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Inhibition of Estrogen-Induced Increases in Uterine Guanosine 3′,5′-Cyclic Monophosphate Levels by Inhibitors of Protein and RNA Synthesis†

Susan E. Nicol and Nelson D. Goldberg*

ABSTRACT: Uterine guanosine 3′,5′-cyclic monophosphate (cyclic GMP) levels are elevated significantly from 2 to 12 h after a single injection of estradiol-17β or diethylstilbestrol to mature, ovariectomized, or immature rats. The accumulation of cyclic GMP is greater in endometrial- than myometrial-enriched uterine tissue. The estrogen-induced increase in cyclic GMP can be prevented by administration of the protein synthesis inhibitors, cycloheximide and puromycin, or by relatively large doses of the RNA synthesis inhibitor, actinomycin D, but not by the muscarinic antagonist, atropine. The requirement for a protein with a relatively rapid rate of turnover is suggested by the demonstration that cycloheximide, when administered after estrogen, can within a 3-h period restore the estrogen-elevated levels of cyclic GMP to those of the non-estrogen-treated tissue.

Uterine cyclic GMP[1] levels have been shown to be elevated in normal estrus-cycling rats at proestrus when plasma estrogen levels are also elevated and in ovariectomized rats following the administration of physiological doses of estradiol-17β or after diethylstilbestrol (Kuehl et al., 1974; Johansson and Andersson, 1975). From these and other related observations, it has been suggested that cyclic GMP may be involved in the expression of estrogen action in uterine tissue.

There are two characteristics of the uterine accumulation of cyclic GMP inducible by estrogen which distinguish it from the increases in the cyclic nucleotide brought about by non-steroidal agents, such as acetylcholine. One is that the increase following estrogen exhibits a lag period of 60–90 min. The second is that the increase is relatively stable, persisting even after the organ is excised and maintained in vitro for at least 30 min. These features of the estrogen-induced increase in uterine cyclic GMP suggest a requirement for RNA and protein synthesis, such as has been demonstrated for a number of other effects of the steroid in estrogen-responsive tissues (Jensen and DeSombre, 1972; O'Malley and Means, 1974). This possibility is examined in the present report along with the alternative that the effect of estrogen to elevate cyclic GMP levels may be brought about indirectly through the release of a neurohumor, such as acetylcholine. The results demonstrate that RNA and protein synthesis appear to be required for estrogen-promoted elevation of uterine cyclic GMP, since the effect can be prevented by cycloheximide, puromycin, or actinomycin D but not by the cholinergic-antagonist atropine.

Materials and Methods

Mature 200–250 g, ovariectomized (1–3 weeks), or immature (21–25 day old) female Sprague-Dawley rats were obtained from Zivic-Miller Laboratories, Allison Park, Pa. Estradiol-17β dissolved in ethanol was diluted with poly(ethylene glycol) 400 and water (1:4.5:4.5, v/v) and administered by ip injection; DES was administered sc in 0.1 ml of sesame oil. Actinomycin D, cycloheximide, and puromycin dissolved in saline and atropine sulfate dissolved in phosphate-buffered saline were administered ip. Studies of uterine contractility were conducted in vitro with uteri suspended in Hanks' balanced salt solution.

Preparation of Tissue and Cyclic Nucleotide Analysis. Tissues were prepared for cyclic nucleotide analysis (Figures

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[1] Abbreviations used are: cyclic GMP, guanosine 3′,5′-cyclic monophosphate; cyclic AMP, adenosine 3′,5′-cyclic monophosphate; DES, diethylstilbestrol; sc, subcutaneously; ip, intraperitoneally; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; IP, estrogen-induced protein.

2, 3, and 5,) as previously described (Kuehl et al., 1974). Modification of these procedures (Figures 1, 4, and 6) included: use of a cyclic GMP antiserum (produced in goats by our laboratory), which required 20 mM $CaCl_2$ for optimal sensitivity in the radioimmunoassay, and acetylation[2] (Harper and Brooker, 1975) before measurement by radioimmunoassay (Steiner et al., 1972a). Succinyl-cyclic GMP-tyrosine methyl ester, purchased from Sigma Chemical Co., was iodinated with $Na^{125}I$ according to a modification of the procedure of Steiner et al. (1972b). Proteins were determined by the method of Lowry et al. (1951).

Preparation of Endometrial- and Myometrial-Enriched Uterine Samples. Uteri were removed, cut lengthwise, flattened on plastic disks, and frozen in Freon 12 cooled in liquid nitrogen. Each uterine horn was fixed with the aid of Tissue Tek II embedding medium to a brass disk with the endometrial side up. Histology of the serial sections (10–12 μm) was monitored by examining representative sections with hematoxylin and eosin stain. When the sections showed primarily myometrium, sectioning was terminated and the sections representing endometrium and the remaining unsectioned tissue representing the myometrium were extracted separately. The combined frozen sections of endometrial-rich tissue and the frozen myometrial tissue (after powdering in liquid nitrogen in a −20 °C room) were layered above frozen 3 M perchloric acid. All the tissue samples were extracted, purified, and assayed at the same time. Cyclic nucleotide standard curves determined in pooled, phosphodiesterase-treated tissue extract of myometrial, endometrial, and whole horn samples were virtually identical. No significant difference in cyclic nucleotides was observed between uterine horns of the same animal when one horn was frozen immediately and the other was cut longitudinally and flattened before freezing.

Results

Since maximum increases in uterine cyclic GMP levels have been shown to occur from 2 to 5 h after intraperitoneal administration of physiological doses of estradiol-17β (Goldberg et al., 1975b), the majority of the experiments with agents used to block the effect of estrogen (Figures 2, 3, 5, and 6) have been conducted within this time frame with an ip route of administration. In studies (Figures 1, and 4) where a more prolonged increase in uterine cyclic GMP levels was desirable, relatively high doses of DES were injected subcutaneously in oil and the animals were sacrificed during the period (12–36 h) where maximum accumulation of uterine cyclic GMP has been found to occur with this type of treatment (Kuehl et al., 1974).

Localization of Cyclic GMP Changes in Endometrial and Myometrial Tissue. Because of the marked effects estrogen promotes in endometrial tissue and the greater concentration of the steroid receptor in endometrium (Feherty et al., 1970), a comparison was made of the changes in endometrial- and myometrial-enriched uterine tissue cyclic GMP concentration following estrogen administration. In this series of experiments, the mean cyclic GMP value for whole uterine horns was increased threefold over control levels 24 h after DES treatment of ovariectomized rats (Figure 1). The increase in cyclic GMP levels 24 h after DES was greatest (fivefold) in the endometrial-enriched tissues and least (twofold) in the myometrial-

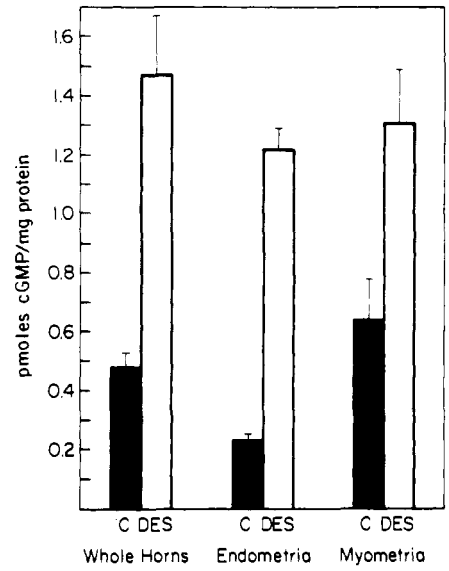

FIGURE 1: Changes in cyclic GMP levels of endometrial- and myometrial-enriched uterine tissue following the administration of DES. Ovariectomized, 250-g, rats were injected with 100 μg of DES or vehicle sc 24 or 72 h before sacrifice. Endometrial and myometrial enriched uterine samples were obtained as described under Methods. DES refers to 24 h after DES injection. Control (C) refers to 24 h after vehicle or 72 h after DES injection. The increase in cyclic GMP levels in the tissues of the 24-h DES-treated animals when compared with controls was significant in the whole horn, endometrial, and myometrial preparations ($P < 0.05$). Control endometrial levels of cyclic GMP were significantly lower than levels in whole horn and myometrial preparations ($P < 0.05$). Each value is the mean ± SEM of cyclic GMP determinations on tissues from four rats.

enriched tissues. The disproportionate changes reflected a difference in control cyclic GMP levels in the two histologically distinguishable tissues. The concentration of cyclic GMP in the endometrium was only 36% of the concentration in myometrial tissue and only half of the level found in the whole uterine horns from control animals. Comparable results were obtained when unfrozen uterine horns were dissected into a portion comprised of endometrium and circular smooth muscle (endometrial enriched) and another containing longitudinal smooth muscle (myometrial enriched) (Nicol et al., 1974).

In the experiments in Figure 1, the controls consisted of two vehicle-injected rats and two 72-h DES-treated rats, since previous work (Kuehl et al., 1974) had shown that uterine cyclic GMP levels return to, or slightly below, the level of vehicle-injected rats 72 h after a single injection of DES and the uteri from DES-treated animals provide more endometrial tissue for study. Endometrial cyclic GMP levels in the uteri of these two treatment groups were found to be very similar (0.22 pmol/mg of protein for oil injected and 0.24 pmol 72 h after DES). The cyclic GMP levels in the whole horns were lower for the 72-h DES-treated rats (0.44 pmol/mg of protein) than in the nonsteroid controls (0.52 pmol/mg of protein), which may reflect the greater proportion of endometrium in the DES-treated animals.

Endometrial levels of cyclic AMP were also lower (by 35–50%) than myometrial levels both prior to and following DES treatment (data not shown); some of the apparent decrease in cyclic AMP levels of the whole uterine horns at later times after estrogen treatment may reflect a greater proportion of endometrial tissue in these samples.

Effect of Atropine. To study whether the increase in uterine cyclic GMP following estrogen administration may derive from an interaction of released acetylcholine with muscarinic re-

[2] The acetylation method was developed through the combined efforts of M. K. Haddox, D. B. Glass, J. H. Stephenson, C. E. Zeilig, and N. D. Goldberg at the University of Minnesota and E. A. Ham at the Merck Institute for Research. A similar assay method was developed independently by Harper and Brooker (1975).

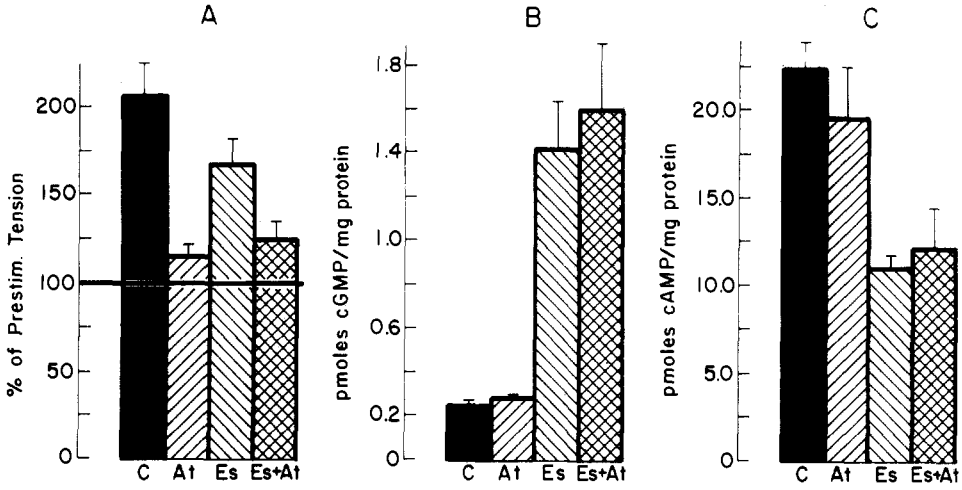

FIGURE 2: Effect of atropine (At) on the estrogen (Es)-induced chages in uterine cyclic nucleotide levels and on the uterine contractile response to methacholine. Ovariectomized, 250-g, rats were administered 1 μg of estradiol-17β or vehicle (C) injections ip and 500 μg of atropine or saline ip at zero time; animals received an additional injection of atropine or saline at 1 and 2 h and were sacrificed at 3 h. Each value is the mean ± SEM of determinations in uteri from 6 to 8 rats. (A) Contractile response to 10^{-5} M methacholine: uteri from rats receiving atropine or estrogen plus atropine in vivo developed significantly less tension in response to methacholine than uteri from nonatropinized animals ($P < 0.01$). (B) Cyclic GMP levels in uteri from estrogen-treated animals whether or not injected with atropine were significantly greater than either non-estrogen-treated group ($P < 0.001$). (C) Uterine cyclic AMP levels were significantly lower in the estrogen and estrogen plus atropine-treated groups than in either group not treated with estrogen ($P < 0.01$).

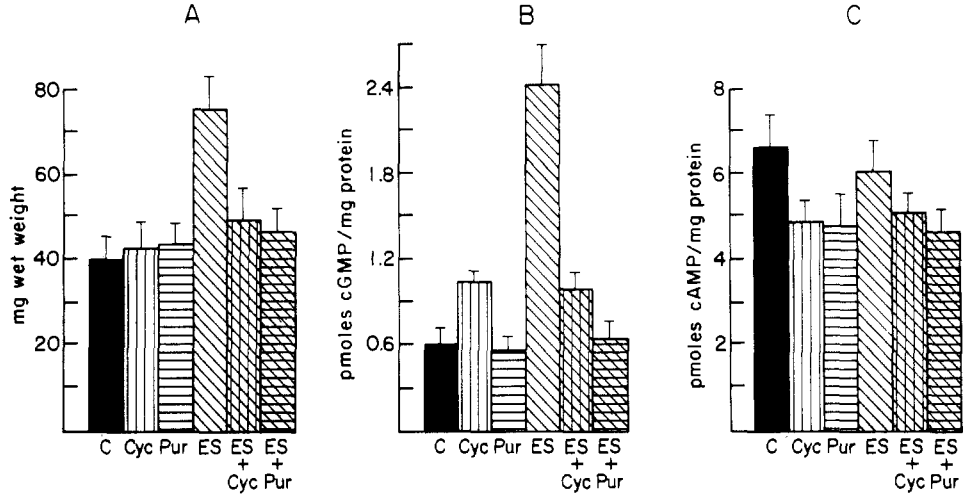

FIGURE 3: Effect of cycloheximide (Cyc) and puromycin (Pur) on estrogen (ES)-induced changes in uterine cyclic nucleotide levels and uterine wet weight. Immature rats (60 g) received 800 μg of cycloheximide, 8 mg of puromycin, or saline (C) by ip injection 30 min before 5 μg of estradiol-17β ip. Additional puromycin (8 mg) was administered 30 and 90 min after estrogen. Animals were sacrificed 3 h after estrogen or vehicle injection. (A) Uterine wet weight increased significantly over control values ($P < 0.01$) 3 h after estrogen given alone but not with any other treatment or combination. (B) Uterine cyclic GMP levels were increased significantly over controls by cycloheximide, estrogen, and estrogen plus cycloheximide ($P < 0.05$). The levels in uteri from the cycloheximide and estrogen plus cycloheximide groups were not significantly different from each other but were significantly lower than the cyclic GMP values in uteri from animals treated with estrogen alone ($P < 0.001$). (C) None of the apparent changes in uterine cyclic AMP levels with different treatments was significant. The values shown represent the mean ± SEM of four to six determinations.

ceptors, ovariectomized rats were treated with 500 μg of atropine at the time of the estrogen injection and again 1 and 2 h later. Animals were sacrificed 3 h after estrogen treatment (1 h after the last atropine injection). One of the uterine horns from each of these animals was frozen immediately for cyclic nucleotide determination and the other was suspended in a tissue bath at constant tension to examine the effectiveness of the muscarinic blockade. The criterion used to establish muscarinic receptor blockade of the tissues was the inhibition by the in vivo atropine treatment of the response in vitro to a supramaximal contractile concentration (10^{-5} M) of methacholine. As shown in Figure 2A, the uterine tissues from nonatropine- or non-estrogen-treated animals responded to methacholine by a doubling of the prestimulation tension. There was less than a 20% increase in tension in uteri from atropinized animals, indicating that there had been extensive blockade of muscarinic receptors. The methacholine response of uteri from rats receiving estrogen alone, while only 168% of the prestimulation tension, was not significantly different from the control tissue response to methacholine.

Atropine alone did not alter the cyclic GMP or cyclic AMP levels (Figure 2B,C) and, in combination with estrogen, atropine did not block either the sixfold increase in uterine cyclic GMP or the 50% decrease in cyclic AMP levels observed 3 h after estrogen treatment. Atropine also had no effect on the increase in uterine wet weight produced by estrogen (data not shown).

Inhibition of Estrogen-Induced Accumulation of Uterine

Cyclic GMP by Inhibitors of Protein Synthesis. The effect of the protein synthesis inhibitors, cycloheximide or puromycin, to prevent the increases in uterine cyclic GMP concentration inducible by estradiol-17β (5 μg, ip to immature rats) is shown in Figure 3. In agreement with previous studies (Gorski and Axman, 1964; Mueller et al., 1961), cycloheximide and puromycin prevented the estrogen-induced increase in uterine wet weight (Figure 3A). Cycloheximide alone raised uterine cyclic GMP levels significantly (73%) but the greater than fourfold increase in cyclic GMP levels produced by estrogen was reduced to only the 64% increase, accountable by the effect of cycloheximide, when cycloheximide was given prior to estrogen. Puromycin had no effect to alter cyclic GMP levels but, like cycloheximide, inhibited the fourfold increase in uterine cyclic GMP induced by estrogen (Figure 3B).

In the results shown in Figure 3C, cycloheximide, puromycin, and estrogen lowered uterine cyclic AMP levels 27, 28, and 9%, respectively. The decreases in this case were not statistically significant and the effect of estrogen and the protein synthesis inhibitors to diminish cyclic AMP levels did not appear to be additive (Figure 3C). In a number of experiments conducted with cycloheximide, an effect of the protein synthesis inhibitor to lower tissue cyclic AMP levels 40 to 50% was observed. These observations with cyclic AMP are consistent with those of Sheppard and Prasad (1973) and Kram et al. (1973) who have shown that cycloheximide can lower cyclic AMP levels in mouse neuroblastoma and BALB/c 3T3 cells, respectively. Whether the cyclic AMP lowering effect is directly or indirectly related to the inhibition of protein synthesis or to another action of cycloheximide remains to be established.

The fact that two mechanistically contrasting inhibitors of protein synthesis (Gottesman, 1967; Ennis and Lubin, 1964) both blocked the estrogen-induced increases in uterine cyclic GMP concentration, which exhibits a lag period of 60–90 min, strongly suggests that the synthesis of new protein is required for the estrogen-induced accumulation of uterine cyclic GMP.

To investigate whether continued protein synthesis is necessary to maintain the elevated uterine cyclic GMP levels following estrogen treatment, cycloheximide was administered to immature rats 18 h after the animals had received DES (Figure 4). In these experiments, the cyclic GMP levels of uteri from immature rats treated with DES were increased significantly over control values at 18 (167%) and 21 (218%) h. The administration of cycloheximide at 18 h resulted, at 21 h, in a decline of the cyclic GMP levels in the DES-treated animals to values found in the vehicle and cycloheximide-treated controls. These results support the conclusion that continued prtein synthesis is required in order to maintain increased uterine cyclic GMP levels following estrogen treatment of immature or mature ovariectomized rats.

Inhibition of the Estrogen-Induced Increase in Uterine Cyclic GMP by Relatively High Doses of Actinomycin D. When actinomycin D was given to ovariectomized rats at a commonly used dose of 3.2 mg/kg, which has been shown to inhibit uterine RNA synthesis by greater than 90% (Ui and Mueller, 1963), the uterine response to estradiol-17β with respect to increase in wet weight was blocked (Figure 5A). However, the estrogen-induced increase in uterine cyclic GMP was unaffected by this dose of actinomycin D (Figure 5B). Cyclic AMP levels were not significantly altered by actinomycin D in this experiment (Figure 5C), but the estrogen-induced decrease in cyclic AMP levels to 40% of control values was partially reversed by this dose of the RNA synthesis inhibitor. This partial reversal may result from the blockade of the uterine wet weight increase.

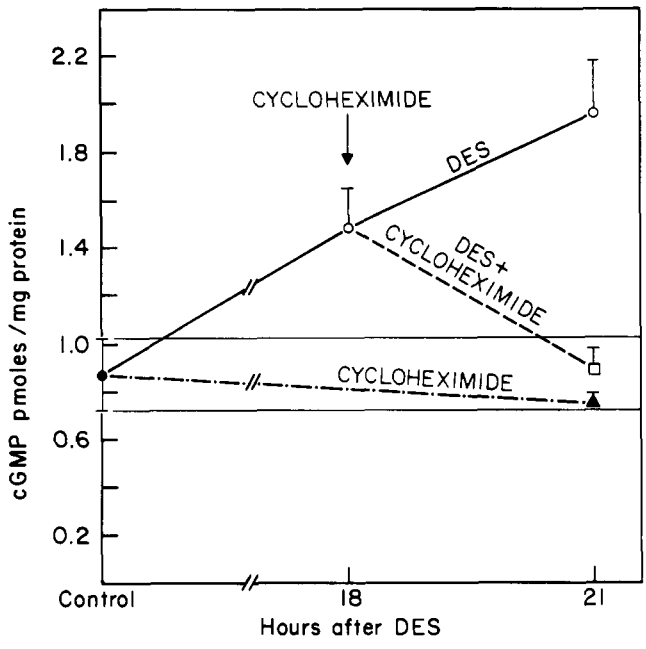

FIGURE 4: Effect of cycloheximide to reverse the DES-induced increase in uterine cyclic GMP levels. Immature rats were administered 50 μg of DES or oil sc at zero time and cycloheximide (1 mg) or saline at 18 h; animals were sacrificed at 18 and 21 h after DES or oil. Control values (●) of uterine cyclic GMP were 0.87 ± 0.15 pmol/mg of protein. Only the cyclic GMP values following DES treatment alone (○) at 18 and 21 h were significantly greater than control levels ($P < 0.05$). Other symbols are (▲) cycloheximide alone and (□) DES + cycloheximide. Each value represents the mean ± SEM of four to six determinations.

Since DeAngelo and Gorski (1970) found that inhibition of the synthesis of the early appearing estrogen-induced uterine protein (designated IP) required concentrations of actinomycin D greater than those necessary to inhibit the bulk of the increase in RNA synthesis observed 2–4 h after estrogen, the effect of higher doses of actinomycin D were examined on the estrogen-induced increase in uterine cyclic GMP (Figure 6). The greater than fourfold increase in uterine cyclic GMP observed 2.5 h after estrogen administration to immature rats in this experiment was prevented when the animals received 10 mg/kg of actinomycin D either 1 h before estrogen or both prior to and at the time of estrogen treatment. Neither regimen of actinomycin D altered uterine cyclic GMP levels significantly when given alone (Figure 6).

Discussion

The results of the experiments reported indicate that the increased accumulation of uterine cyclic GMP following estrogen administration is dependent on the synthesis of a protein inducible by estrogen that exhibits a relatively rapid rate of turnover (<3 h). The dependence on protein synthesis for this effect is consistent with a number of other manifestations of this steroid hormone action and constrasts with the action of other hormonal agents, such as acetylcholine which produce rapid increases in the level of this cyclic nucleotide without any apparent requirement for the synthesis of protein. The definitive proof that such a protein is indeed involved in the action of estrogen must await its isolation and characterization with respect to the role it may play in influencing cyclic GMP metabolism. However, the information that the different protein synthesis inhibitors and an inhibitor of RNA synthesis can

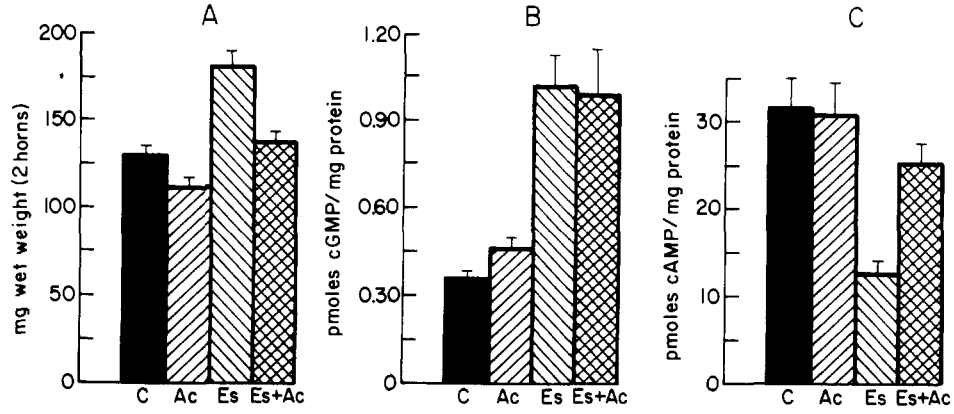

FIGURE 5: Effect of actinomycin D (3.2 mg/kg) on the estrogen-induced changes in uterine wet weight and cyclic nucleotide levels. Ovariectomized, 250-g, rats were injected (ip) with 800 μg of actinomycin D (Ac) or saline (C) 30 min before 10 μg of estradiol-17β (Es) or vehicle was given. Animals were sacrificed 3 h after the estrogen or vehicle injection. (A) Uterine wet weight increased significantly over control values ($P < 0.001$) only after estrogen treatment alone. (B) Uterine cyclic GMP levels were increased significantly over controls in both the estrogen and estrogen plus actinomycin D treated groups ($P < 0.01$). (C) Uterine cyclic AMP levels were decreased significantly only after treatment with estrogen alone ($P < 0.001$). Each value represents the mean ± SEM of six to eight animals.

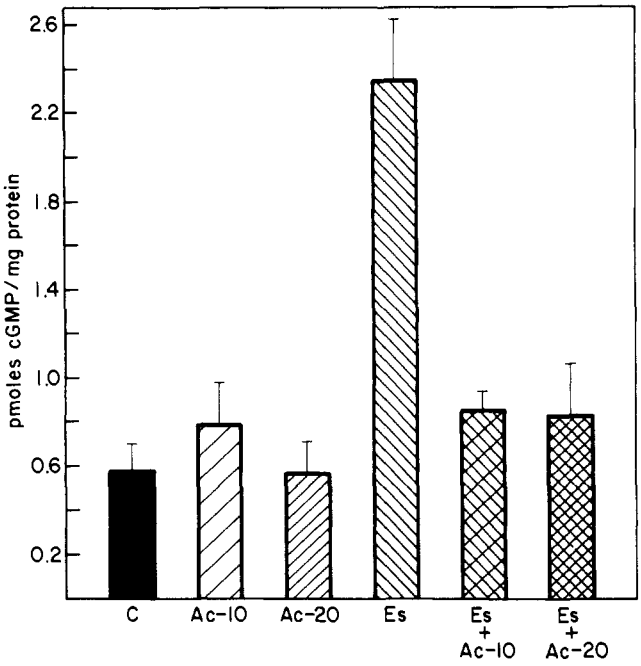

FIGURE 6: Effect of relatively high doses of actinomycin D on the estrogen (ES)-induced changes in uterine cyclic nucleotide levels. Immature rats (60 g) designated Ac-10 received 10 mg/kg of actinomycin D or saline 1 h before either estradiol-17β or vehicle (C) was injected. Rats designated Ac-20 received a second 10 mg/kg injection of actinomycin D 1 h after the estrogen or vehicle injection. Animals were sacrificed 2.5 h after the estrogen or vehicle injection. Uterine cyclic GMP values were elevated significantly by the estrogen treatment ($P < 0.001$) but were not significantly different from control values when actinomycin D was given. Each value represents the mean ± SEM of five to nine determinations.

block the estrogen-induced increase in uterine cyclic GMP indicates that the influence of this hormone on cyclic GMP metabolism may occur by way of the presently recognized pathway for estrogen action, which involves binding of hormone to intracellular receptors, transfer of hormone–receptor complex to the nucleus, and increased RNA and protein synthesis (Jensen and DeSombre, 1972; O'Malley and Means, 1974).

The possibility that estrogen action may promote the generation of additional neurohumoral receptors cannot be entirely ruled out, but an involvement of muscarinic receptors is virtually eliminated by the results reported here. Also, Ham et al. (1975) have shown that the estrogen-induced increase in cyclic GMP is not prevented by treatment with indomethcin, which blocks the enhanced synthesis of $PGF_{2\alpha}$ that appears to be promoted by estrogen. $PGF_{2\alpha}$, like acetylcholine, has been shown to increase uterine contractility and cyclic GMP accumulation (Goldberg et al., 1973). The possibility that the increases in uterine cyclic GMP levels may result from estrogen-induced release of other neurohumoral agents has not been ruled out but the nature of the lag period, and evidence for the requirement of RNA and protein synthesis minimizes these alternatives.

How the synthesis of new protein may result in an increased steady-state level of cyclic GMP concentrations in uterine tissue is only a matter of conjecture at this time. Potential mechanisms include: (1) an increase in guanylate cyclase activity from the generation of more enzyme or an activator of this cyclase; (2) a decrease in cyclic GMP phosphodiesterase activity, resulting either from enhanced degradation or decreased synthesis of the enzyme or the synthesis of an inhibitor of phosphodiesterase; (3) an enhanced synthesis of a cyclic GMP binding protein which protects against enzymic degradation; or (4) a combination of these effects. With respect to guanylate cyclase, it was found that the specific activity of the enzyme in whole homogenate, soluble, or particulate fractions did not increase after estrogen treatment if it was the whole uterine horn (i.e., endometrial and myometrial tissue) that was examined. However, a significant increase in guanylate cyclase activity after estrogen treatment can be demonstrated in endometrial enriched tissues (R. C. Kraska and N. D. Goldberg, unpublished observations). It is not possible to say with certainty at this time whether more enzyme or an enzyme activator is induced.

Decreased specific activity (40–50%) of soluble cyclic GMP phosphodiesterase has been observed in uteri from estrogen-treated animals (Kuehl et al., 1974; Kraska et al., 1974). However, there is good reason to believe that a decrease in cyclic GMP phosphodiesterase activity is not the sole mechanism underlying the increases in uterine cyclic GMP levels promoted by estrogen. The specific activities (on a uterine protein or wet weight basis) of the phosphodiesterases, which

promote the hydrolysis of cyclic GMP as well as cyclic AMP, are apparently decreased following estrogen treatment at times when the uterine cyclic GMP levels are elevated and the cyclic AMP levels are depressed. Also, there is no change in phosphodiesterase activity with estrogen treatment when total enzyme activity per uterine horn is considered, which would indicate only a dilution of preexisting enzyme by newly synthesized protein and by increase in water uptake. The decreases in phosphodiesterase activity, indeed, mirrored the uterine wet weight increases, not the increases in uterine cyclic GMP levels. This is exemplified by the observation that, at doses of actinomycin D which block the uterine wet weight response but not the uterine cyclic GMP increase after estrogen, cyclic GMP phosphodiesterase activity is unchanged by estrogen (R. C. Kraska, S. E. Nicol, and N. D. Goldberg, unpublished observations).

Although an increase in guanylate cyclase activity appears at this time to be the most likely mechanism by which estrogen increases uterine cyclic GMP levels, the possible contribution of an estrogen-stimulated cyclic GMP binding protein has not been eliminated. A cyclic nucleotide-dependent protein kinase with greater affinity for cyclic GMP than cyclic AMP (Greengard and Kuo, 1970) has been demonstrated in uterine cytosol.

If a protein with a rapid turnover is required for the estrogen-induced increase in uterine cyclic GMP, a question that arises is whether the protein involved may be one that has already been identified as estrogen responsive in uterine tissue. Results of studies on the mechanism of estrogen action in rat uterine tissue suggest an amplification system in which a limited number of specific proteins are synthesized during the first 2 h after estrogen with a requirement for the continued presence of these proteins in order to facilitate the larger increases in RNA and protein synthesis seen 2–6 h after estrogen administration (Nicolette and Mueller, 1966; Gorski and Morgan, 1967; Nicolette and Babler, 1974).

Of the early appearing, estrogen-induced proteins that have been described in rat uterine tissue, IP (induced protein), which has received the most extensive study (Notides and Gorski, 1966; Mayol and Thayer, 1970; Barnea and Gorski, 1970; Katzenellenbogen and Gorski, 1972; Somjen et al., 1974), shares certain of the properties that seem to characterize the protein that appears to be involved in the enhanced accumulation of cyclic GMP promoted by estrogen. IP is first detectable early (30–45 min) after estrogen administration and, like the estrogen-induced increases in cyclic GMP, can be prevented from appearing only with relatively large doses of actinomycin D. The rapidity with which mRNA is transcribed for the early estrogen-specific proteins and the relatively greater resistance of RNA polymerase II than polymerase I to inhibition by actinomycin D may explain why higher doses of the inhibitor are needed to block IP formation as well as the enhanced cyclic GMP accumulation than are necessary to inhibit the majority of RNA and protein synthesized 2–6 h after estrogen treatment. From the work of Vokaer et al. (1974), there is no indication that IP is involved in any aspect of cyclic nucleotide metabolism. It is not known whether any of the estrogen-induced nuclear proteins (Teng and Hamilton, 1970; Barker, 1971; Glasser et al., 1972; King et al., 1974) bind cyclic nucleotides but it is interesting that the time course for the appearance of acidic proteins in rat uterine nuclei is similar to that for increases in uterine cyclic GMP concentrations when allowances are made for the different routes of estrogen administration (i.e., intravenous in the study by Glasser et al. (1972) vs. intraperitoneal in the studies by Kuehl et al. (1974) and Goldberg et al. (1975b). Furthermore, the appearance of some estrogen-induced nuclear proteins is relatively resistant to actinomycin D (Barker, 1971).

It should be pointed out that another of the effects of estrogen reported here is to decrease uterine cyclic AMP levels. The decrease was seen in all of the experiments conducted but the magnitude of the effect varied considerably. Chew and Rinard (1974) have reported small (20–40%) but significant increases in uterine cyclic AMP levels 6, 48, and 72 h after estrogen administration. The reason for the contrasting results with those reported here is not known.

Another question that remains to be resolved is the role that cyclic GMP may play in the expression of estrogen action in uterine endometrial tissue of the rat. RNA polymerase I activity, which is enhanced after estrogen treatment and exhibits a requirement for the early synthesis of protein, has been shown to be activated in lymphocyte nuclei exposed to submicromolar concentrations of cyclic GMP (Johnson and Hadden, 1975). It is also of interest that Steiner et al. (1975), using immunofluorescent localization techniques, found that in adrenal tissue high levels of cyclic GMP are present in the nucleolus, which is the locale of polymerase I.

The finding that estrogen-induced accumulation of cyclic GMP occurs primarily in endometrial tissue is consistent with the greater concentration of estrogen receptors in this tissue and an involvement of cyclic GMP in the enhanced proliferation that characterizes estrogen action in the endometrium. Elevated levels of cyclic GMP have been associated with stimulation of cell proliferation in a number of systems (Hadden et al., 1972; Goldberg et al., 1974, 1975a). There are, however, numerous changes that occur in endometrial cell function as a result of the influence of estrogen. It may be more appropriate to view the relatively stable enhancement of endometrial cyclic GMP concentration inducible by estrogen as a change required to support the new functional mode signaled by the steroid.

Although the questions still unanswered regarding the involvement of cyclic GMP in the expression of estrogen action are considerable, the results of the present study strongly support the concept that a cyclic GMP component is present in the overall action of this steroid hormone in uterine tissue.

Acknowledgments

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