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Steroid hormone regulation of free secretory component in the rat uterus

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Summary. The present studies analysed the uterine free secretory component (SC) response to steroid hormones, and correlated effects on SC with those on IgA. Administration of oestradiol for 3 days to ovariectomized rats significantly increased the levels of SC in uterine secretions, when compared to those in saline-injected controls. This response was dose-dependent and specific for oestrogens, since progesterone, testosterone and glucocorticoids had no effect.

The oestradiol-induced elevation in SC levels occurred in parallel with that of IgA. Time course studies of SC and IgA in uterine secretions indicated that both proteins accumulated in nearly identical patterns following oestradiol administration. The oestradiol-stimulated accumulation of SC, however, appears to be independent of IgA since dexamethasone treatment with oestradiol decreased IgA but not SC levels in uterine secretions. In contrast to dexamethasone, progesterone antagonized the oestradiol effect on both uterine IgA and SC.

The uterine SC response to oestradiol was also observed *in vitro*. Incubation of uterine tissue following oestradiol exposure *in vivo* resulted in a significant accumulation of SC in the culture medium, when compared to levels from control uteri. Addition of either cycloheximide or colchicine to uterine incubation media significantly decreased the effect of oestradiol on SC accumulation. These results suggest that oestrogen regulation of uterine SC may involve stimulation of its synthesis. In addition, our findings indicate that oestradiol control of SC in uterine secretions may be responsible for the movement of IgA from uterine tissues to lumen.

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INTRODUCTION

Sex steroid hormones play a central role in regulating the secretory immune system in the rat uterus (Wira & Sullivan, 1982; Wira, Sullivan & Sandoe, 1982a). The principal hormone, oestradiol, has been shown to stimulate the accumulation of both IgA and IgG in uterine secretions of ovariectomized rats. This response appears to be specific for oestrogens, since progesterone, cortisol or dihydrotestosterone had no effect (Wira & Sandoe, 1977). Progesterone administration with oestradiol, however, prevents the oestrogen-induced increase in IgA and IgG in the uterine lumen (Wira & Sandoe, 1980). In addition, oestradiol has also been shown to stimulate the appearance of IgA- but not IgG-positive cells in both the endome-

trium and myometrium, as well as the intraepithelial accumulation of IgA (Wira *et al.*, 1980; Wira, Sullivan & Sandoe, 1982b).

Recently, we have found that, in addition to cellular migration into the uterus, oestradiol induces the rapid transfer (hours) of both IgA and IgG from blood into tissue through plasma transudation (Sullivan & Wira, 1983a). The movement of IgA and IgG from tissue to lumen, however, appears to involve two distinctly different mechanisms (Sullivan & Wira, 1982a; Wira *et al.*, 1983b). Maximal accumulation of IgG in uterine secretions occurs within 3–6 h after hormone treatment and parallels the early, oestradiol-induced increase in tissue IgG levels. The IgG transferred into the lumen, though, represents only a small fraction of total tissue IgG. In contrast, IgA accumulation in uterine fluid is gradual and requires several days of hormone treatment. Furthermore, when IgA does increase, it is against an apparent concentration gradient.

Since IgA in uterine secretions is polymeric (Wira & Sullivan, 1982) and bound to secretory component (Sullivan & Wira, 1981), these results suggest that oestrogen regulation of IgA movement in the uterus may be mediated through hormonal control of secretory component (SC). Consistent with this hypothesis are our preliminary findings that oestradiol treatment of ovariectomized rats increases SC levels in uterine secretions (Sullivan & Wira, 1981). Moreover, SC appears to regulate the movement of polymeric IgA into external secretions at other mucosal sites (Brandtzaeg, 1974; Brown *et al.*, 1977; Fisher *et al.*, 1979; Goodman *et al.*, 1981; Lemaitre-Coelho *et al.*, 1981).

The purpose of the present study was three-fold: (i) to evaluate quantitatively the nature of hormonal control of uterine SC *in vivo*; (ii) to determine whether oestradiol effects on SC occur in parallel with those on IgA in the presence or absence of oestrogen antagonists; and (iii) to examine whether the uterine SC response to oestradiol can be observed *in vitro*.

MATERIALS AND METHODS

General procedures

Adult female Sprague-Dawley rats (Charles River, Wilmington, Mass.; 150–200 g) were maintained in temperature-controlled rooms with light/dark intervals 12 hr long. Ovariectomies were performed 7–9 days before each experiment, except in those rats that had ligatures placed on their uteri at the cervical end (Sullivan & Wira, 1982b). These animals were ovariectomized 7 days prior to ligation and then used 7 days later.

Uterine secretions were obtained by flushing lumina with saline (0·9%), according to the procedure of Wira & Sandoe (1980). Following centrifugation at 10,000 ***g*** for 4 min, supernatants were lyophilized and stored at −20°. Samples were reconstituted with double distilled H_2O before assay.

Protein levels in mucosal samples were measured by the Hartree method (Hartree, 1972) and utilized bovine plasma albumin (BPA; Calbiochem) as the standard. Significance of all data was determined by the Student's *t* test.

Uterine tissue incubation studies

Uterine tissue, prior to incubation *in vitro*, was perfused *in situ* with saline to remove residual blood, as previously described (Wira & Sandoe, 1980; Sullivan & Wira, 1983a). Tissues were cleared of adherent debris, slit lengthwise, halved, rinsed in saline, blotted on surgical gauze and weighed. Single uteri or uterine segments were then placed in 20 ml glass vials containing 2 ml of pre-warmed incubation medium, which consisted of RPMI 1640 (Gibco) supplemented with 2 mM glutamine (Sigma), 50 μg/ml gentamycin (Schering) and 10% foetal calf serum (Sterile Systems Inc.). Vials were gassed with 95% O_2–5% CO_2, capped and placed in a shaking Dubnoff water bath (65 oscillations/min) at 37°. At various times during incubation, and with intermittent gassing, 100 μl aliquots of media were removed and centrifuged at 10,000 ***g*** for 2 min. Supernatants were frozen at −20°. To prepare uterine cytosols after tissue incubation, uteri were homogenized in 6 volumes of ice-cooled TKM buffer (50 mM Trizme-HCl, 25 mM KCl, 5 mM MgCl, pH 7·5) with a Polytron PT-10 (3 × 10 sec bursts at rheostat setting 3). Homogenates were centrifuged at 10,000 ***g*** for 8 min at 3°, and supernatants (cytosol) were stored at −20°.

Measurement of SC, IgA and IgG

Free SC, IgA and IgG levels in uterine secretions, incubation media and tissue cytosols were measured by previously characterized radioimmunoassays (RIA; Sullivan & Wira, 1982a, b).

Assay components included labelled and unlabelled standards, rabbit anti-rat SC, goat anti-rat IgA (from Dr H. Bazin, Brussels) and rabbit anti-rat IgG (Miles Laboratories) as first antibodies, and goat anti-rabbit IgG (Miles) and rabbit anti-goat (Miles) as the second

antibodies. The rat standards were SC, IgA (from Dr Bazin; source was IR22 immunocytoma serum) and IgG (Miles). Rat SC and rabbit anti-rat SC were prepared as previously described (Fisher *et al.*, 1979). The sample of anti-rat SC used in this study recognized free SC but not SC bound to IgA (Sullivan & Wira, 1983b). Immunoglobulins and SC were iodinated by the IODO-GEN method (Markwell & Fox, 1978), as previously described (Sullivan & Wira, 1983a). For each of the RIAs, first antibody levels bound approximately 70% of the radiolabelled standard. Second antibodies were added at concentrations that precipitated all first antibody. Standard curves were run with each assay to determine sample levels of SC, IgA or IgG. Sensitivities for these assays have been reported previously (Sullivan & Wira, 1983a, b).

Steroid Preparations

Oestradiol, progesterone, cortisol and testosterone were purchased from Calbiochem. Dexamethasone was acquired from Steraloids. Oestradiol was solubilized in absolute ethanol, evaporated and resuspended in saline. Progesterone, cortisol, testosterone and dexamethasone were suspended in saline by glass–glass homogenization. Control animals received saline. To correct for the alcohol residue present in the oestradiol preparation, an equivalent amount of ethanol was evaporated in flasks used to prepare saline. All injections were administered subcutaneously, with a 25 gauge needle and glass syringe. Doses, in 0·1 ml, and injection sequences are described in the 'Results' section.

RESULTS

Influence of various hormones on SC levels in uterine secretions of ovariectomized rats

The effects of various steroid hormones on the free SC content in uterine flushings of ovariectomized rats is shown in Fig. 1. Daily administration of oestradiol for 3 days significantly increased uterine SC levels when compared to those measured in saline-injected controls. In contrast, administration of progesterone, testosterone or cortisol, at doses known to elicit physiological responses, failed to increase SC content.

The oestradiol-induced accumulation of free SC in uterine fluid, measured after 3 daily injections, coincided with significant elevations in luminal IgA ($P < 0{\cdot}005$) and protein ($P \leqslant 0{\cdot}001$) and a 2·5-fold increase in uterine weight. Testosterone treatment, which had no effect on SC, doubled uterine weight and increased protein content in uterine secretions ($P \leqslant 0{\cdot}001$), when related to control measurements. This protein effect, however, was 40-fold less than that observed following oestradiol administration.

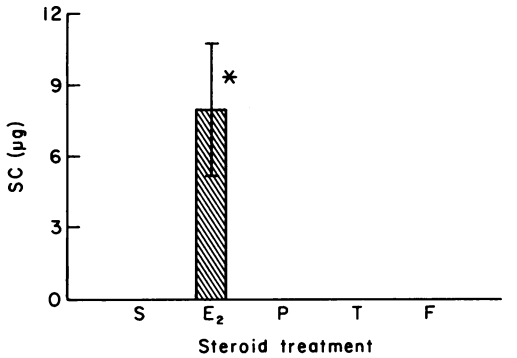

Figure 1. Influence of various steroids on SC levels in uterine secretions of ovariectomized rats. Animals received three daily treatments of progesterone (P, 2 mg), oestradiol (E_2, 2 μg), cortisol (F, 2 mg), testosterone (T, 2 mg), or saline (S, control) and were killed 24 hr after the third injection. Each bar represents the mean value from four to five animals per group, and the vertical lines on the bars indicate the SE. (*) Significantly ($P < 0{\cdot}05$) greater than control.

Uterine SC response to increasing doses of oestradiol

To determine whether the uterine free SC response to oestradiol was dose-dependent, varied amounts of oestradiol were injected daily into ovariectomized rats

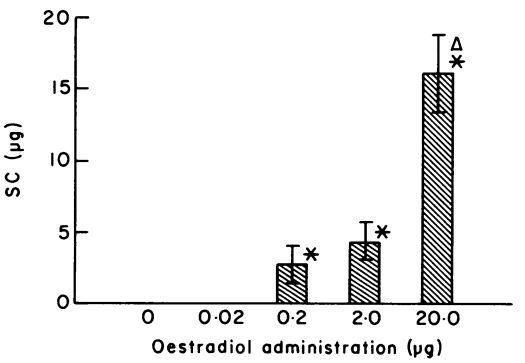

Figure 2. Effect of increasing doses of oestradiol on uterine luminal SC levels. Ovariectomized rats were administered three daily injections of oestradiol or saline and killed 23 hr later. Bars equal the mean ± SE of 5 values. (*) Significantly ($P < 0{\cdot}05$) higher than control; (Δ) significantly ($P < 0{\cdot}005$) greater than all other oestradiol-treated groups.

for 3 days. As illustrated in Fig. 2, luminal SC accumulation depended upon the dose of oestradiol administered. Treatment of rats with 0·02 μg oestradiol had no effect on uterine SC levels. Injection of 0·2 or 2·0 μg, however, signficantly increased SC content. Highest levels of SC were measured after administration of 20 μg oestradiol.

Comparison between the time courses of SC and IgA accumulation in uterine secretions following oestradiol treatment of ovariectomized rats

The time course of IgA accumulation in uterine secretions coincides exactly with the increase in free SC levels that occurs following oestradiol treatment of ovariectomized rats. As shown in Fig. 3, significant ($P \leqslant 0{\cdot}01$) elevations in IgA and SC were first measured 18 hr after the second daily injection of oestradiol, in comparison to levels in saline-treated controls. Both IgA and SC continued to increase in parallel until 10 hr following the third oestradiol administration. This progressive rise in IgA and SC levels was also observed when values were normalized to uterine fluid volume (Table 1). Concentrations of IgA and SC in uterine secretions rose 10- and 4-fold, respectively, from 18 hr after the second to 23 hr following the third injection of oestradiol. Total protein concentrations doubled during this time interval (Table 1).

Antagonism of the uterine SC response to oestradiol by dexamethasone and progesterone

Glucocorticords and progestins are known to anta-

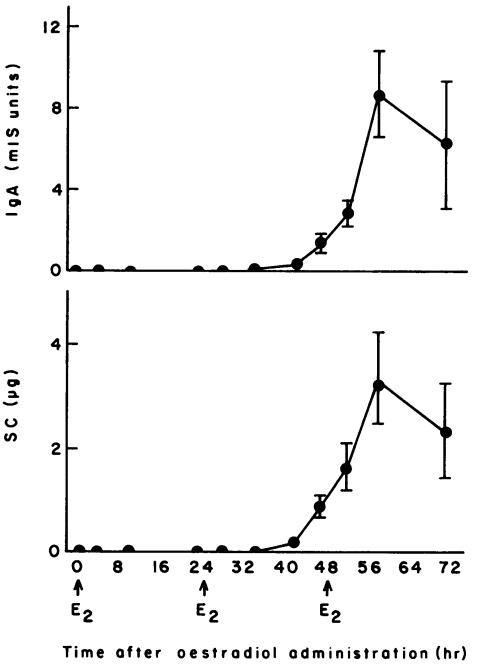

Figure 3. Time course of the effect of 1, 2 or 3 oestradiol treatments on SC and IgA content in uterine secretions of ovariectomized rats. Animals were injected with either oestradiol (E_2, 2 μg/day) or saline (controls, indicated as 0 time point). Each value equals the mean ± SE of 4 (E_2) or 12 (saline) determinations. The levels of IgA are reported as immunocytoma (IS) units, as previously described (Wira & Sandoe, 1980).

Table 1. Concentrations of SC, IgA and total protein in uterine secretions following oestradiol treatment of ovariectomized rats.

Treatment*	Number†	Uterine fluid volume (μl)	SC (μg/ml)	IgA (mIS units/ml)	Total protein (mg/ml)
Two oestradiol injections:					
18 hr	3	46·7 ± 3·3‡	5·5 ± 0·8	6·7 ± 3·7	0·9 ± 0·21
23 hr	4	82·5 ± 6·3	10·4 ± 1·4	14·9 ± 4·5	1·04 ± 0·14
Three oestradiol injections:					
4 hr	4	102·5 ± 12·5	15·2 ± 2·3	29·8 ± 10	1·38 ± 0·16
10 hr	4	190 ± 40·4	17·0 ± 3·4	54·1 ± 16·5	1·22 ± 0·11
23 hr	3	90 ± 26·5	21·7 ± 6·5	77·9 ± 25·5	2·25 ± 0·20

* Number of hours after each oestradiol injection represents the time of sample collection.

† Only those samples with measurable uterine fluid volumes were included. No accurate measurements of volume could be obtained prior to 18 hr after the second oestradiol injection.

‡ All values are the mean ± SEM.

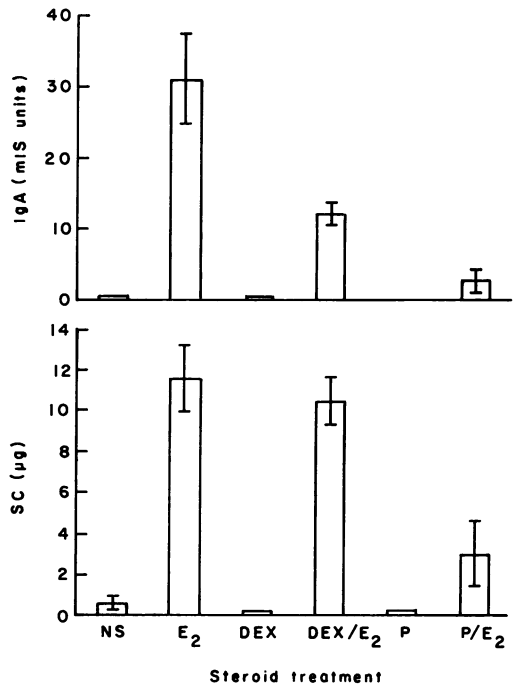

Figure 4. Antagonism by dexamethasone and progesterone of the uterine SC and IgA responses to oestradiol. Ovariectomized rats with uterine ligations received saline (NS), dexamethasone (DEX, 1 mg/day), or progesterone (P, 2 mg/day) approximately 30 min before an injection of saline or oestradiol (E_2, 1 μg/day). Animals were killed 24 hr after the third injection. Numbers represent the mean ± SE of 5–6 values.

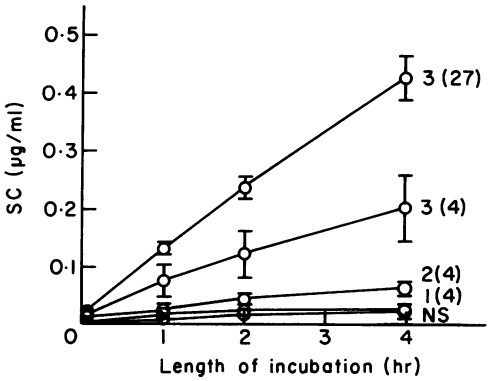

Figure 5. Effect of increasing length of oestradiol exposure *in vivo* on uterine SC output *in vitro*. Ovariectomized rats were administered saline (NS) or one, two or three injections of oestradiol (1 μg) and then killed 4 or 27 hr later. First figure = the number of oestradiol injections; the figure in parentheses = the time of death expressed as the number of hours after the last injection. Whole uteri were perfused and cultured as described in the 'Materials and Methods'. Values equal the mean ± range; N = 2 uterine samples.

gonize a number of uterine responses to oestradiol (Tchernitchin *et al.*, 1975; Campbell, 1978; Koseki *et al.*, 1977; Kelly, Morrison & Green, 1978; Kimura, Obata & Okada, 1978). To assess whether oestrogen-induced increases in uterine IgA and SC were also susceptible to antagonism, dexamethesone or progesterone were administered to rats with uterine ligations 30 min before injection with oestradiol. Uteri were ligated to prevent loss of uterine fluid, which may occur when progesterone is given along with oestradiol (Armstrong, 1968). As illustrated in Fig. 4, progesterone treatment prior to oestradiol significantly reduced the levels of both IgA and SC in uterine secretions, when compared to amounts measured in oestradiol-injected rats. Dexamethasone also diminished the oestrogen effect on uterine IgA, but did not interfere with the oestradiol-stimulated increase in luminal SC levels (Fig. 4). These latter results suggest that oestrogen regulation of uterine SC may be independent of IgA.

Uterine secretion of SC *in vitro* following oestradiol treatment *in vivo*

To analyse further the uterine SC response to oestradiol, uteri were incubated following oestradiol treatment *in vivo*. As seen in Fig. 5, accumulation of SC in uterine incubation media depended upon the length of oestradiol exposure *in vivo*. Uteri from rats that received three daily injections of oestradiol released more SC over a 4 hr interval than did uteri from animals treated with one or two oestradiol injections. Furthermore, the time that uteri were collected following the third oestradiol administration affected the amount of SC released. Secretory component concentrations in incubation media of uteri cultured 27 hr after the third injection of oestradiol were greater than those of uteri cultured after 4 hr (Fig. 5). These results are consistent with the kinetics of the uterine SC response to oestradiol *in vivo* (Fig. 3). In both types of experiments, uterine SC release was positively correlated with length of oestradiol treatment.

To test whether the oestradiol-induced accumulation of SC in uterine incubation media could be reduced by the presence of a protein synthesis inhibitor, uterine horns were placed in media either with or

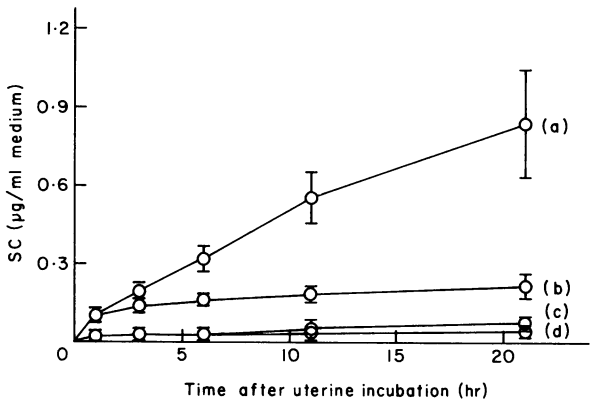

Figure 6. Antagonism by cycloheximide of the oestradiol-induced uterine SC response *in vitro*. Twenty-five hours after the third daily injection of oestradiol (2 μg) or saline to ovariectomized rats, uterine horns were divided and placed in either incubation medium alone or medium with cycloheximide (100 μg/ml; Sigma). (a) Oestradiol; (b) oestradiol/cycloheximide; (c) saline; (d) saline/cycloheximide. Numbers represent the mean ± SE of 3 (saline) or 5 (oestradiol) determinations per time point.

without cycloheximide. Analysis of SC levels at various times after the initiation of uterine cultures demonstrated that cycloheximide significantly decreased the effect of oestradiol on uterine SC output (Fig. 6). Cycloheximide inhibition was not due to blockage of uterine SC release, since uterine tissue content of SC in both oestradiol-treated groups were similar. Furthermore, measurement of IgG levels in the incubation media showed that cycloheximide had no effect on the release of this immunoglobulin from oestradiol- or saline-treated uteri.

Evaluation of the uterine SC response *in vitro* to colchicine is presented in Fig. 7. Colchicine, which is known to retard protein secretion (Redman *et al.*, 1975), reduced by 50% the effect of oestradiol on uterine SC release.

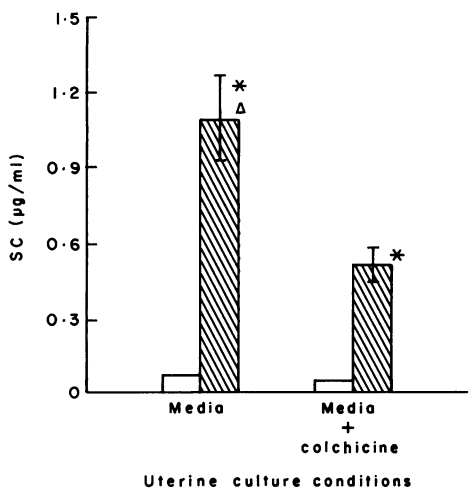

Figure 7. Influence of colchicine on the oestradiol-stimulated secretion of uterine SC *in vitro*. Ovariectomized rats were treated with either oestradiol (2 μg/day) or saline, and killed 26 hr after the third injection. Uterine horns were separated and incubated for 21 hr in the presence or absence of colchicine (1×10^{-4} M; Sigma). Bars equal the mean ± SE of 3 values. (□) Saline; (▨) oestradiol. (*) Significantly ($P < 0.005$) greater than respective control; (Δ) significantly ($P < 0.005$) greater than value from oestradiol-treated uteri in colchicine-containing media.

DISCUSSION

The present studies demonstrate that oestradiol stimulates the accumulation of free SC in rat uterine secretions. This response is dose-dependent and specific for oestrogens, since progesterone, testosterone and glucocorticoids have no effect. These results extend our previous qualitative findings, which suggest that oestrogens regulate the levels of SC in uterine secretions (Sullivan & Wira, 1981). Furthermore, our findings in this study also suggest that oestradiol is most likely responsible for the elevation of uterine SC that occurs during the oestrous cycle (Sullivan & Wira, 1983b). Highest levels of SC in uterine secretions of intact rats were found at proestrus (Sullivan & Wira, 1983b), the stage of the oestrous cycle at which serum oestradiol concentrations are known to be maximal (Nequin, Alvarez & Schwartz, 1979).

The uterine SC response to oestradiol paralleled

that of IgA. Time course analysis of SC and IgA levels in uterine secretions showed that both proteins increased at the same time following oestradiol administration, and that their patterns of accumulation were nearly identical. These data suggest that oestrogen control of SC regulates IgA movement from tissue to lumen. Several observations support this interpretation. First, tissue IgA levels increased rapidly after one (Sullivan & Wira, 1982a) or two (unpublished observation) injections of oestradiol. SC, however, did not increase at this time and IgA was not transferred into the lumen. Second, when IgA did increase in uterine secretions, its presence coincided exactly with the luminal accumulation of SC. Third, after oestradiol treatment, only the polymeric form of IgA is found in the uterine lumen (Wira & Sullivan, 1982). Fourth, IgA in the uterine lumen is bound to SC (Sullivan & Wira, 1981).

An intriguing finding with the present study was that following the third injection of oestradiol, the concentration of IgA in uterine fluid rose more rapidly than did free SC. One explanation for this is that tissue IgA, which is known to increase significantly at this time, binds to SC and thereby effectively lowers the amount of free SC available for transfer into the lumen.

At other mucosal sites, SC is thought to mediate the transport of IgA. With immunofluorescent analysis, this process appears as secretory IgA staining in the basolateral margins and apical cytoplasm of epithelial cells (Brandtzaeg, 1974; Brown *et al.*, 1977; Nagura, Nakane & Brown, 1979). We have observed a similar accumulation of IgA in uterine epithelial cells following 3 days of oestradiol administration to ovariectomized rats (Wira *et al.*, 1982b).

The uterine SC and/or IgA responses to oestradiol were antagonized by progesterone and dexamethasone. We have previously reported that progesterone blocks IgA accumulation in uterine secretions after oestradiol treatment (Wira & Sandoe, 1980). However, since progesterone also causes uterine-fluid loss through cervical relaxation (Armstrong, 1968), our findings were inconclusive. In the present study, uteri were ligated at their cervical attachment to prevent the loss of uterine secretions. Under these conditions, progesterone still interferred with the action of oestradiol on uterine IgA. In addition, progesterone significantly reduced SC content in the uterine lumen of oestradiol-treated rats.

In contrast to progesterone, the potent glucocorticoid dexamethasone diminished the oestrogen-induced increase in uterine IgA, but had no effect on free SC. This decrease in luminal IgA might be due to interference by dexamethasone with uterine tissue stores of IgA available for transfer. Glucocorticoids prevent the oestrogen-stimulated movement of serum IgG into the uterus after a single injection of each hormone (Sullivan & Wira, 1982a). However, whether analogous responses apply for multiple treatments or for IgA movement from serum to tissue remains to be determined. Another possibility is that glucocorticoids retard the migration of IgA-positive cells into the uterus, an infiltration known to occur under oestradiol influence (Wira *et al.*, 1980). The lack of effect by dexamethasone on the oestradiol-stimulated increase in free SC levels suggests that the SC response is independent of IgA. This hypothesis is supported by the findings of others (Mullock, Jones & Hinton, 1980; Nagura, Nakane & Brown, 1980).

The increase in uterine SC by oestradiol could be due either to increased release of pre-formed SC into the uterine lumen, or to increased synthesis of SC resulting from the proliferation of epithelial cells induced by oestradiol and/or the increased synthesis of SC by each cell. The inhibition observed with cycloheximide suggests that the increase in uterine SC results from increased synthetic capacity of the uterus. Experiments to distinguish between epithelial cell proliferation and/or a direct effect of oestradiol on SC synthesis are currently underway in our laboratory. The presence of some SC in cycloheximide-containing media of oestradiol-treated uteri probably represents a tissue washout of pre-formed SC. Similar tissue release has been seen in other systems (Oldham *et al.*, 1979). In the present studies, cycloheximide had no effect on the accumulation of IgG in uterine incubation media. This result suggests that IgG was washed out of uteri during incubation *in vitro* and was not synthesized. In support of this conclusion, we have previously reported that following oestradiol administration, the source of uterine IgG appears to be plasma (Sullivan & Wira, 1982a) and not local synthesis (Wira *et al.*, 1982b).

Inclusion of colchicine in the uterine incubation media significantly reduced the effect of oestradiol on uterine SC release. Since colchicine is known to disrupt microtubules (Redman *et al.*, 1975), these may be involved in uterine SC secretion. Our findings of a reduction in SC release is similar to that reported by Nagura *et al.* (1979), who demonstrated that the same dose of colchicine effectively interferred with SC secretion from neoplastic colon cells.

In summary, these studies indicate that oestradiol

regulates uterine SC and that, through this action, may possibly mediate the movement of IgA from tissue to lumen. Furthermore, oestrogen control of SC may involve stimulation of its synthesis. These findings, when considered with our previous results, underline the central role of oestrogens in uterine immunity.

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