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MODULATION OF QUAIL OVIDUCT ADENYLATE CYCLASE ACTIVITY BY ESTRADIOL AND PROGESTERONE

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Summary—Oviduct adenylate cyclase activity of the quail was measured by radiochemical analysis following different hormonal treatments. A single injection of estradiol benzoate (EB) to immature female quails resulted in a prereplicative surge of adenylate cyclase activity. A second surge of enzyme activity was observed during the proliferative phase induced by EB. Estradiol-17α, estrone, estriol and testosterone were ineffective. Tamoxifen completely inhibits the growth-promoting effect of EB and the second surge of adenylate cyclase activity but does not inhibit the prereplicative increase of enzyme activity. This prereplicative increase of adenylate cyclase activity was also observed, even in the absence of increased plasma estradiol, when estradiol-17β (E_2) was perfused through the hepatic portal vein. Moreover, E_2 had no effect on enzyme activity when added directly to the oviduct homogenate preparation, at concentrations ranging from 10^{-9} to 10^{-7} M. In response to progesterone injection, oviduct adenylate cyclase activity followed a different pattern, beginning its increase after 3 h and remaining elevated up to 24 h.

The activation by estradiol was independent of the presence of guanylylimidodiphosphate. Moreover, the enzyme was more sensitive to forskolin at submaximal concentration in estradiol treated birds than in control. These results demonstrate that transient activation of adenylate cyclase at the early stages of the action of estradiol does not occur through the classic nuclear receptor-gene activation pathway or a membrane receptor mediated process, but involves an indirect pathway, yet to be defined.

INTRODUCTION

The role of cAMP in cell proliferation has been widely explored in a variety of cells in culture or in intact animals (for review see [1]). Increase in cAMP concentration following estradiol injection was first described in the rat uterus by Szego and Davis[2] and since then, several reports have suggested that cAMP was involved in the proliferative activation of estrogen target cells in mammals [3–10]. In addition, the ability of steroid hormones to influence adenylate cyclase activity has been reported in rat and mice uteri [11–14] and in human endometrium [15].

In birds, the effects of estrogen and progesterone on the adenylate cyclase system of the oviduct were first investigated by Rosenfeld and O'Malley[16]. They showed that progesterone produced a delayed and progressive activation of adenylate cyclase and an increase in concentration of oviduct cAMP whereas diethylstilbestrol was inactive from 30 s to 120 min following administration to chicks, suggesting that growth and differentiation of this target tissue was not mediated by cAMP. More recently, we have demonstrated that estradiol indirectly stimulates the proliferation of quail oviduct epithelial cells *in vivo* [17] and that this indirect mechanism involves the cAMP regulatory systems [18–19]. Estradiol induces first a transient surge in oviduct cAMP concentration within the prereplicative period and then a sustained decrease. This drop in oviduct cAMP content is due to the rise in cAMP phosphodiesterase activity. Moreover, the activation of this enzyme is estrogen specific and closely linked to the proliferative response. However, activity of this enzyme does not significantly change between 0 and 12 h after estradiol injection, indicating that regulation of adenylate cyclase activity can not be excluded to explain the early rise in cAMP level. On the other hand, progesterone induces a sustained increase in oviduct cAMP concentration without any significative effect on cAMP phosphodiesterase activity [18–19].

In the light of these findings, we investigated the properties of adenylate cyclase in the quail oviduct and the ability of estradiol, progesterone and other steroid hormones to modulate its activity.

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EXPERIMENTAL

Materials

The reagents used and their sources were: ATP disodium salt, cAMP, creatine kinase and 5'-guanylyl imidodiphosphate (Boehringer); neutral alumina activity I (70–230 mesh), sodium fluoride and $MgCl_2$ (Merck); creatine phosphate and forskolin (Calbiochem, La Jolla, Calif.); imidazole (Fluka) and sodium dodecylsulfate (Sigma). Estradiol-17β (E_2), estradiol benzoate (EB), estradiol-17α (E-17α), estriol (E_3), estrone (E_1), progesterone (P_4) and testosterone (T) were purchased from Sigma Chemical Company (St Louis, Mo., U.S.A.). Tamoxifen [TAM: Trans-1-(4β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] was a gift generously provided by ICI Ltd Pharmaceutical division (Alderley Park, Macclesfield, England). [α-^{32}P]ATP (sp. act.: 0.37–1.85 TBq/mmol) and [^{3}H]cAMP (sp. act.: 1.08 TBq/mmol) were purchased from the Radiochemical Center (Amersham, England).

Animals and tissue collection

Immature female quails 15–23-day-old (*Coturnix coturnix japonica*) were used for these experiments. They were raised in groups at a constant temperature (23°C) in a daily cycle of light and darkness (14 h of light). Chicken powder chow (UAR 115, UAR Villemoisson, France) and water were provided *ad libitum*. Steroid hormones were injected i.m., dissolved in olive oil, in a total volume of 0.1 ml. Control animals received only the vehicle.

For perfusion experiments, 30-day-old ovariectomized quails were used and estradiol-17β (E_2: 4 μg/ml dissolved in 5% ethanol in saline) was perfused through the jugular or the hepatic portal vein (perfusion rate: 30 μl/h) as described previously [17]. E_2 plasma concentrations were determined by radioimmunoassay [17].

Animals were sacrificed by decapitation. The oviduct was quickly removed, weighed and homogenized in cold 25 mM Tris–HCl buffer (pH = 7.5) containing 1 mM EDTA (1 ml for 25 mg tissue) using a glass Dual homogenizer (Kontes) for 20 s at 4°C. Aliquots of the homogenate were used to determine adenylate cyclase activity and DNA concentration according to the method of Labarca and Paigen [20].

Adenylate cyclase assay

Adenylate cyclase activity was measured according to White and Zenser [21] and Ramachandran [22] using the rate of conversion of [α-^{32}P]ATP to [^{32}P]cAMP as a measure of the enzymatic activity. The assay mixture contained, in a final volume of 50 μl: 50 mM Tris–HCl (pH = 7.5), 5 mM $MgCl_2$, 1 mM cAMP, 20 mM creatine phosphate, 150 U/ml creatine kinase, 0.1 mM ATP, [α-^{32}P] ATP (1.2×10^6 cpm), [^{3}H]cAMP (10^4 cpm) as a recovery standard and the indicated concentrations of drugs or reagents. Each assay mixture was preincubated for 30 s at 33°C, and the reaction was initiated by the addition of 50 μl of the homogenate and proceeded for 10 min at 33°C. The reaction was stopped by adding 100 μl of a solution containing 40 mM ATP, 10 mM cAMP and 20 mg/ml SDS and boiling for 5 min. The [^{32}P]cAMP formed during incubation was isolated by adsorption chromatography using a 2 g neutral alumina column: the assay was diluted with 250 μl of distilled water, deposed on the column; cAMP was then eluted with 3 ml of 0.1 M imidazole (pH = 7.5) and counted. Analysis of the imidazole eluate by HPLC showed that no other [^{32}P] product than cAMP was detectable. In control assays, conducted in the presence of 50 μl boiled homogenate, the ^{32}P-radioactivity found in the chromatography eluate was very reproducible and never exceeded 10% of the radioactivity formed during the corresponding assay. Recovery of the [^{3}H]cAMP was checked to be greater than 80%.

Statistical analysis

Non parametric statistics (Mann–Whitney, Kruskal–Wallis tests) were used.

RESULTS

Characterization and properties of oviductal adenylate cyclase

Samples of quail oviduct homogenates incubated with ATP and an ATP regenerating system catalysed the synthesis of cAMP. After centrifugation of the homogenate at 2000 *g* for 1 h at 4°C, more than 90% of the activity was recovered in the supernatant. After centrifugation of this low-speed supernatant at 105,000 *g* for 1 h at 4°C, only marginal activity was detected in the supernatant indicating that, as in most tissues, oviduct adenylate cyclase is a membrane-bound enzyme.

The time-course of the assay was determined using oviduct homogenates of low (20 mg/ml) and high (40 mg/ml) tissue concentrations for incubation periods of 0–30 min. The reaction rate was linear with time in both systems for the first 10 min (data not shown). Moreover, during a 10-min incubation period, adenylate cyclase activity increased linearly with tissue concentrations in the homogenate between 5 and 50 mg/ml (data not shown). Thus a tissue concentration of 25 mg/ml and a 10-min incubation period were used for all subsequent assays.

Maximal enzymatic activity was measured using 2 mM ATP (Fig. 1). When ATP concentration was greater than 2 mM enzyme activity was inhibited. This inhibition was overcome by increasing the Mg^{2+} concentration [23]. As calculated from Fig. 1, the apparent K_m and the maximum velocity (V_{max}) values for ATP in the presence of 5 mM Mg^{2+} were 0.26 mM and 10.37 pmol cAMP/min/assay respectively. The effect of Mg^{2+} concentration is shown in Fig. 2. In the absence of Mg^{2+}, no activity was detected. The K_a for Mg^{2+}, estimated from Fig. 2 by Lineweaver and Burk

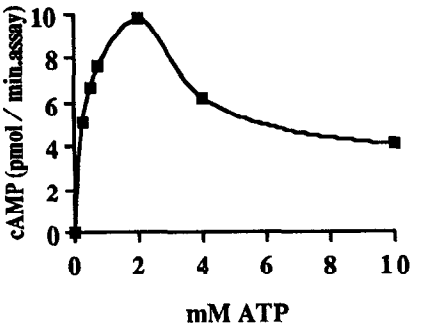

Fig. 1. Oviduct adenylate cyclase activity versus ATP concentration. A pooled sample of oviducts from immature quails was used. All values are the means of triplicate determinations. Other details are as given in Experimental.

analysis, was 1.7 mM. Several activators were then used: NaF and Gpp(NH)p known to act through an interaction with the regulatory G/F subunit [24–26] and forskolin, a direct activator of the catalytic subunit of adenylate cyclase [27]. The results are presented in Table 1. The activity was very sensitive to the addition of Gpp(NH)p, NaF and forskolin. The response to NaF was concentration dependent, and maximal activation was measured at 10 mM NaF. Forskolin also stimulated the activity in a concentration-dependent relationship, leading to an 8-fold increase in the rate of synthesis of the cyclic nucleotide at 100 μM forskolin.

Hormonal sensitivity of oviductal adenylate cyclase

A single injection of estradiol benzoate (EB: 0.1 mg/kg, i.m.) to immature 21-day-old female quails resulted in a proliferative response of the oviductal cells. DNA content (Fig. 3) of the oviduct increased between 12 and 24 h after the injection, remained high between 24 and 48 h and then declined to return to its initial value after 72 h.

Following EB administration, a prereplicative surge of adenylate cyclase activity was observed (Fig. 3). The adenylate cyclase activity started to increase after 3 h, peaked at 6 h and then dropped to control level at 12 h. A second surge of enzyme activity was observed during the proliferative phase. When 21-day-old quails were injected with only 0.01 mg/kg EB, oviduct DNA content and adenylate cyclase activity were not affected by the treatment. Moreover, when 15-day-old animals were injected with 0.01 mg/kg or 0.1 mg/kg EB, no proliferative activity or increase in adenylate cyclase activity was observed (data not shown).

To clarify further the effect of estradiol, basal, Gpp(NH)p stimulated, forskolin stimulated and

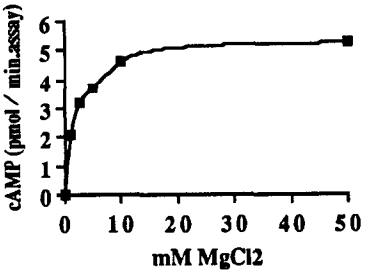

Fig. 2. Oviduct adenylate cyclase activity versus Mg^{2+} concentration. A pooled sample of oviducts from immature quails was used. All values are the means of triplicate determinations. Other details are as given in Experimental.

Table 1. Basal and stimulated adenylate cyclase activity of quail oviduct

Conditions		Adenylate cyclase activity (pmol cAMP/min·mg tissue)
Basal		1.936 ± 0.065
Gpp(NH)p	0.1 mM	3.262 ± 0.153
NaF	1 mM	1.932 ± 0.070
NaF	2.5 mM	1.934 ± 0.042
NaF	5 mM	2.996 ± 0.073
NaF	10 mM	3.268 ± 0.067
NaF	20 mM	3.266 ± 0.047
NaF	50 mM	2.125 ± 0.042
Forskolin	0.1 μm	1.913 ± 0.072
Forskolin	1 μm	4.354 ± 0.349
Forskolin	5 μm	7.355 ± 0.739
Forskolin	10 μm	10.097 ± 0.157
Forskolin	100 μm	16.078 ± 0.474

Five different pooled samples of oviducts from immature quails were used. Values are the means ± SEM.

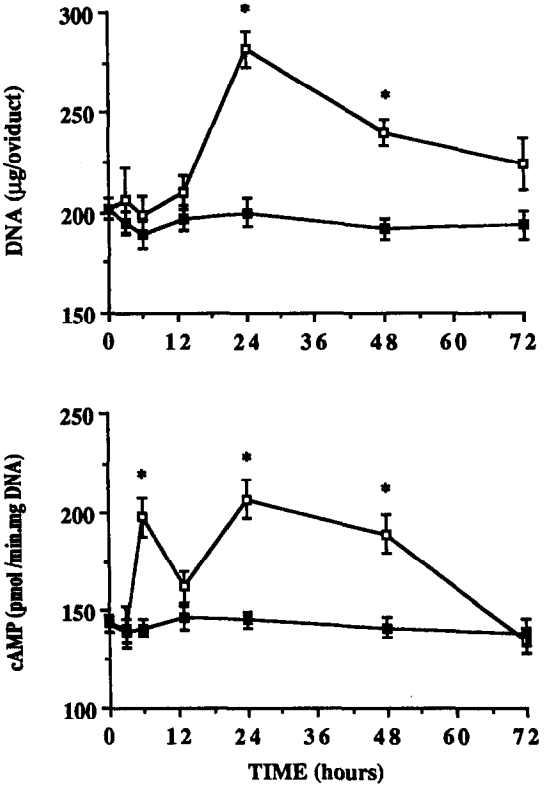

Fig. 3. Effect of EB on oviduct DNA content and adenylate cyclase activity. Immature quails were injected with either 0.1 mg/kg EB (□) or vehicle (■) and oviducts were excised at the designated times. All animals were 21-days-old when killed. Oviduct DNA content (upper panel) and adenylate cyclase activity (lower panel) were measured as described in Experimental. All values are the means ± SEM of at least 6 animals per group. *Significantly different from control ($P < 0.01$).

Table 2. Effect of estradiol benzoate on adenylate cyclase activity in the presence and absence of Gpp(NH)p, forskolin or Gpp(NH)p + forskolin

Conditions	Adenylate cyclase activity (pmol cAMP/min · mg DNA) Control	EB-treated
Basal	145 ± 10	202 ± 18*
+ Gpp(NH)p	224 ± 10	325 ± 27*
+ Forskolin (1 μM)	359 ± 31	627 ± 51*
+ Gpp(NH)p + forskolin (1 μM)	484 ± 40	823 ± 69*
+ Forskolin (100 μM)	1327 ± 270	1386 ± 288
+ Gpp(NH)p + forskolin (100 μM)	1151 ± 66	1205 ± 156

Results are mean ± SD of 4 duplicate independent determinations for each group.
21-day-old quails were injected with either 0.1 mg/kg EB (EB treated) or the vehicle (control) 6 h before sacrifice.
Activators were added to the assay mixture at a final concentration of 100 μM for Gpp(NH)p and 1 μM or 100 μM for forskolin. *Significantly different from the corresponding control ($P < 0.05$).

Gpp(NH)p + forskolin stimulated adenylate cyclase activities were measured in 21-day-old control and 0.1 mg/kg EB-treated animals (Table 2). In the EB-treated group, basal activity was increased by 39%, which agreed with previous results. With a concentration of forskolin (100 μM) which maximally activated the enzyme, the activity was the same in control and EB-treated animals, indicating that estradiol does not increase the concentration of enzyme molecules per cell. In contrast, forskolin used at submaximal concentration (1 μm) was more active in the EB-treated group than in the control group (+147%, +210% respectively). The same effect was observed when Gpp(NH)p and forskolin (1 μM) were used together (+233%, + 307% for EB-treated and control group respectively) whereas Gpp(NH)p alone increased the activity of both groups to the same degree (+54% for control, + 60% for EB-treated). In each group, additive effects of Gpp(NH)p and forskolin (1 μM) were evident.

In order to examine the hormonal specificity of the estrogen-induced prereplicative surge of adenylate cyclase activity, other steroids were tested (Table 3). E-17α, E_2, E_1 and T neither induced a significant increase in weight and DNA content of the oviduct, nor stimulated adenylate cyclase activity. The effect of P_4 was quite different. P_4 increased enzyme activity

Table 3. Effect of different steroids on oviduct growth and adenylate cyclase activity

Treatment[a]	DNA (μg/oviduct) 6 h	24 h	Adenylate cyclase activity (pmol cAMP/min · mg DNA) 6 h	24 h
Control (7)	173 ± 8	180 ± 9	174 ± 8	167 ± 9
EB (10)	170 ± 10	241 ± 12*	241 ± 12*	252 ± 9*
E-17α (5)	206 ± 19	171 ± 9	180 ± 10	176 ± 10
E_3 (5)	191 ± 11	206 ± 11	195 ± 7	148 ± 12
E_1 (5)	194 ± 10	202 ± 10	211 ± 13	163 ± 10
T (5)	162 ± 7	169 ± 5	186 ± 12	165 ± 10
P_4 (10)	180 ± 9	186 ± 12	269 ± 14*	213 ± 12*

Values are means ± SEM. The number of quails for each group (6 or 24 h) is in parentheses. [a] Immature quails (21-day-old) were injected with either EB (0.1 mg/kg) or E-17α (0.1 mg/kg) or E_3 (0.1 mg/kg) or E_1 (0.1 mg/kg) or T (0.1 mg/kg) or P_4 (10 mg/kg) or the vehicle (control). Oviducts were excised 6 or 24 h after the injection. *Significantly different from control ($P < 0.05$).

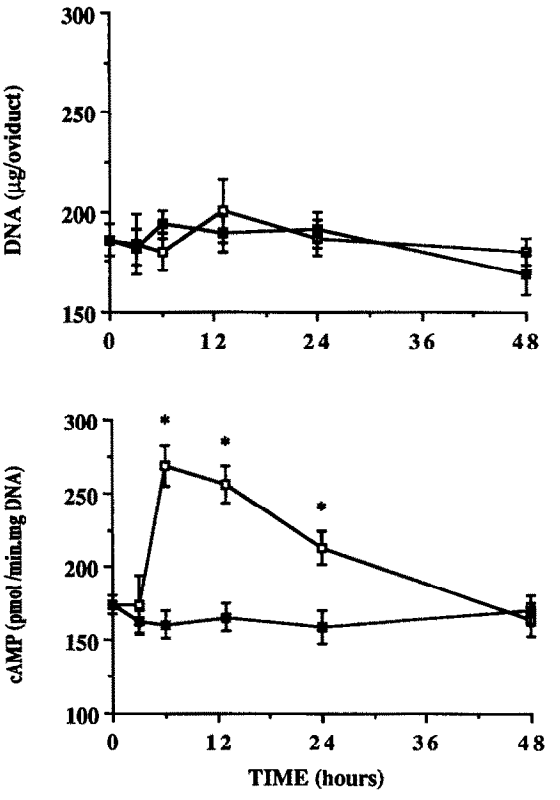

Fig. 4. Effect of P_4 on oviduct DNA content and adenylate cyclase activity. Immature quails were injected with either 10 mg/kg of P_4 (□) or vehicle (■) and oviducts were excised at the designated times. All animals were 21-days-old when killed. Oviduct DNA content (upper panel) and adenylate cyclase activity (lower panel) were measured as described in Experimental. All values are the means ± SEM of at least 6 animals per group. *Significantly different from control ($P < 0.01$).

without any trophic effect on the oviduct. To clarify this point, a more detailed analysis of oviduct DNA content and adenylate cyclase activity up to 48 h after P_4 injection (10 mg/kg i.m.) was performed (Fig. 4). Enzyme activity started to increase after 3 h, peaked at 6 h but, in contrast to what was observed after EB injection, remained elevated up to 24 h and then returned to control value at 48 h. No significative change in oviduct DNA content occurred during this period.

To clarify the mechanism by which estrogens increase adenylate cyclase activity during the prereplicative period, E_2 perfusion experiments and *in vitro* experiments were performed.

Ovariectomized quails were perfused with E_2 (2 ng/min) through either the jugular or the hepatic portal vein. Animals were killed after 6 h of perfusion and oviduct adenylate cyclase activity was measured (Table 4). Perfusion of E_2 (2 ng/min) through the jugular vein provides a constant level of plasma E_2 (~70 pg/ml) while when E_2 is perfused through the portal vein, plasma E_2 level does not significantly increase. In both groups, adenylate cyclase activity was significantly increased (36% and 30% after

Table 4. Effect of E_2 perfusion on oviduct adenylate cyclase activity

Perfusion route	Oviduct wet wt (mg)	Plasma E_2 (pg/ml)	Adenylate cyclase activity (pmol cAMP/min · mg DNA)
Jugular: vehicle (5)	18.5 ± 1.5	8.9 ± 1.8	169 ± 7
Portal: vehicle (5)	20.1 ± 1.4	8.5 ± 2.2	163 ± 6
Jugular: E_2 (5)	21.3 ± 2.0	70.3 ± 11.2*	231 ± 10*
Portal: E_2 (5)	22.5 ± 0.5	9.1 ± 1.5	213 ± 4*

15-day-old quails were ovariectomized and E_2 perfusion was performed when they were 30-day-old. E_2 perfusion rate was 2 ng/min and the time of perfusion 6 h. Control animals were perfused with 5% ethanol in saline (vehicle). Values are the means ± SEM. The number of quails is in parentheses. *Significantly different from the corresponding control ($P < 0.01$).

jugular and portal perfusion respectively). It is noteworthy that, as we have demonstrated previously [17], an increase in oviduct DNA content was observed after 24 h regardless of the route of administration, while an increase in progesterone receptor content was only apparent in the animals perfused through the jugular vein.

The enzyme sensitivity to estradiol *in vitro* was also determined. Samples of 21-day-old quail oviduct homogenates were incubated in the presence of different concentrations of E_2 (10^{-9}–10^{-7} M). The hormone was dissolved in ethanol and added to the assay mixture, taking care to keep the final ethanol concentration at 0.01% (v/v), the addition of ethanol alone at the same final concentration being without any effect on the basal activity. No significant variation of adenylate cyclase activity was observed no matter what concentration of E_2 was used (results not shown).

Finally TAM, a synthetic non steroidal antiestrogen, injected with EB to immature quails does not inhibit the increase of adenylate cyclase activity observed in EB-treated quails for 6 h but completely inhibits the growth-promoting effect of EB and the second surge of enzyme activity observed in EB-treated animals for 24 h (Fig. 5).

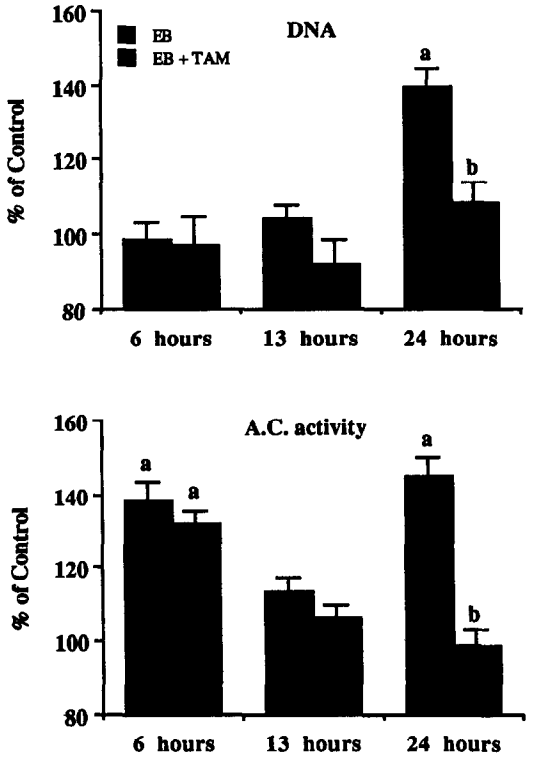

Fig. 5. Effect of EB and TAM on oviduct growth and adenylate cyclase activity. 21-day-old quails were injected with either 0.1 mg/kg EB or 0.1 mg/kg EB + 1 mg/kg TAM or the vehicle. Oviducts were excised at the designated times. Oviduct DNA content (upper panel) and adenylate cyclase activity (lower panel) were measured as described in Experimental. At least 6 animals per group were used and results are expressed as % of the corresponding vehicle injected group (control). a—significantly different from the corresponding control group ($P < 0.01$). b—significantly different from the corresponding EB injected group ($P < 0.01$).

DISCUSSION

The data presented in this report give clear evidence for the existence of a membrane-bound form of adenylate cyclase in quail oviduct. The apparent K_m for ATP (0.26 mM) of the oviduct enzyme is in the same order of magnitude as that reported for the rat uterus cyclase (0.3 mM, [12]). The activity of the enzyme is affected by the usual activators, acting either at the level of the regulatory G/F subunit (Gpp(NH)p, NaF) or directly on the catalytic subunit (forskolin).

A single injection of estradiol benzoate to immature female quail resulted in a prereplicative surge of oviduct adenylate cyclase activity. The transient activation of adenylate cyclase at early stages of the action of estradiol is undoubtedly responsible for the rise in oviduct cAMP concentration that we have previously described [18] since oviduct cAMP phosphodiesterase activity was left unchanged for the first 12 h following the administration of the hormone [19]. The activation of oviduct adenylate cyclase is estrogen specific since estradiol-17α, estrone, estriol and testosterone were ineffective, as they were on oviduct cAMP concentration [18]. This activation seems to be coupled with further DNA synthesis as attested by the absence of adenylate cyclase activity stimulation and the lack of proliferative response in the oviduct of 15-day-old quails injected with EB. Moreover, as previously shown, forskolin administration to immature quails pretreated with EB stimulated DNA synthesis but not P_4 receptor synthesis [18].

The following hypotheses can be suggested as an explanation to estrogen stimulation of oviduct adenylate cyclase activity:

(1) Estradiol can bind to the nuclear estrogen receptor and directly increase gene transcription rates ([28], for review). It is clear that these effects on gene expression induced by estrogen result in increased activity of many enzymes involved in cell growth.

(2) Estradiol can bind to a membrane receptor linked to adenylate cyclase. Estrogen receptors have been detected in the plasmatic membrane of the endometrial cells of the rat uterus [29] and of estradiol-dependent tumour cells [30], and reports have been published that demonstrate the ability to estradiol to influence adenylate cyclase activity by a mechanism independent of the nuclear estrogen receptor [14, 15].

(3) Adenylate cyclase system consists of a hormone-sensitive receptor, regulatory proteins and the catalytic subunit. Estradiol can also act directly at the regulatory or catalytic subunits level.

Results presented in this study demonstrate that enzyme activation does not occur through the classic nuclear receptor-gene activation pathway (hypothesis 1) since adenylate cyclase activity was significantly increased even in the absence of a detectable increase in plasma E_2 concentration, when E_2 is perfused through the hepatic portal vein of castrated animals. We have demonstrated previously [17], that, in animals perfused through the portal vein, the nuclear estrogen receptor concentration in the oviduct remained unchanged compared to ovariectomized control. Moreover TAM, a synthetic antiestrogen with a large spectrum of biological properties ([31], for review) but with pure estrogen-antagonist activity in birds (32), does not inhibit the prereplicative surge of adenylate cyclase activity induced by EB. In contrast, the increase in oviduct DNA content and the second surge of enzyme activity, observed in EB-treated quails for 24 h were completely inhibited by TAM.

Our results also exclude the hypothesis of a membrane receptor mediated process since: (1) as explained previously portal perfusion of E_2 was as effective as jugular perfusion in increasing adenylate cyclase activity; (2) E_2 (10^{-9}–10^{-7} M) does not activate oviduct adenyl cyclase during *in vitro* incubation. This latter point confirms the previous results of Rosenfeld and O'Malley [16] on the effects of diethylstilbestrol on chick oviduct adenyl cyclase activity. A direct effect of E_2 on human endometrium adenylate cyclase has been recently described, [15] but it was selective for membranes prepared from endometria in the secretory phase, and absent in membranes prepared from endometria in the proliferative phase. (3) In the ovariectomized rat, uterine cAMP significantly increase within the first minutes after intravenous administration of physiological doses of E_2 [2]. The absence of such an acute effect of estradiol in chicks [16] and the delayed effect observed in quails [18] are incompatible with a membrane receptor mediated process as suggested in the rat [29].

The stimulation of adenylate cyclase is also independent of the regulatory subunit since it takes place in the absence of Gpp(NH)p and the addition of this nucleotide increases to the same extent the basal activity in control and EB-treated animals. The increase in enzyme activity does not result from an increase in the concentration of the catalytic subunit as attested by the effect of forskolin at 100 μM (Table 3) but more likely from a change in the catalytic subunit, making it more sensitive to forskolin at submaximal concentration (Table 2).

Adenylate cyclase activity has been shown to be dependent upon the membrane lipids [33] and sensitive to phosphorylation [34]. As estrogens have been reported to affect lipid metabolism in target tissues [35, 36], it is reasonable to expect that the mechanism responsible for enzyme activation following *in vivo* estradiol treatment would include modifications of the composition of cell membrane lipids and/or stimulation of the synthesis of inositol containing phospholipids. Experiments are in progress to investigate the relationship between oviduct lipid metabolism and estrogen action.

It should be noted that in progesterone treated quails, in contrast to estradiol treated animals, a more prolonged activation of the oviduct adenylate cyclase system is observed. This agrees with the sustained increase in oviduct cAMP we saw previously in progesterone treated quails. Similar results have been reported in the chick oviduct [16]. Whether this observation is related to the mechanism of progesterone action is not clear at present, however, the possibility of the regulation of progesterone binding through the cAMP-dependent phosphorylation of the receptor [37] deserves consideration.

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