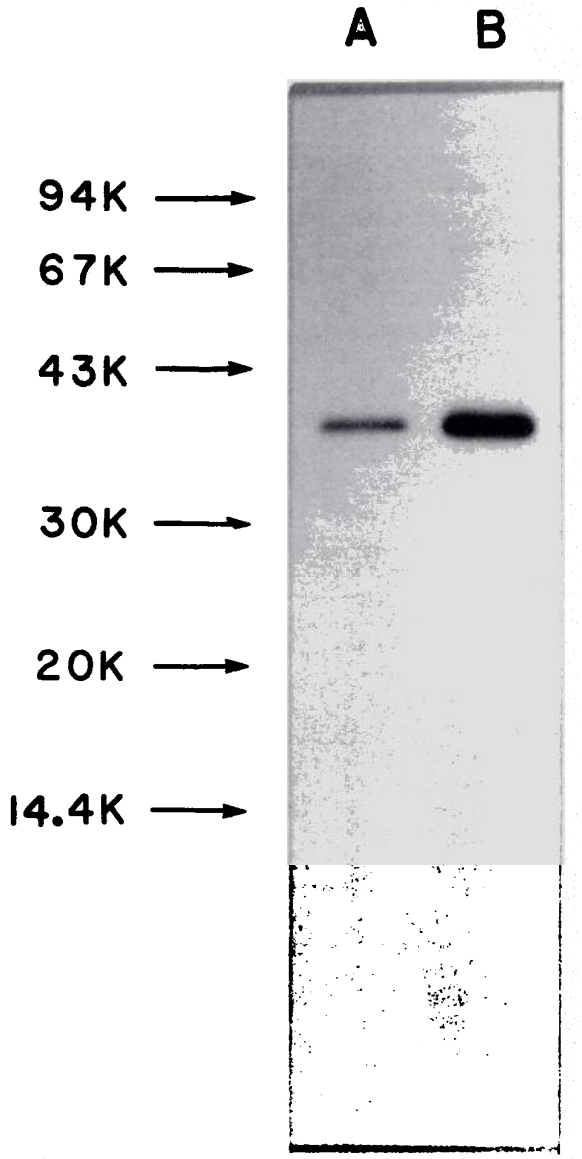

FIG. 4. *The extent of ADP-ribosylation of N_i in intact oocytes*

Oocytes were incubated in the presence or absence of 0.4 μg/ml IAP for 2.5 hr prior to membrane isolation, as described under Experimental Procedures. *In vitro* ADP-ribosylation of the membrane samples was then carried out with IAP and [α-^{32}P]NAD followed by gel electrophoresis and autoradiography. *A*, membranes from IAP-treated oocytes; *B*, control membranes preincubated in the absence of IAP. Excision and counting of the radiolabeled band showed that over 80% of the radioactivity associated with the 41,000-D protein (865 cpm) in control oocytes was absent in IAP-incubated cells.

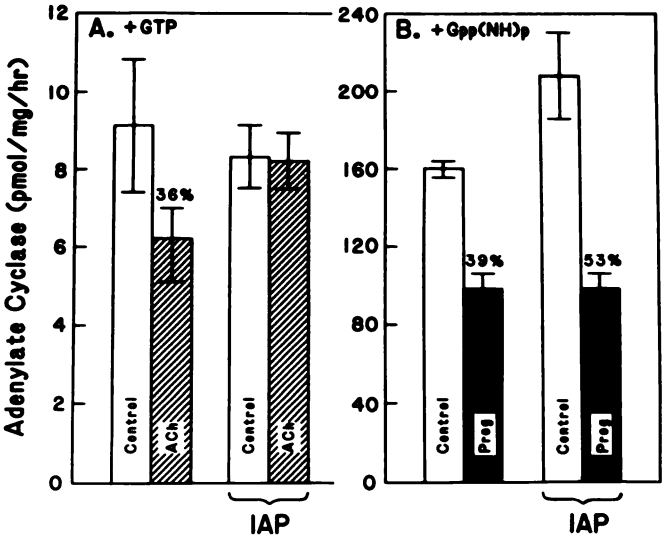

FIG. 5. *Effect of IAP on inhibition of oocyte adenyate cyclase by acetylcholine*

Groups of oocytes were preincubated for 2.5 hr in the presence or absence of 0.4 μg/ml IAP, as indicated. Membranes were isolated and assayed in the presence of 0.15 mM GTP (panel A) or 0.15 mM Gpp(NH)p (panel B) with no further additions to the assay mixture (*CONTROL*), or in the presence of 5 μM acetylcholine (*ACh*, panel A), or 20 μM progesterone (*PROG*, panel B). The percentage of inhibition caused by each drug treatment relative to their respective controls is also shown.

IAP pretreatment also affected the time course of GVBD induced by suboptimal concentrations of progesterone, as shown in Fig. 6. In Fig. 6C, 0.01 μM progesterone stimulated a maximum level of 40% GVBD within 9 hr. IAP pretreatment both reduced the maximum level of GVBD by more than 50% and slowed the time course of maturation. Increasing concentrations of progesterone were able to overcome the effects of IAP. The maximal GVBD response elicited by 0.1 μM progesterone was only reduced 30% by IAP treatment (Fig. 6B), and IAP treatment had no significant effect upon either the time course of GVBD or the maximum response to 10 μM progesterone (Fig. 6A). The ability of IAP to slow the time course of GVBD and reduce the maximum physiological response of the oocytes to suboptimal concentrations of progesterone was consistent with the elevation of basal enzyme activity induced by IAP (Fig. 1).

DISCUSSION

The results reported here demonstrate that the amphibian oocyte membrane contains a functional N_i com-

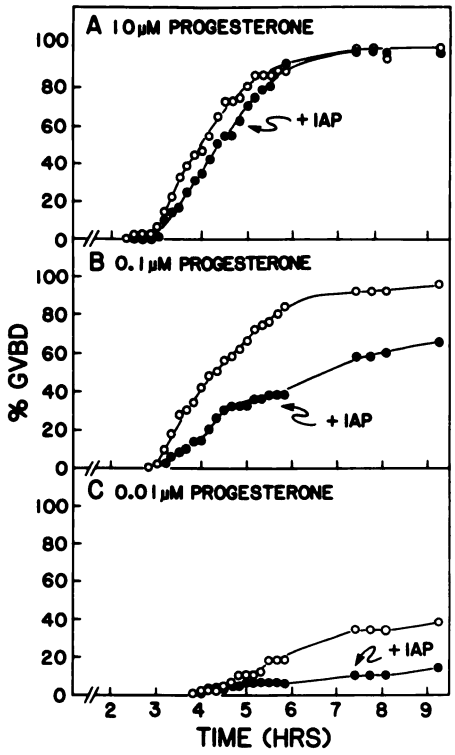

FIG. 6. *Effect of IAP on progesterone-induced germinal vesicle breakdown*

Groups of oocytes were preincubated for 2 hr in the presence (●) or absence (○) of 0.4 μg/ml IAP. The cells were then washed in Buffer B, and groups of 50 oocytes were subsequently treated with various concentrations of progesterone and scored for GVBD at various times after steroid addition, as described under Experimental Procedures. The results are expressed as the percentage of oocytes which displayed a white spot (GVBD) as a function of time after progesterone addition.

ponent of the adenylate cyclase complex. The presence of the N_i protein is indicated by the existence of a 41,000-D protein in the oocyte membrane that is effectively ADP-ribosylated by incubation of oocyte membranes in the presence of [α-^{32}P]NAD and IAP (Fig. 2). This 41,000-D protein substrate for IAP-catalyzed NAD-dependent ADP-ribosylation is presumably identical to the N_i protein described in other cell systems (1–6). The functionality of the oocyte N_i protein is demonstrated in Fig. 1 in which treatment of intact cells with IAP resulted in increased levels of adenylate cyclase activity measured in the presence of either GTP or Gpp(NH)p, and in Fig. 5 in which inhibition of enzyme activity by acetylcholine was blocked by IAP pretreatment. The ability of IAP to increase oocyte adenylate cyclase activity presumably reflects relief from a tonic suppression of catalytic activity by the N_i component. The increased level of adenylate cyclase activity that results from IAP pretreatment is sensitive to progesterone inhibition, as shown by the increased percentage inhibition seen in progesterone-treated samples following IAP treatment (Figs. 3 and 5). It is worth noting that the percentage inhibition of adenylate cyclase by progesterone and 2′,5′-DDA is increased in membranes from IAP-treated oocytes (Figs. 3 and 5). This finding is consistent with the similar mechanism of inhibition observed with progesterone and P site agonists (15) and the observation in several laboratories that agents which activate N_s increase the percentage inhibition seen with P site agonists (26, 31).

Certain parallels can be drawn between the oocyte adenylate cyclase system and other cell systems. In contrast to 3T3 fibroblasts (3) and rat adipocytes (4) where treatment of cells with IAP did not affect Gpp(NH)p-stimulated levels of enzyme activity, IAP did elevate the level of Gpp(NH)p-stimulated activity in the oocyte membrane preparation (Figs. 1, 3, and 5). Murayama and Ui (4) reported a monophasic dose-response curve for Gpp(NH)p stimulation of rat adipocyte membrane adenylate cyclase in the presence or absence of isoproterenol that was not affected by IAP. The oocyte membrane adenylate cyclase is also stimulated by Gpp(NH)p in a monophasic manner with an EC_{50} of 10 μM, but IAP treatment elevates the maximum level of stimulated activity without altering the EC_{50} for Gpp(NH)p.[3] IAP also affects oocyte adenylate cyclase activity measured in the presence of GTP (Fig. 1). In this way, oocytes are similar to 3T3 fibroblasts (3) and NG108-15 cells (24) in which IAP treatment elevated the level of activity measured in the presence of GTP without combined addition of receptor agonists. According to the scheme for dual signal transduction described by Murayama and Ui (4), this suggests that the oocyte adenylate cyclase activity is balanced between N_s-induced activation and N_i-induced inhibition of enzyme activity, and abolition of the inhibitory influence of N_i by IAP results in enhancement of GTP-dependent activity similar to that observed in other cell types (3, 24).

The inhibitory effect of IAP treatment upon progesterone-induced GVBD (Fig. 6) might be predicted in view of the previously established role of cyclic nucleotide in triggering the physiological response. It has been demonstrated that intracellular levels of cyclic AMP decrease to 40–60% of basal within minutes after progesterone is added to the oocytes (32), and microinjection of both the catalytic and regulatory subunits of cyclic AMP-dependent protein kinase demonstrated that the decrease in cyclic AMP was both necessary and sufficient to trigger oocyte maturation (33). In addition, cholera toxin was shown to be a potent inhibitor of progesterone-induced maturation (32, 34). More recently, this decrease in the intracellular level of oocyte cyclic AMP that triggers oocyte maturation has been shown to be due, at least in part, to progesterone-induced inhibition of adenylate cyclase activity (9–12). The increased levels of adenylate cyclase activity caused by IAP treatment (Fig. 1) would result in increased levels of cyclic AMP in the intact cell that would counteract the inhibitory action of progesterone and lead to a diminished physiological response to the steroid (GVBD), as shown in Fig. 6.

In contrast to other cell systems in which inhibitory hormone action is blocked by IAP treatment (3–5), the inhibitory action of progesterone upon the oocyte adenylate cyclase is not abolished by IAP (Fig. 3). The IAP pretreatment protocol was sufficient to modify a majority of the 41,000-D subunit in the oocyte plasma membrane, since it blocked 80% of subsequent radiolabeling of mem-

[3] S. E. Sadler and J. L. Maller, unpublished data.

brane samples (Fig. 4) and also blocked the inhibitory action of acetylcholine (Fig. 5). In parallel with the ability of high concentrations of progesterone to inhibit oocyte adenylate cyclase activity in membranes from cells pretreated with IAP (Fig. 3), 10 μM progesterone was also able to overcome the inhibitory effects of IAP upon steroid-induced GVBD (Fig. 6). Thus, it appears that progesterone inhibition of oocyte adenylate cyclase is not mediated via the action of N_i, even though the inhibitory action of acetylcholine is mediated via N_i. Similar considerations apply to inhibition of the oocyte adenylate cyclase activity by the P site adenosine agonist, 2′,5′-DDA. The lack of effect of IAP on both progesterone and P site inhibition provides another indication of the similarity in the inhibitory mechanisms which mediate the actions of these two hormones. Progesterone inhibition of oocyte adenylate cyclase has been shown to involve a guanine nucleotide-binding protein (9, 16), and the actions of progesterone and 2′,5′-DDA are correlated with decreased levels of guanine nucleotide exchange (16). Combined with the results presented here and elsewhere (12, 35), this suggests that progesterone inhibition of oocyte adenylate cyclase may be mediated by either an altered rate of activation of N_s by guanine nucleotide or a unique interaction with N_i that is not abolished by IAP. Further investigation is required to clearly elucidate this novel mechanism for inhibition of adenylate cyclase.

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