Progesterone and Estradiol Interaction in the Regulation of Rat Uterine Weight and Estrogen Receptor Concentration (43690)

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Abstract. **Autologous down-regulation of hormone receptors has been shown for several steroid hormones. We have previously shown estradiol (E_2) regulates estrogen receptor (ER) in ovariectomized (OVX) rats. These studies have been extended to investigate the interaction between progesterone (P) and E_2 in the regulation of ER and uterine weight. We implanted Silastic capsules containing varying concentrations of E_2 (0.0005 mg E_2/ml to 5.0 mg E_2/ml of sesame oil) into adult female Sprague-Dawley rats one week after OVX. Simultaneously with implantation, P injections were started (sc in sesame oil) at doses of 1–40 mg/day for three days. In the absence of P, the 0.05 mg E_2/ml implant significantly increased total ER levels (measured by cytosol and nuclear exchange assays) by 25%, while the two highest concentrations of E_2 (0.5 and 5.0 mg E_2/ml) significantly decreased cytosol and total ER levels by at least 40%. No P dose altered ER levels in OVX rats or in rats given E_2 implants of 0.01 mg E_2/ml or lower. At E_2 implant concentrations of 0.05 mg E_2/ml and higher, P decreased total ER levels 30%–50% compared to the appropriate E_2-only controls. P increased uterine weight by 25% in OVX controls and in rats treated with E_2 implants of 0.01 mg E_2/ml and below. In contrast, P inhibited uterine weight gain induced by 0.05–5.0 mg/E_2 ml implants by 20%–30%; maximal inhibition occurred at 5 mg/day of P and above. These data demonstrate that P increases uterine weight but does not alter ER concentration in rats with low E_2 levels (OVX or low E_2 concentration implants) but decreases uterine weight and down-regulates ER at higher E_2 levels, regardless of whether ER is up-regulated or down-regulated by E_2.** [P.S.E.B.M. 1994, Vol 205]

Studies of the down-regulation of the estrogen receptor (ER) in intact animals are important both because ER is an example of a steroid-regulated gene product and because of the physiological significance of altered ER concentration in target tissues. Natural and synthetic estrogens have been shown to down-regulate the ER (1–3). In the uterus, the ER concentration can be maximally and reversibly reduced by 50%, whether measured by hormone binding or immunoassay of ER, demonstrating a true autologous down-regulation (1). This down-regulation of ER was subsequently suggested (3) to be the same as the phenomenon earlier termed "ER processing" (2).

Progesterone is also capable of down-regulating ER (4–6) but only in an estrogen-primed uterus (5, 6). Estradiol (E_2) administration results in an increase in progesterone receptor (PR) (7, 8), and increased PR synthesis appears related to the nuclear ER concentration (7, 9). In several cell lines, E_2 increased PR mRNA levels more than 100-fold (10). The effect of progesterone on ER down-regulation was first thought to be due to inhibition of synthesis of the "cytosolic" ER (5, 11). Later studies reported that progesterone effects a reduction in the occupied nuclear ER (12–14).

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Received April 29, 1993. [P.S.E.B.M. 1994, Vol 205]
Accepted October 13, 1993.

0037-9727/94/2052-0146$10.50/0

It has been suggested that progesterone induces a regulatory factor which blocks ER acceptor sites and leads to increased receptor degradation (13). ER synthesis is suppressed following the decrease in ER concentration (13). In T-47D cells, there was a decrease in ER mRNA and subsequently ER level following treatment with a synthetic progestin (15).

Since both estrogens and progestins down-regulate ER, and these hormones display predictable changes in cycling animals, their combined effects are of interest. The purpose of this study was to examine the consequence of a number of doses of E_2 and progesterone, separately and together, on regulation of ER concentration in adult rat uteri.

Materials and Methods

Adult (60-day-old) female Sprague-Dawley rats, raised on site, were ovariectomized under light ether anesthesia one week before treatment. Groups of animals were either not implanted or were implanted (dorsally and subcutaneously) with Silastic implants (1) containing 50 μl of 0.0005, 0.005, 0.01, 0.05, 0.1, 0.5, or 5.0 mg E_2/ml in sesame oil. Implants remained in place for three days. These groups either received no other treatment or were injected daily with 1, 5, 10, 20, or 40 mg of progesterone in sesame oil for three days. The first progesterone dose was administered when the Silastic capsules were implanted. Three hours after the last progesterone injection, the animals were anesthetized with ether, then killed by cervical dislocation. Uteri were removed, trimmed of fat and mesentery, weighed and homogenized in cold TE buffer (10 m*M* Tris, 1.5 m*M* EDTA, pH 7.4) at a concentration of 30 mg tissue/ml of buffer. The homogenates were processed as described previously with cytosolic and nuclear ER levels being determined by the [^{3}H]-E_2 exchange assay (2). "Cytosolic" is used here to indicate ER found in the supernatant fraction after a 30,000*g* centrifugation and does not imply actual localization.

To determine plasma E_2 and progesterone concentrations, blood was drawn at sacrifice into Sarstedt monovette NH_4-heparin AH/10 tubes (Numbrecht, West Germany) via the descending aorta while the rats were under light ether anesthesia. Following collection, the tubes were centrifuged at 1,000*g* for 10 min at 4°C. Plasma was withdrawn and stored at −70°C until radioimmunoassays (RIAs) for E_2 and progesterone could be run using kits from ICN Biomedicals, Inc. (Carson, CA).

Statistical analyses were conducted using a two-way ANOVA. Results are presented as means ± SEM with a level of significance of $P \leq 0.05$ (significance was determined by Duncan's multiple range test).

Diethylstilbestrol and 17β-E_2 were obtained from Research Plus Steroid Laboratories, Inc. (Denville, NJ), while [^{3}H] 17β-E_2 (specific activity 90–114 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Progesterone and dithiothreitol were obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA). All other chemicals used were reagent grade.

Results

In the absence of progesterone, the two lowest doses (0.0005 and 0.005 mg E_2/ml) of E_2 caused no increase in uterine weight compared to the ovariectomized (OVX) controls (Fig. 1A). However, when progesterone (5 mg/rat or greater) was injected daily at the same E_2 doses, there was a 25% increase in uterine weight, equal to that seen for the OVX controls injected with progesterone. At the 0.01 mg E_2/ml dose, uterine weight increased by 25% compared with ovariectomized controls and progesterone at 5 mg/rat or greater induced a further 25% increase in weight. Doses of 0.05 mg E_2/ml or greater resulted in a 2-fold or greater increase in uterine weight (Fig. 1B and C). Injection with the lowest dose of progesterone (1 mg/rat) reduced uterine weight about 15%–20% in all of these latter E_2 implant dose groups.

The increased uterine weight response to progesterone at 0.01 mg E_2/ml and decreased weight at higher doses of E_2 represents a very significant interaction between E_2 and progesterone ($P \leq 0.0001$). The major effect of progesterone with E_2 is seen at the two lowest doses of progesterone (Fig. 1A–C).

In OVX animals with no progesterone treatment, only the 0.05 mg E_2/ml implant dose caused a significant increase in the total (nuclear plus cytosolic) ER levels (Fig. 2A and B). When the E_2 dose was increased to 0.1 mg E_2/ml, the total ER levels dropped back to control values and were reduced significantly below control values at the two highest doses (Fig. 1C).

The regulation of ER levels due to the combination of E_2 and progesterone is complex. In OVX animals and those implanted with the two lowest E_2 doses, progesterone was without effect (Fig. 2A). E_2 plus progesterone decreased the ER concentration further than that seen in the four highest E_2-only groups (Fig. 2B and C). Interestingly, progesterone decreased ER levels at the 0.05 mg E_2/ml dose (which by itself increased ER levels), at the 0.1 mg E_2/ml dose (which by itself decreased ER back to OVX control levels), and at the 0.5 and 5 mg E_2/ml doses (which by themselves decreased ER levels significantly below controls). There is a significant effect of both E_2 ($P \leq 0.0001$) and progesterone ($P \leq 0.0001$) on total ER level as well as a significant interaction between the two hormones ($P \leq 0.0001$).

Most of the reduction in the total ER levels was due to reduction in the cytosolic ER (Fig. 3A–C).

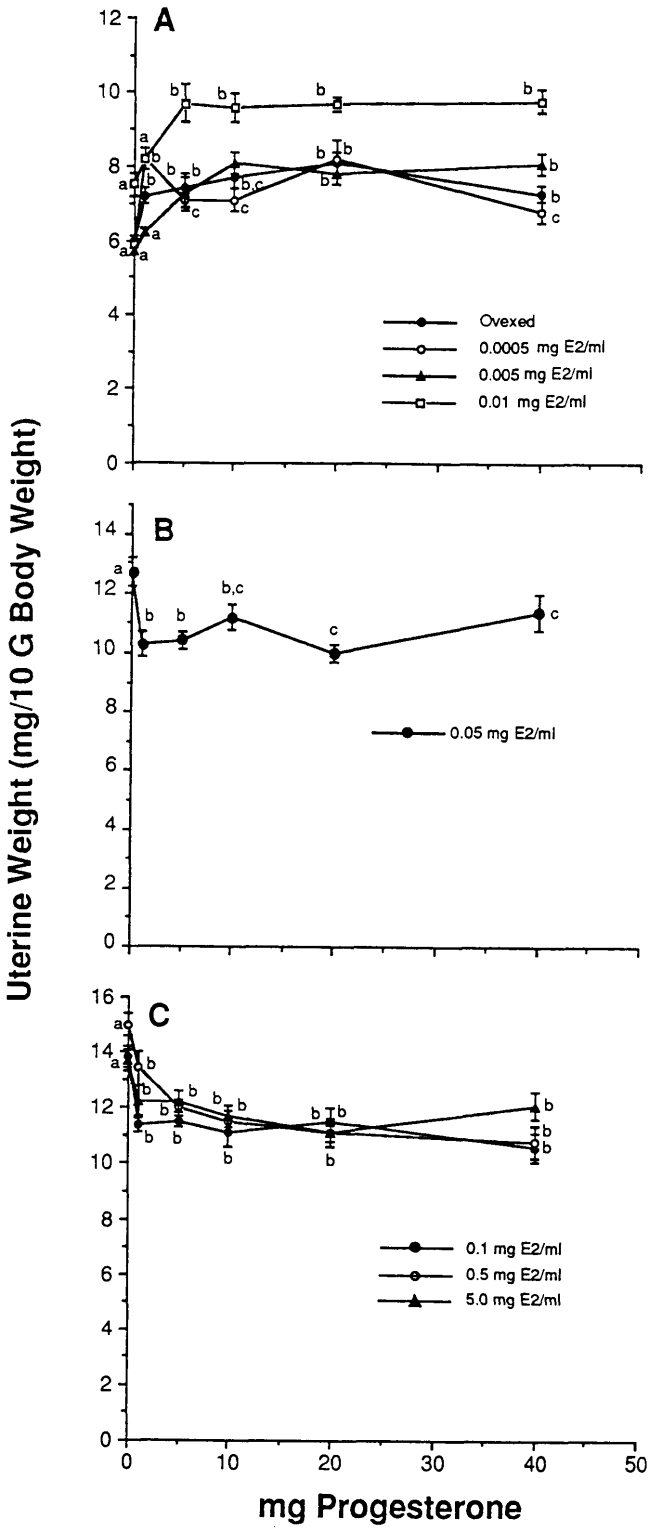

Figure 1. The effects of varying doses of E_2 ([A] untreated controls, 0.0005, 0.005, and 0.1 mg E_2/ml; [B] 0.05 mg E_2/ml; [C] 0.1, 0.5, and 5 mg/E_2/ml) and progesterone on uterine weight in ovariectomized adults. The data are presented as means ± SEM. At the 0 mg dose of progesterone, the significant differences between the E_2 doses are as follows: 0, 0.0005 & 0.005 < 0.01 < 0.05 < 0.1 & 5.0 < 0.5 where < indicates "significantly lower than" ($P \leq 0.05$). Within each of the eight E_2 implant groups, data points with different letters are significantly different.

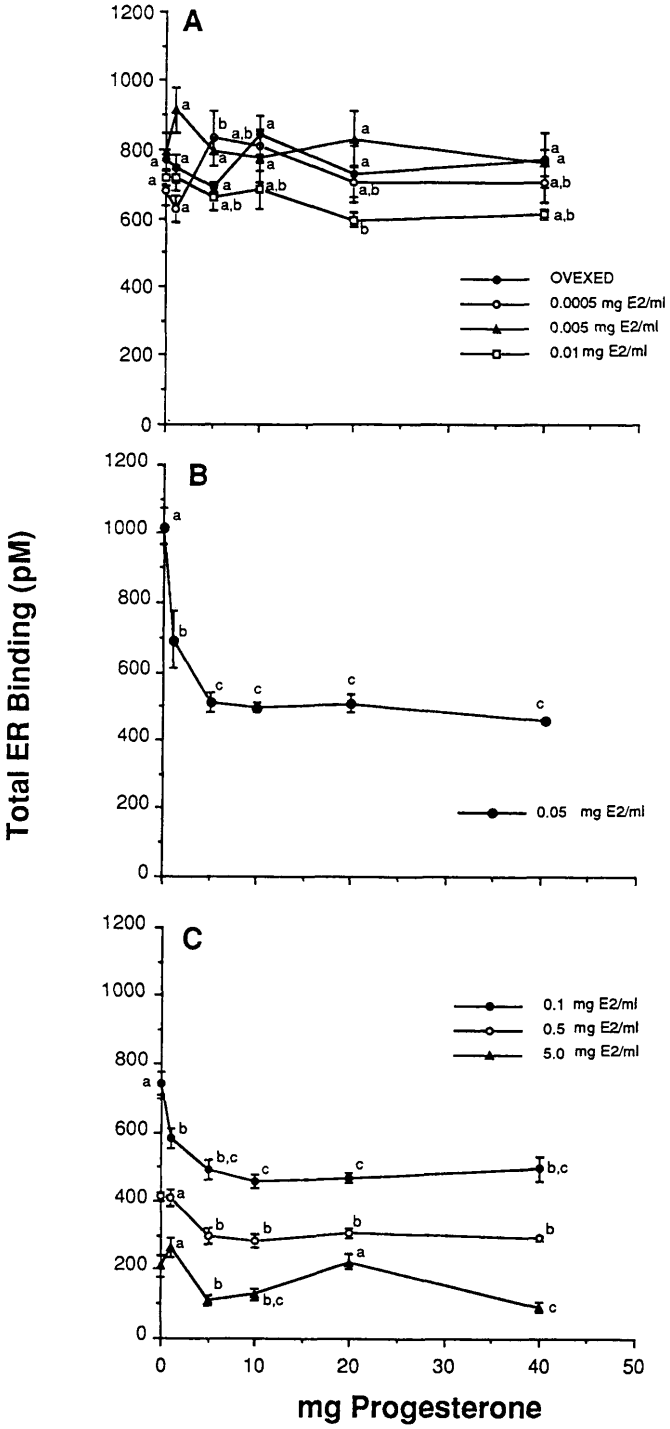

Figure 2. The effects of varying doses of estradiol and progesterone on the total ER concentration in ovariectomized adults. Panels A, B, and C are defined in Figure 1. The data are expressed as means ± SEM. At the 0 mg dose of progesterone, the significant difference between the E_2 doses are as follows: 5.0 < 0.5 < 0.1, 0.01, 0.005, 0.0005, 0 < 0.05 where < indicates "significantly lower than" ($P \leq 0.05$). Within each of the eight E_2 implant groups, data points with different letters are significantly different.

Thus, for cytosolic ER, the individual effects of E_2 or progesterone as well as their interactive effects were essentially the same as that seen for the total ER levels. In the nuclear fraction (Fig. 4A and B), the E_2-

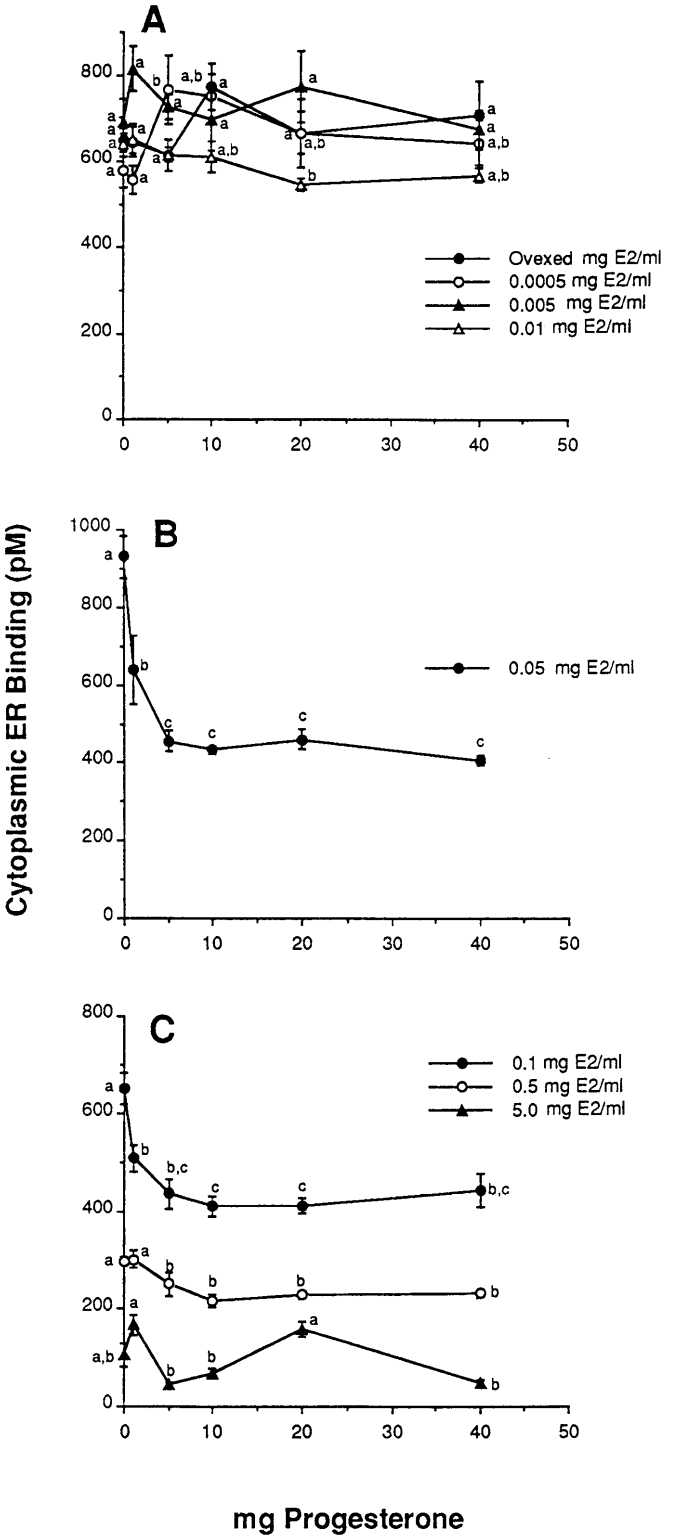

Figure 3. The effects of varying doses of estradiol and progesterone on cytosolic ER concentration in ovariectomized adults. Panels A, B, and C are defined in Figure 1. The data are expressed as means ± SEM. At the 0 mg dose of progesterone, the significant difference between the E_2 doses are as follows: 5.0 < 0.5 < 0.0005, 0.01, 0.1, 0, 0.005 < 0.05 where < indicates "significantly lower than" ($P \leq 0.05$). Within each of the eight E_2 implant groups, data points with different letters are significantly different.

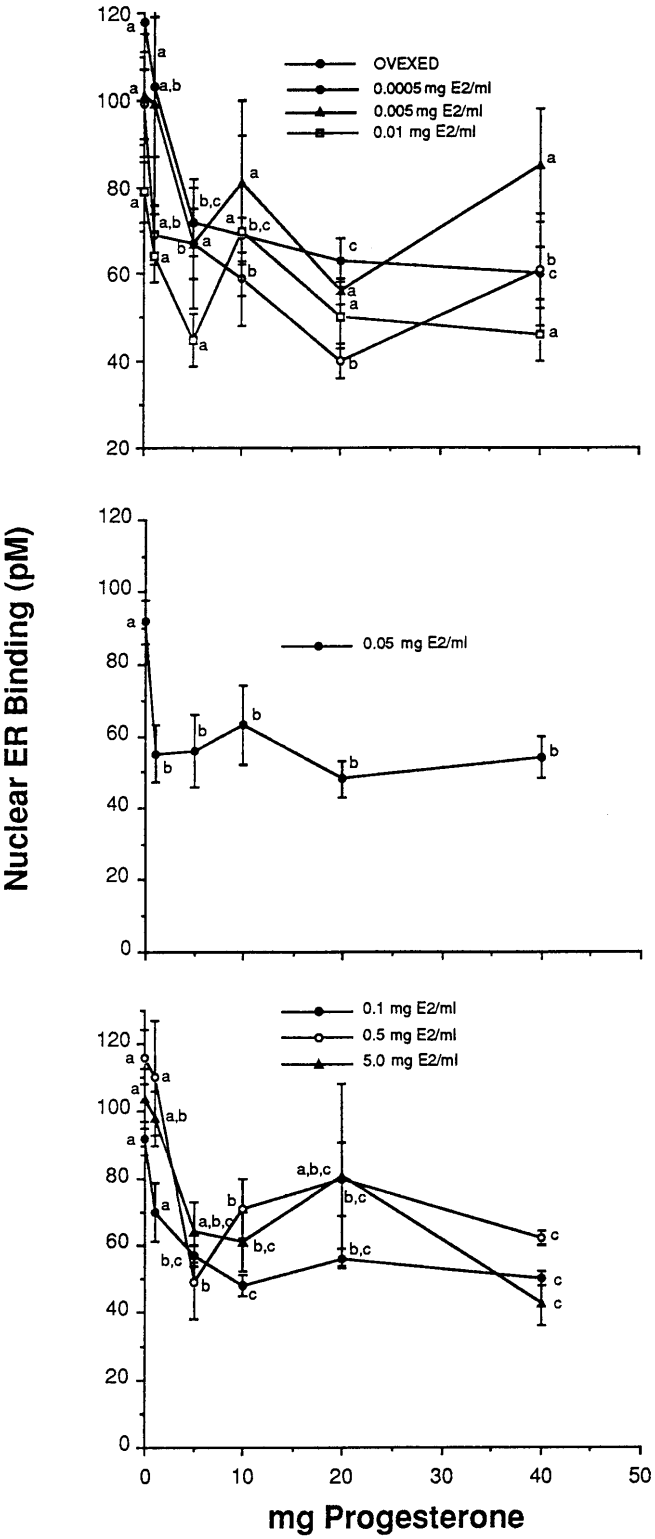

Figure 4. The effects of varying doses of estradiol and progesterone on nuclear ER concentration in ovariectomized adults. Panels A, B, and C are defined in Figure 1. The data are expressed as means ± SEM. At the 0 mg dose of progesterone, the significant differences between the E_2 doses are as follows: 0.01 < 0, 0.0005, 0.005, 0.05, 0.1, 0.5 and 5.0 where < indicates "significantly lower than" ($P \leq 0.05$). Within each of the eight E_2 implant groups, data points with different letters are significantly different.

implant doses and the progesterone doses each had a significant effect ($P \leq 0.002$) on the ER levels. Progesterone generally reduced nuclear ER levels, particularly at E_2 doses of 0.05 mg E_2/ml or above, but there were large error terms in these determinations. No significant interaction between E_2 and progesterone was observed ($P \geq 0.7$).

Visual inspection of the binding affinity of the cytosolic and nuclear ER suggested that neither E_2 nor progesterone had an effect (Tables I and II). However, statistical evaluation of the data showed that the binding affinity (K_D) of the cytosolic ER (Table I) was significantly increased by the E_2-implant dose ($P \leq 0.001$) and by E_2-progesterone interaction ($P \leq 0.02$). For nuclear ER, there is an E_2 dose-dependent reduction in the K_D ($P \leq 0.0001$) but there is no effect of added progesterone ($P \geq 0.3$) (Table II).

The E_2 dose has a significant effect on the plasma E_2 concentration (Table III) whether progesterone is present or absent ($P \leq 0.0001$). When combining all the progesterone doses, there was no significant progesterone dose effect on plasma E_2 concentration although this statistic is marginal ($P \leq 0.06$). When progesterone plasma levels were collapsed across E_2 doses, progesterone had a significant effect on the plasma progesterone concentration ($P \leq 0.0001$) (Table IV). Additionally, the increase in the plasma level of progesterone as a function of progesterone dose was not altered by either the E_2 dose ($P \geq 0.6$) or by a progesterone-E_2 interaction ($P \geq 0.4$).

Table I. Effect of E_2 and Progesterone Dose on the Binding Affinity (K_D) of [^{3}H]-E_2 for the Cytosolic Estrogen Receptor[a]

Progesterone (mg/rat)	Implant dose (mg E_2/ml of sesame oil)							
	0	0.0005	0.005	0.01	0.05	0.1	0.5	5
0	0.76 ± 0.08	0.72 ± 0.10	1.04 ± 0.17	0.83 ± 0.17	1.32 ± 0.25	1.03 ± 0.23	0.91 ± 0.05	1.08 ± 0.29
1	1.04 ± 0.14	1.53 ± 0.59	1.50 ± 0.35	0.66 ± 0.16	1.44 ± 0.25	0.97 ± 0.31	1.54 ± 0.47	0.76 ± 0.16
5	0.60 ± 0.15	0.89 ± 0.42	1.03 ± 0.08	0.75 ± 0.03	0.87 ± 0.05	1.16 ± 0.19	2.12 ± 0.59[b,c]	1.18 ± 0.27
10	1.26 ± 0.36	0.73 ± 0.24	1.24 ± 0.12	0.74 ± 0.10	0.95 ± 0.05	0.90 ± 0.26	1.16 ± 0.14	2.57 ± 0.72[b,c]
20	0.66 ± 0.09	1.52 ± 0.19	1.62 ± 0.36[c]	0.58 ± 0.11	1.44 ± 0.38	0.92 ± 0.37	0.96 ± 0.33	1.19 ± 0.28
40	1.26 ± 0.76	0.67 ± 0.15	1.38 ± 0.30	0.72 ± 0.04	1.06 ± 0.20	0.52 ± 0.05[c]	0.81 ± 0.06	1.98 ± 0.49

[a] Values are means ± SEM presented as n*M*.
[b] Different from 0 progesterone value for the particular E_2 dose.
[c] Different from 0 E_2 value for the particular progesterone dose.

Table II. Effect of E_2 and Progesterone Dose on the Binding Affinity (K_D) of [^{3}H]-E_2 for the Nuclear Estrogen Receptor[a]

Progesterone (mg/rat)	Implant dose (mg E_2/ml of sesame oil)							
	0	0.0005	0.005	0.01	0.05	0.1	0.5	5
0	5.28 ± 0.79	3.56 ± 0.54[c]	3.95 ± 1.00[c]	2.94 ± 0.53[c]	1.97 ± 0.46[c]	1.49 ± 0.26[c]	1.40 ± 0.31[c]	1.60 ± 0.44[c]
1	4.79 ± 1.57	2.84 ± 0.19	5.42 ± 1.64	3.62 ± 0.96	1.49 ± 0.57[c]	1.35 ± 0.34[c]	1.66 ± 0.52	0.72 ± 0.12[c]
5	3.87 ± 0.88	5.24 ± 1.10	2.09 ± 0.36[c]	2.87 ± 0.49[c]	2.55 ± 0.64[c]	1.67 ± 0.48[c]	0.63 ± 0.22[c]	1.49 ± 0.25[c]
10	4.76 ± 0.48	3.10 ± 0.77	4.33 ± 1.11	6.97 ± 3.33	2.87 ± 1.11	1.31 ± 0.17[c]	1.48 ± 0.39[c]	1.84 ± 0.46[c]
20	3.57 ± 0.70	2.60 ± 0.74	3.88 ± 0.91	4.96 ± 0.74	1.59 ± 0.36[c]	2.47 ± 0.64	2.62 ± 1.32[b]	2.94 ± 2.03
40	4.68 ± 0.95	3.61 ± 1.08	8.44 ± 2.29[b,c]	3.87 ± 0.76	3.10 ± 0.68	1.23 ± 0.28	1.20 ± 0.47	0.86 ± 0.15

[a] Values are means ± SEM presented as n*M*.
[b] Different from 0 progesterone value for the particular E_2 dose.
[c] Different from 0 E_2 value for the particular progesterone dose.

Table III. Effect of E_2 and Progesterone Dose on the Plasma E_2 Concentration[a]

Progesterone (mg/rat)	Implant dose (mg E_2/ml sesame oil)							
	0	0.0005	0.005	0.01	0.05	0.1	0.5	5
0	37 ± 15	57 ± 6	68 ± 9	66 ± 6	72 ± 11	91 ± 12[b]	147 ± 12[b]	238 ± 27[b]
1	—	—	66 ± 11	78 ± 6	83 ± 13	108 ± 36	155 ± 19	249 ± 10
5	106 ± 8[c]	89 ± 10	45 ± 13[b]	75 ± 6	100 ± 14	109 ± 8	176 ± 15[b]	—
10	—	94 ± 7	72 ± 6	63 ± 3	95 ± 12	94 ± 6	155 ± 10	—
20	83 ± 4[c]	—	80 ± 24	77 ± 6	94 ± 13	175 ± 55[c]	168 ± 30	203 ± 10[b]
40	87 ± 11[c]	117 ± 30[c]	214 ± 95[c]	84 ± 7	76 ± 10	71 ± 8	118 ± 7	—

[a] Values are means ± SEM presented as pg/ml.
[b] Different from 0 E_2 value for the particular E_2 dose.
[c] Different from 0 progesterone value for the particular progesterone dose.

Table IV. Effect of E_2 and Progesterone Dose on the Plasma Progesterone Concentration

Progesterone (mg/rat)	Implant dose (mg E_2/ml sesame oil)							
	0	0.0005	0.005	0.01	0.05	0.1	0.5	5
0	6 ± 1	6 ± 1	10 ± 4	4 ± 1	5 ± 1	9 ± 3	21 ± 8[a]	3 ± 1
1	—	—	97 ± 34	58 ± 4	73 ± 14	53 ± 10	60 ± 9	49 ± 7[b]
5	137 ± 26	112 ± 19	270 ± 41[a,b]	153 ± 18[b]	150 ± 20	171 ± 44[b]	123 ± 24	—
10	—	289 ± 37[b]	279 ± 37[b]	205 ± 37[b]	244 ± 41[b]	285 ± 62[b]	216 ± 65	—
20	370 ± 90	—	354 ± 27[b]	386 ± 49[b]	390 ± 87[b]	279 ± 86[b]	493 ± 67[b]	195 ± 16[a,b]
40	730 ± 201[b]	598 ± 108[b]	577 ± 65[b]	738 ± 113[b]	478 ± 59[b]	634 ± 72[b]	742 ± 209[b]	—

Values are means ± SEM presented as ng/ml.
[a] Different from 0 E_2 value for the particular E_2 dose.
[b] Different from 0 progesterone value for the particular progesterone dose.

Discussion

A role for progesterone in the regulation of ER is well established (5, 12). In order for progesterone to reduce ER levels, the target tissue must be estrogen-primed (6). Estrogens are known to both up-regulate and down-regulate ER (1–3). ER down-regulation by injected E_2 is dose-dependent and occurs within the first 3–4 hr after E_2 administration (1, 12). These are the same characteristics we reported for down-regulation of uterine ER by E_2-containing Silastic implants, but with implants ER levels remain low until implant removal (1) while with E_2 injections, down-regulation is transient. Progesterone down-regulation of ER levels has also been reported to be E_2 dose-dependent (5, 14).

Our data demonstrate that progesterone can elicit a reduction in uterine ER levels at E_2 implant concentrations which up-regulate as well as those which down-regulate ER levels. Even at the highest E_2 dose (providing the greatest ER down-regulation), progesterone can further down-regulate ER. We found experimental conditions which provided identical ER levels in OVX and E_2-implanted animals (0.01 mg E_2/ml dose group). However, progesterone had no effect on ER levels in the OVX group (while increasing uterine weight) but significantly reduced ER levels and uterine weight in the E_2 group. Likewise, the significantly increased uterine ER levels and weight in the 0.05 mg E_2/ml group were reduced by progesterone. These findings suggest that different rates of synthesis and degradation of ER (16, 17) may result in the same ER level, but provide different sensitivity of ER to progesterone regulation. While cytosolic and total ER showed a complex pattern of ER regulation by E_2 and progesterone, nuclear ER displayed a different pattern in two respects. First, nuclear ER levels were not up- or down-regulated by E_2 in the absence of progesterone. Second, progesterone down-regulated nuclear ER to about the same extent regardless of the estrogen status of the animal. These observations suggest different regulatory mechanisms for cytosolic and nuclear ER for both E_2 and progesterone. Although no changes were seen in nuclear ER with three days of E_2 treatment, we earlier reported slight but significant increases at 12–24 hr following a 3.5-fold ER increase at 1 hr (3). Thus, there may be a further decrease by three days to levels indistinguishable from controls. Additionally, conditions leading to changed rates of synthesis and degradation of ER may provide identical nuclear ER concentrations in OVX and E_2 implanted animals while having different effects on responses, an argument similar to that suggested for progesterone regulation of ER.

The knowledge that ovariectomy reduces the major source of progesterone suggests that down-regulation of ER by E_2 is not mediated by endogenous progesterone. The possibility that adrenal progesterone may down-regulate ER is minimal, since there is no increase in the plasma progesterone concentration at any of the E_2-implant doses after three days of continuous exposure. Additionally, the further reduction of ER levels by progesterone, even at the highest E_2 dose, suggests independent mechanisms for E_2 and progesterone down-regulation of ER, consistent with a conclusion that E_2 down-regulation of ER is not progesterone-mediated.

Progesterone has both an agonistic and an antagonistic effect on uterine weight. At doses of E_2 which are uterotropic, progesterone is antagonistic in that the uterine weight gain is decreased with progesterone. The possibility that this decrease in uterine weight is due to progesterone-induced reduction of ER levels is confounded by the increase in uterine weight caused by E_2 doses which caused the ER down-regulation. In OVX controls, or at doses of E_2 which are not uterotropic, progesterone seems to act as an agonist with a maximum increase in uterine weight at 5 mg progesterone/rat. Although statistical analysis showed that the K_D of the cytosolic ER increased with E_2 dose, this was not a strong effect, and there does not appear to be a pattern for the statistically significant E_2-progesterone interaction. Earlier, we had seen no significant effect of E_2 implant dose on cytosolic ER K_D (1). However, during a 24-hr time-course study comparing E_2 injections with E_2 implants, the cytosol K_D

increased to a maximum by 3 hr following treatment (3). In injected animals, the K_D subsequently decreased to control values by 12 hr, while in implanted animals the K_D remained constant (3). These data suggest that E_2 treatment can increase K_D, and a reasonable suggestion is that E_2 present in the cytosol as a result of E_2 injection or implant acts as a competitive inhibitor in the binding assay.

The nuclear K_D decreased 3–4-fold in an E_2 dose-dependent manner whether progesterone was present or absent. Earlier, we reported this effect for E_2 only, and found it to be reversible upon implant removal (1) and within 24 hr following an E_2 injection (3). Other investigators have shown that the K_D of ER binding of E_2 is a function of ER phosphorylation (18, 19) which may be the explanation in this case.

Even though the lower E_2-implant doses (0–0.05 mg E_2/ml) resulted in plasma E_2 concentrations similar to those seen in early proestrus (20) and lower progesterone doses gave progesterone levels similar to those seen in late pregnancy (21), it is difficult to relate in detail these hormone levels or other parameters to those observed in either the estrous cycle or pregnancy, since the constant E_2 level results in stable parameters (*i.e.*, ER levels, uterine weight, *etc.*) following the initial events elicited by E_2 implant or injection (1). Such stability does not model the changing absolute and relative concentrations of E_2 and progesterone during the estrous cycle. However, we earlier suggested that E_2 may contribute to down-regulation of ER during the estrous cycle (1). Because the data presented here demonstrate that in the presence of E_2, progesterone can further down-regulate ER, both hormones may act together to regulate ER during the estrous cycle.

The experimental advantages of E_2 implants lie in their ability to maintain constant ER levels and K_Ds across time once the ER is up- or down-regulated, compared to the transitory changes in ER concentration and K_D seen following injection of E_2. Such transitory changes appear due to the pattern of increase and decrease in E_2 following injections and complicate experimental design and interpretation, particularly when combined with a second hormone injection such as progesterone.

In summary, this study demonstrates that both E_2 and progesterone can down-regulate ER levels as well as cause uterine weight gain. Progesterone can down-regulate ER which has been up-regulated by E_2. Progesterone down-regulation of ER is not a function of initial ER level but rather of plasma E_2 concentration. Likewise, progesterone's agonistic versus antagonistic effects on uterine weight are a function of plasma E_2 level and not of initial uterine weight. There appear to be different mechanisms of regulation of nuclear and cytosolic ER as well as different mechanisms of ER regulation by E_2 and progesterone. Progesterone and E_2 interact synergistically to down-regulate ER. This latter finding may be important in expanding our understanding of hormonal control of uterine ER levels in estrous and menstrual cycles.

The authors gratefully acknowledge the contribution of Mary Lee Leamons for doing all necessary surgeries in a timely manner and for her technical assistance in conducting the ER assay. The authors also wish to thank Ms. Loetta Bradford for manuscript preparation; General Services Branch for preparation of figures; and Vickie Dollar, Bob Harmon, Tina Johnson, Charles Law, Marsha Law, John Smith, Hazel Carpenter, and Alice Crumpton for their care and attention in animal maintenance.

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