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STUDIES OF A PLASMA MEMBRANE STEROID RECEPTOR IN *XENOPUS* OOCYTES USING THE SYNTHETIC PROGESTIN RU 486

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Summary—A steroid binding protein ($M_r = 110{,}000$) has previously been identified in the plasma membrane of *Xenopus laevis* oocytes by photoaffinity labeling with [^{3}H]R5020. In order to further characterize this steroid receptor, the photoaffinity labeled receptor protein was solubilized with 0.1% Brij 35. The solubilized labeled receptor yielded an approximate mol. wt of 102,000 ± 2,000 by sucrose density gradient centrifugation, suggesting that the solubilized receptor exists as a monomer. RU 486, a synthetic progestin antagonist for mammalian cytosolic receptor systems, inhibited up to 70% of [^{3}H] R5020 photoaffinity binding to the 110,000-Dalton receptor with an IC50 of 5 μM and induced germinal vesicle breakdown (GVBD) with an EC50 of 9.0 ± 0.6 μM. GVBD induced by RU 486 was slower than with progesterone, and RU 486 was less powerful than progesterone. Micromolar concentrations of RU 486 also potentiated GVBD induced by sub-optimal concentrations of progesterone or R5020. Furthermore, RU 486 inhibited oocyte plasma membrane adenylate cyclase with an apparent IC50 of 7.5 ± 2.5 μM. The close correlation of the EC50 value for RU 486 induction of GVBD with the IC50 values for inhibition of [^{3}H]R5020 photoaffinity labeling of the 110,000-Dalton receptor and inhibition of adenylate cyclase activity further supports the physiological significance of the oocyte plasma membrane steroid receptor.

INTRODUCTION

During meiotic maturation, large *Xenopus laevis* oocytes (Stage VI by the criteria of Dumont [1]), which are physiologically arrested in prophase I, are stimulated to undergo meiotic division and proceed to metaphase II upon treatment with progesterone or certain other steroids. An abundance of evidence has suggested that meiotic maturation (germinal vesicle breakdown, GVBD) in amphibian oocytes is initiated at the cell surface [2, 3]. Early evidence for the surface action of steroids in *Xenopus* oocytes includes the inability of microinjected progesterone to induce oocyte maturation [4, 5] and the ability of polymer-bound steroid to elicit the response when applied extra-cellularly [6, 7]. In addition, high concentrations of a number of different local anesthetics and cationic drugs can mimic the action of progesterone [8]. More recently, our laboratory demonstrated that progesterone inhibits oocyte plasma membrane adenylate cyclase by a mechanism that involves the guanine nucleotide regulatory protein [9], and this plasma membrane steroid action has been confirmed by other investigators [10, 11]. The oocyte system is the first example of a direct effect of steroids on a target cell enzyme system without a need for RNA or protein synthesis. The inhibition of oocyte adenylate cyclase by an inducing hormone like progesterone is consistent with previous experiments which demonstrated that the activity of cAMP-dependent protein kinase regulates the initiation of amphibian oocyte maturation [12].

Since hormonal regulation of adenylate cyclase is usually receptor mediated, the adenylate cyclase data suggested that a steroid receptor might be present in the oocyte plasma membrane. Equilibrium steroid binding studies using rapid filtration were difficult to interpret because of high levels of nonspecific binding to both the oocyte membrane sample and the glass fiber filters. As another method of investigation, [^{3}H]R5020 (17,21-dimethyl-19-norpregn-4,9-diene-3,10-dione, New England Nuclear) was previously used to photoaffinity label a steroid binding protein in oocyte plasma membrane samples [13]. After samples of oocyte plasma membranes were photoaffinity labeled with [^{3}H]R5020 a steroid receptor with an apparent mol. wt of 110,000 by SDS-polyacrylamide gel electrophoresis was identified [13]. In addition, the amount of [^{3}H]R5020 bound to the 110,000-Dalton steroid receptor after photolysis was correlated with the level of inhibition of adenylate cyclase activity and the EC50 for GVBD [13]. This suggested that the photolabeled receptor protein might be a specific and

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Abbreviations used: EC50, concentration of drug that causes 50% of the maximal response; GVBD, germinal vesicle breakdown; IC50, concentration of drug that causes 50% of the maximum level of inhibition of response; R5020, 17,21-dimethyl-19-norpregn-4,9-diene-3,10-dione (New England Nuclear); RU 486, 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)-estra-4,9-diene-3-one (Roussel Uclaf).

physiologically relevant steroid receptor on the surface of the oocyte.

One important criterion for a specific drug–receptor interaction is the demonstration that various receptor agonists and antagonists compete for available binding sites on the target cell. Recently, a new synthetic steroid, RU 486 (17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)estra-4,9-diene-3-one, Roussel Uclaf), has been developed. RU 486 has been shown to display binding affinity for both glucocorticoid and progestin receptors, and the steroid antagonizes the physiological actions of both types of receptor in the mammalian systems that have been studied. The affinity of RU 486 for glucocorticoid receptors in adrenalectomized rats is 2- to 3-fold higher than that of dexamethasone [14]. RU 486 has also been shown to affect ACTH secretion from rat adenohypophyseal cells both *in vivo* and *in vitro* [15]. Similar effects were demonstrated in humans [16, 17] and in cultured L-929 mouse fibroblasts [18]. In addition to the antiglucocorticoid activity of RU 486, it has also been shown to have a high affinity for the progesterone receptor. In mammalian cytosolic receptor systems, RU 486 has been described as a potent progestin antagonist that lacks any agonist activity [19, 20]. The powerful antiprogestin activity of RU 486, which causes uterine bleeding and induction of menses by direct action on endometrial tissue, has suggested the potential for clinical use of RU 486 as a post-coital or once-a-month contraceptive [21].

It was of interest to characterize the effect of RU 486 on the oocyte plasma membrane steroid receptor with respect to [^{3}H]R5020 binding, adenylate cyclase activity, and the biological response of the oocyte to steroid. The results indicate that RU 486 is a weak agonist in the oocyte membrane receptor system.

EXPERIMENTAL

Plasma membrane preparation

Sexually mature *Xenopus laevis* were primed with pregnant mare's serum gonadotropin (Sigma) and oocytes were manually dissected using watchmaker's forceps under a stereomicroscope, as previously described [22]. After incubating small pieces of ovary for 10 min in Buffer A (110 mM NaCl, 1 mM EDTA, 10 mM Hepes, pH 7.9) at room temperature, large (Stage VI) oocytes were manually dissected and stored in Buffer B (83 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM KCl, 10 mM Hepes, pH 7.9) until either membrane isolation, as previously described [9], or subsequent experimental manipulation, as described below. Each figure represents experimental results using oocytes from a separate animal, and each sample routinely contained plasma membranes from 10 oocytes (50 μg of total protein as measured by the method of Lowry *et al.* [23] using bovine serum albumin as a standard).

Photoaffinity labeling

Plasma membrane samples were collected in Pyrex centrifuge tubes (10 × 75 mm), centrifuged at 10,000 *g* for 10 min, and resuspended in 1 ml vol of Buffer B containing 2×10^{-8} M [^{3}H]R5020 (17α-methyl-[^{3}H]promegestone, 87 Ci/mmol, New England Nuclear) in the presence or absence of various concentrations of RU 486, as indicated in the figure legends. The samples were then photolyzed, as previously described [13]. The resulting photolabeled membrane samples were collected by centrifugation (10,000 *g* for 10 min), washed in ice-cold Buffer B, and subsequently analyzed by SDS-polyacrylamide gel electrophoresis or extracted with detergents, as described below.

SDS-Polyacrylamide Gel Electrophoresis

Washed membrane samples were resuspended in SDS-sample buffer (10% glycerol, 5% β-mercaptoethanol, 3% sodium dodecyl sulfate, 70 mM Tris, pH 6.8), warmed in a boiling water bath for 4 min, and analyzed by electrophoresis by the method of Laemmli [24] on either disc or slab gels of 8.5% polyacrylamide. Disc gel profiles were determined by serial sectioning, and the photolabeling of the 110,000-Dalton protein was quantitated, as previously described [13]. Slab gels were analyzed by slicing 1 cm sections of each sample lane centered around the position of the 110,000-Dalton protein as determined by Coomassie Blue staining of companion lanes containing molecular weight standards. Radioactivity in gel slices was measured by liquid scintillation spectrometry, and the results are expressed as the total amount of [^{3}H] R5020 covalently bound to the 110,000-Dalton protein.

Detergent extraction

Photolabeled membrane preparations were extracted by gentle shaking for 1 h at 4°C in 100 mM NaCl, 10 mM Tris, pH 7.4, containing various detergents, as indicated in the legend to Fig. 1. Samples were then centrifuged for 15 min at 23,000 *g* and the resulting supernatants, referred to below as the detergent extracts, were used for molecular weight determination by sucrose density gradient centrifugation or analyzed by SDS-polyacrylamide gel electrophoresis. Prior to gel electrophoresis, detergent extracts were dialyzed overnight against 2 l of 10 mM Tris, pH 7.4.

Sucrose density gradient centrifugation

Detergent extracts from photolabeled membranes were layered onto sucrose step gradients (5–20% sucrose in 100 mM NaCl, 4 mM NaH_2PO_4, 1 mM Na_2HPO_4, 10% glycerol, pH 7.4 at 4°C) prepared 16 h prior to use. Following centrifugation at 4°C for 22 h at 45,000 rpm (Beckman SW 50.1 rotor), the gradients were fractionated using a peristaltic pump.

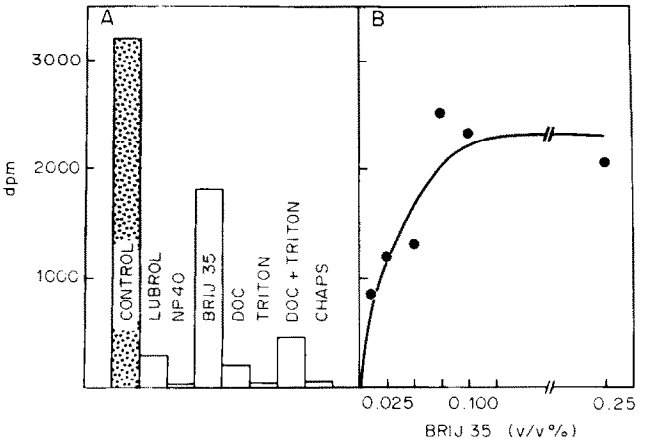

Fig. 1. Detergent extraction of the photolabeled 110,000-Dalton protein. Plasma membrane samples were prepared as outlined in Experimental, resuspended in 1 ml vol of Buffer A containing 2×10^{-8} M [^{3}H]R5020 and photolysed for 10 min. After detergent solubilization, the amount of extracted photolabeled protein was subsequently determined by SDS-polyacrylamide gel electrophoresis of lyophilized detergent extracts. A. Selective solubilization of the 110,000-Dalton photolabeled protein with Brij 35. Photolabeled membrane samples (20 membranes/tube, 100 μg total protein) were extracted for 1 h on ice with shaking in 0.25 ml vol of 100 mM NaCl, 10 mM Tris, pH 7.4, containing 0.1% solutions of various detergents. After centrifugation (15,000 *g* for 20 min), the resulting supernatants were dialyzed and analyzed by SDS-polyacrylamide gel electrophoresis using disc gels to determine the total amount of photolabeled 110,000-Dalton protein extracted by each detergent treatment. (Control, photolabeled membrane sample prior to detergent extraction; CHAPS, 3((3-cholamido-propyl) dimethylammonio)propane sulfonate (Pierce); DOC, deoxycholate (Sigma); Lubrol, Lubrol PX (Sigma); NP40, Nonidet p40 (Particle Data Laboratories, Ltd); Triton, Triton X-100 (Sigma)). B. Concentration dependent solubilization of the 110,000-Dalton protein with Brij 35. Photolabeled membrane samples were combined, homogenized in Buffer A and extracted for 1 h on ice with 0.25 ml of 100 mM NaCl, 10 mM Tris, pH 7.4, containing various concentrations of Brij 35. The resulting supernatants were analyzed by SDS-polyacrylamide gel electrophoresis using slab gels, and the results are plotted as the amount of radioactivity associated with the 110,000-Dalton protein as a function of Brij 35 concentration.

Radioactivity in aliquots of each fraction was measured by liquid scintillation spectrometry, and the relative protein content of each fraction was determined by spectrophotometric measurement of absorbance at 280 nm. Fractions containing the peak of radioactivity were pooled, lyophilized, and analyzed by SDS-gel electrophoresis to verify that the peak of radioactivity identified by sucrose gradient centrifugation co-migrated with the 110,000-Dalton protein.

Steroid induced maturation (GVBD)

Groups of 20 oocytes were exposed to various steroids, as indicated in the figure legends, in 3 ml vol of Buffer B. At progressive time points after steroid addition, each group of oocytes was scored for meiotic maturation. The criterion for cell division was the appearance of a white spot on the animal pole, an indicator of GVBD, and all responses were verified by manual dissection of oocytes after fixation in 10% trichloroacetic acid.

Adenylate cyclase assay

Adenylate cyclase activity in oocyte membrane samples was measured, as previously described [9], with the exception that 0.15 mM Gpp(NH)*p* was included in the assay mixture in place of GTP. The assay was initiated by addition of [α-^{32}P]ATP, and the assay time was 60 min at 30°C.

RESULTS

In order to determine the molecular weight of the photolabeled oocyte plasma membrane steroid receptor by sucrose density gradient centrifugation, the receptor was first solubilized with detergent. A number of different detergents, either alone or in combination, were tested for their abilities to solubilize the 110,000-Dalton protein from photolabeled oocyte plasma membrane samples. As shown in Fig. 1A, approx 45% of the photolabeled 110,000-Dalton protein was extracted from the oocyte membrane preparation with a 0.1% solution of Brij 35 in 100 mM NaCl, 10 mM Tris, pH 7.4. By comparison, none of the other detergents tested were as effective as Brij 35. To optimize the efficiency of detergent extraction with Brij 35, the concentration dependence of Brij 35 extraction was tested, and the results are shown in Fig. 1B. The maximum level of receptor extraction was achieved with 0.075–0.1% Brij 35, and subsequent samples of solubilized receptor were prepared using 0.1% Brij 35.

Using sucrose gradient centrifugation, an approximate molecular weight for the solubilized, labeled receptor was calculated [25] to be 102,000 $\pm$ 2,000 ($n = 10$, $\bar{x} \pm$ SEM) by comparison with known molecular weight standards. This molecular weight value is based upon the assumption that the solu-

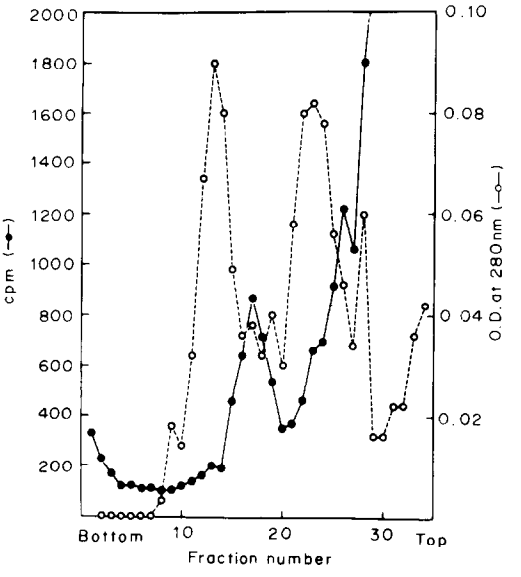

Fig. 2. Sucrose gradient centrifugation of detergent solubilized steroid receptor. Plasma membrane samples were prepared, photoaffinity labeled with [^{3}H]R5020, and detergent extracted with 0.1% Brij 35, as described under Experimental. The detergent extract was then combined with molecular weight standards (Pharmacia, aldolase and ovalbumin, mol. wt 158,000 and 45,000, respectively) and analyzed by sucrose density gradient centrifugation on a 5–20% sucrose gradient, as described in Experimental. Shown here is a representative gradient profile for the radioactivity (●) and the optical density (A at 280 nm, ○).

bilized receptor is a globular protein. When sucrose gradient fractions corresponding to the peak of radioactivity (fractions 16–18 in Fig. 2) were pooled and analyzed by SDS-polyacrylamide gel electrophoresis, the radioactivity comigrated with the 110,000-Dalton protein from photolabeled membrane samples that had not undergone detergent extraction (data not shown). Furthermore, in order to verify that the 5–20% sucrose density gradients used for the molecular weight determinations were isokinetic, the migration profiles of the molecular weight standards and the labeled receptor were measured after two different times of centrifugation (16 and 22 h). After both periods of centrifugation, the molecular weight of the photolabeled receptor was calculated to be approx 102,000. Figure 2 shows a representative gradient profile after 22 h of centrifugation.

If the 110,000-Dalton steroid receptor that has been identified in the oocyte plasma membrane by photoaffinity labeling is the progestin receptor that triggers maturation, then an "antiprogestin" like RU 486 that has been shown to inhibit steroid binding in other cell systems might be predicted to inhibit both photoaffinity labeling of the steroid surface receptor and the steroid induced response (GVBD). Competitive binding studies revealed that RU 486 did inhibit photolabeling of the 110,000-Dalton receptor in a concentration dependent manner. As shown in Fig. 3, RU 486 inhibited up to 70% of [^{3}H]R5020 photolabeling of the 110,000-Dalton protein with an IC50 of 5 μM. Possible explanations for the ability of RU 486 to inhibit only 70% of the photoaffinity labeling are the limited solubility of the steroid in aqueous solution and the low affinity of the oocyte membrane receptor for the steroid. Nonetheless, it appears that RU 486 interacts with the membrane receptor in the amphibian oocyte, since it antagonizes photoaffinity labeling of the 110,000-Dalton protein with [^{3}H]R5020.

Since in mammalian systems RU 486 has been described as a progestin antagonist devoid of any agonist activity [19,20], it was hypothesized that RU 486 might antagonize the steroid induced response in the oocyte (GVBD). This proved not to be the case. Even though RU 486 inhibited photoaffinity labeling of the 110,000-Dalton receptor, RU 486 induced GVBD in a dose dependent manner, as shown in Fig. 4. The maturation response elicited by extracellularly applied RU 486 was extremely variable with the percent maximum response ranging from 30 to 95%, and in oocyte populations from some animals RU 486 did not induce GVBD. This observed variability in response could be explained by either between-animal variation, since oocytes from different donor frogs were used in each experiment, or by impurities in the drug preparation. In oocyte populations that did respond to treatment with RU 486, the mean maximum response to Ru 486 was $65 \pm 10\%$ ($\bar{x} \pm$ SEM, $n = 4$). This compares to a mean maxi-

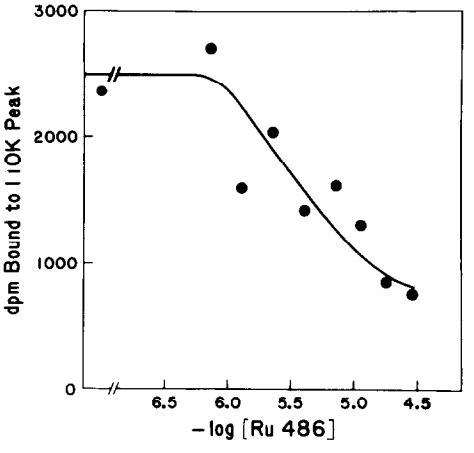

Fig. 3. Inhibition of photoaffinity labeling of the 110,000-Dalton protein with RU 486. Aliquots of homogenized oocyte plasma membrane were preincubated for 15 min at room temperature in the presence of various concentrations of RU 486 (Roussel Uclaf). After addition of 2×10^{-8} M [^{3}H]R5020, the samples were incubated for 15 min at room temperature in the dark and photolyzed for 2 min. After centrifugation (15,000 *g* for 15 min), the resulting membrane pellet was washed once with ice cold Buffer B, and covalent binding to the 110,000-Dalton protein was quantitated by SDS-polyacrylamide gel electrophoresis. The results are expressed as the amount of radioactivity bound to the 110,000-Dalton protein as a function of the concentration of RU 486 in the preincubation mixture.

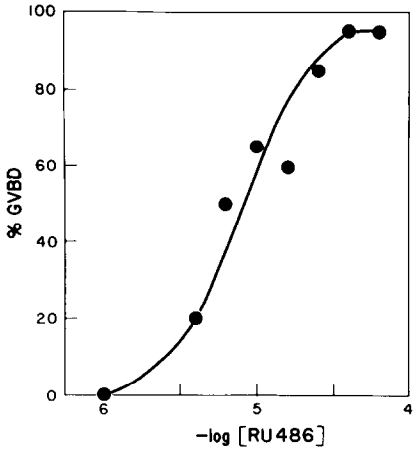

Fig. 4. Dose–response curve for RU 486 induction of meiotic maturation. Groups of 20 oocytes were treated with various concentrations of RU 486, as described in Experimental, and each group was scored for meiotic maturation by manual dissection of acid fixed cells 20 h after steroid addition. The results are expressed as the percentage of the cell population that exhibited a white spot and lacked an intact germinal vesicle (%GVBD) as a function of the concentration of RU 486.

mum response of $97 \pm 3\%$ GVBD in companion oocyte samples that were treated with progesterone. Even though the maximum response to treatment with RU 486 in different experiments was variable, the EC50 for GVBD was highly reproducible. RU 486 stimulated oocyte maturation with an EC50 of $9.0 \pm 0.6\,\mu M$ ($\bar{x} \pm$ SEM, $n = 4$). Furthermore, oocyte maturation in response to RU 486 was slower than the progesterone-induced response. In oocyte populations that did respond to RU 486, GVBD was not seen until after control cells treated with progesterone had completed GVBD (see Fig. 5). Routinely, dose–response curves for RU 486 induction of GVBD were obtained by manual dissection of steroid treated oocytes that were fixed with 10% trichloroacetic acid 20 h after steroid addition. Figure 4 shows a representative dose–response curve for a responsive population of oocytes.

Since RU 486 acted as a weak agonist in the oocyte system, it was conceivable that RU 486 might inhibit germinal vesicle breakdown induced by suboptimal concentrations of more potent inducing steroids. It was hypothesized that this inhibitory effect might be displayed as either a slowing or inhibition of the maturation response, and the experiments shown in Figs 5 and 6 were designed to test this hypothesis. The effect of $14.5\,\mu M$ RU 486 on the time course of progesterone induced maturation is shown in Fig. 5A. In this oocyte population, RU 486 alone stimulated GVBD in a small fraction of the oocytes at relatively late time points during the time course shown. The maturation response elicited by $10\,\mu M$ progesterone (an optimal concentration) was complete by 4 h 45 min, and the time course for GVBD stimulated by a suboptimal concentration of progesterone ($0.1\,\mu M$) was intermediate between the optimal progesterone induced response and the slow RU 486 induced response. When RU 486 was combined with the sub-optimal concentration of progesterone ($0.1\,\mu M$), the GVBD response was potentiated rather than inhibited. Ethinyl estradiol has been shown previously to act as a weak agonist in the oocyte system [26], and the estrogen had a similar effect on the maturation response (Fig. 5B). This potentiation of the maturation response was not due to displacement of inducing steroid from nonspecific binding sites in the incubation dishes, since $20\,\mu M$ triamcinolone acetonide, a non-inducing steroid, did not potentiate the maturation response, even though it would be expected to cause a similar displacement of progesterone from nonspecific steroid binding sites on the incubation dish (data not shown).

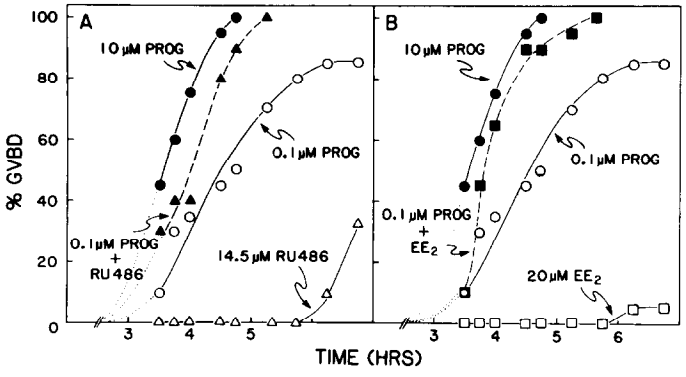

Fig. 5. Potentiation of the time course of GVBD induced by a sub-optimal concentration of progesterone. Groups of 20 oocytes were treated with various steroids either alone or in combination, as described in Experimental. At various time points after steroid addition, the oocyte samples were scored for response (%GVBD) and the results are expressed as the percentage of the oocytes responding as a function of time. A. Effect of RU 486 on progesterone induced oocyte maturation. (●, $10\,\mu M$ progesterone (PROG); 0, $0.1\,\mu M$ progesterone; △, $14.5\,\mu M$ RU 486; ▲, $0.1\,\mu M$ progesterone + $14.5\,\mu M$ RU 486). B. Effect of ethinyl estradiol on progesterone induced oocyte maturation. (●, $10\,\mu M$ progesterone (PROG); 0, $0.1\,\mu M$ progesterone; □, $20\,\mu M$ ethinyl estradiol (EE_2); ■, $0.1\,\mu M$ progesterone + $20\,\mu M$ EE_2).

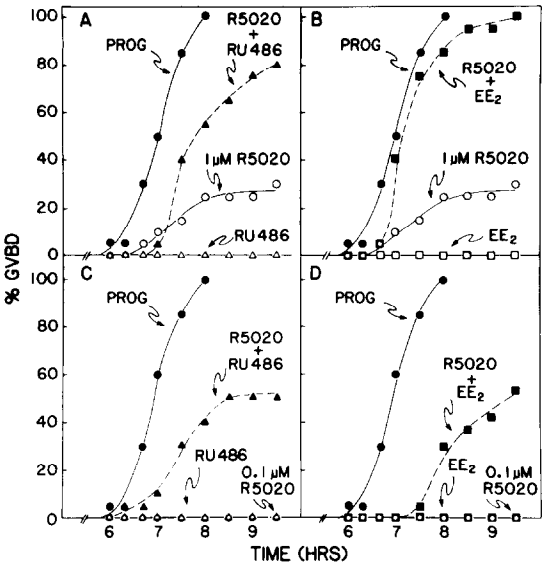

Fig. 6. Potentiation of the time course of GVBD induced by sub-optimal concentrations of R5020. Groups of 20 oocytes were treated with various steroids, as described for Fig. 5. A. Effect of RU 486 on oocyte maturation induced by 1 μM R5020. (●, 10 μM progesterone (PROG); ○, 1 μM R5020; △, 14.5 μM RU 486; ▲, 1 μM R5020 + 14.5 μM RU 486). B. Effect of ethinyl estradiol on oocyte maturation induced by 1 μM R5020. (●, 10 μM progesterone (PROG); ○, 1 μM R5020; □, 20 μM ethinyl estradiol (EE_2); ■, 1 μM R5020 + 20 μM EE_2). C. Effect of RU 486 on oocyte maturation induced by 0.1 μM R5020. (●, 10 μM progesterone (PROG); ○, 0.1 μM R5020; △, 14.5 μM RU 486; ▲, 0.1 μM R5020 + 14.5 μM RU 486). D. Effect of ethinyl estradiol on oocyte maturation induced by 0.1 μM R5020. (●, 10 μM progesterone (PROG); ○, 0.1 μM R5020; □, 20 μM ethinyl estradiol (EE_2); ■, 0.1 μM R5020 + 20 μM EE_2).

The effects of RU 486 and ethinyl estradiol on the time course of oocyte maturation induced by a sub-optimal concentration of R5020 were very similar to their effects upon maturation induced by progesterone, as shown in Fig. 6.

In this population of oocytes, 1 μM R5020 stimulated only 30% GVBD, and there was no GVBD measured in response to either RU 486 or ethinyl estradiol (Figs 5A and 5B). When either RU 486 or ethinyl estradiol was combined with the sub-optimal concentration of R5020, the time course of GVBD approached that seen in the control group of oocytes treated with 10 μM progesterone. This potentiating effect was also seen with lower concentrations of R5020. When oocytes were treated with 0.1 μM R5020 alone, there was no maturation response; but, when 0.1 μM R5020 was combined with either RU 486 or ethinyl estradiol, the cells were stimulated to undergo GVBD, as shown in Figs 6C and 6D. Once again, the potentiation was not due to displacement of inducing steroid from nonspecific binding sites on the plastic dishes, since potentiation of the maturation response was not seen with triamcinolone acetonide, a non-inducing steroid (data not shown). Thus, RU 486 did not antagonize steroid induced oocyte maturation, but instead potentiated maturation stimulated by sub-optimal concentrations of other inducing steroids.

Since RU 486 inhibited [^{3}H]R5020 binding (Fig. 3) and acted as a weak agonist for GVBD (Fig. 4), it was of interest to measure the effect of RU 486 on oocyte adenylate cyclase. Previously, inducing steroids like progesterone [9–11] and R5020 [13] have been shown to inhibit oocyte plasma membrane adenylate cyclase activity. Since RU 486 binds to the 110,000-Dalton receptor and induces the physiological response, it was predicted that RU 486 would also have an inhibitory effect on the oocyte adenylate cyclase. Concentration dependent inhibition of oocyte plasma membrane adenylate cyclase by RU 486 is shown in Fig. 7. RU 486 maximally inhibited 50% of Gpp(NH)*p*-stimulated adenylate cyclase activity. This level of inhibition is comparable to the maximum levels of inhibition reported previously for progesterone [9, 13]. The apparent IC50 for inhibition of oocyte adenylate cyclase activity by the synthetic steroid, i.e. the concentration of drug that caused 50% of the maximum observed level of inhibition, was 7.5 ± 2.5 μM RU 486 ($\bar{x}$ ± half-range, $n = 2$). This value is similar to the concentration of RU 486 required for induction of GVBD or displacement of R5020.

DISCUSSION

It has previously been demonstrated that progesterone and other inducing steroids inhibit the oocyte plasma membrane adenylate cyclase [9–11]. In comparison to other cell systems in which inhibitory regulation of adenylate cyclase has been studied, one unique aspect of the oocyte system is the ability of progesterone to inhibit Gpp(NH)*p*-stimulated enzyme activity [9, 27]. It has also been shown that

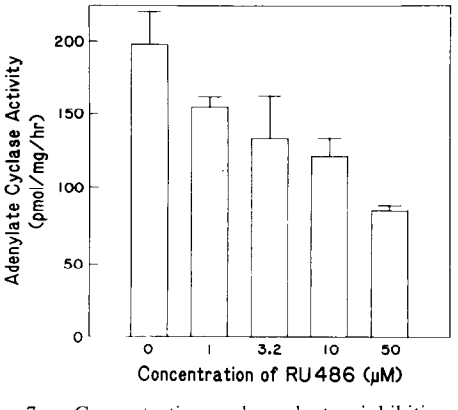

Fig. 7. Concentration dependent inhibition of Gpp(NH)*p*-stimulated oocyte plasma membrane adenylate cyclase by RU 486. Adenylate cyclase activity was measured in triplicate samples of oocyte plasma membrane in the presence of increasing concentrations of RU 486, as described in Experimental ($\bar{x}$ ± SEM). When the results of two separate experiments were pooled, the mean IC50 value was 7.5 ± 2.5 μM RU 486 ($\bar{x}$ ± half-range).

inhibition of oocyte plasma membrane adenylate cyclase by progesterone is associated with slowing of guanine nucleotide exchange [27]. This inhibition of adenylate cyclase appears to be receptor mediated, since a steroid receptor was identified on the oocyte plasma membrane and photoaffinity labeling of the putative steroid receptor was correlated with the level of inhibition of oocyte plasma membrane adenylate cyclase activity [13]. This previous study also suggested a possible cooperativity of steroid binding [13], and cooperativity of steroid binding to *Rana* oocyte plasma membrane–vitelline envelope complexes has also been reported [28]. In order to more fully characterize the nature of the previously identified oocyte plasma membrane steroid receptor and to further substantiate the role of this receptor as a progestin receptor, the molecular weight of solubilized, photoaffinity, labeled receptor was determined by sucrose density gradient centrifugation, and the effects of RU 486 were measured.

When photolabeled steroid receptor was extracted from photoaffinity labeled oocyte plasma membrane preparations with 0.1% Brij 35, the solubilized steroid–receptor complex migrated on 5–20% sucrose density gradients with an approx mol. wt of 102,000 (Fig. 2). This molecular weight is in agreement with the mol. wt of 110,000 previously measured by SDS-polyacrylamide gel electrophoresis [13], and suggests that the solubilized receptor exists as a monomer.

In contrast to mammalian cytosolic steroid receptor systems in which RU 486 has been described as a pure progestin antagonist that lacks any agonist activity [19, 20], the synthetic steroid functions as a weak agonist in the intact amphibian oocyte. RU 486 inhibits [^{3}H]R5020 photoaffinity labeling of the 110,000-Dalton steroid receptor in oocyte plasma membrane samples with an IC50 of 5 μM RU 486 (Fig. 3). Moreover, RU 486 stimulates slow, submaximal levels of oocyte maturation with an EC50 of approx 9 μM (Figs 4 and 5). The EC50 for progesterone induced GVBD was previously reported as 3×10^{-7} M when the oocytes were exposed to steroid for 30 min [13], and upon constant exposure to progesterone the EC50 is approx 3×10^{-8} (data not shown). This difference in EC50 values is a consequence of continuous uptake and metabolism of progesterone prior to GVBD [2]. Since RU 486 is less powerful and less potent than progesterone at stimulating cell division, it can be classified as a weak agonist in the amphibian oocyte. This is in contrast to its characterization as a potent antagonist in mammalian systems and suggests that the steroid binding site in the oocyte plasma membrane is different from that previously characterized in mammalian systems. It is not surprising that high concentrations of a drug that acts as a potent antagonist in one cell system might act as a weak agonist in another cell system. Furthermore, as would be predicted for an inducing steroid, RU 486 also inhibited oocyte plasma membrane adenylate cyclase in a concentration dependent manner with an IC50 of 7.5 μM (Fig. 7).

The close correlation between the IC50 value for inhibition of photolabeling, the EC50 for induction of oocyte maturation, and the IC50 for inhibition of oocyte plasma membrane adenylate cyclase by RU 486 supports the hypothesis that inducing steroids stimulate oocyte maturation via receptor-mediated inhibition of the oocyte adenylate cyclase. This correlation of hormone binding with both the physiological response and inhibition of a regulatory enzyme system further supports the physiological significance of hormone binding to the 110,000-Dalton protein as the trigger for oocyte maturation, but the correlation of hormone binding with response is not conclusive evidence for a cause and effect relationship. A clear demonstration that hormone binding to the 110,000-Dalton receptor is the real trigger for oocyte maturation awaits the development of a progestin antagonist that antagonizes both binding of inducing hormone to the 110,000-Dalton receptor and hormone induction of the physiological response, GVBD.

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