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NUCLEAR RECEPTOR–ESTROGEN COMPLEX: *IN VIVO* AND *IN VITRO* BINDING OF ESTRADIOL AND ESTRIOL AS INFLUENCED BY SERUM ALBUMIN

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SUMMARY

The influence of serum albumin (SA) on the ability of estradiol (E_2) and estriol (E_3) to promote the translocation of the receptor–estrogen complex to the nucleus of uterine cells was examined. Uteri from immature rats were incubated with E_2 or E_3 *in vitro* in the presence or absence of SA and the translocation of the receptor–estrogen complex was measured by the [^{3}H]-estradiol exchange assay. In the absence of SA estradiol is more potent in causing the translocation of the receptor to the nucleus (50% saturation: E_2 at 2 nM, E_3 at 6 nM). However, the reverse is true when SA is present in the incubation medium (50% saturation: E_2 at 18 nM, E_3 at 9 nM). Thus the presence of SA in the incubation medium strongly influences the amount of estrogen available for receptor binding and may explain why the ability of E_3 to cause translocation *in vivo* is equal to or greater than that of E_2. These results suggest that SA may play an important role in determining the relative potencies of estrogens *in vivo* with regard to the translocation process and the subsequent induction of uterine responses. Furthermore, these data indicate that extrapolation of relative potencies for estrogens based on *in vitro* determinations in the absence of SA must be viewed with caution.

INTRODUCTION

Receptor proteins which specifically bind estrogen are localized in estrogen sensitive tissues [1–4]. The interaction of estrogen, E, with the rat uterus is a two step mechanism in which E first binds with a cytoplasmic receptor, R, to form a complex, RE [1–3]. This complex is subsequently transferred from the cytoplasm to the nucleus where it mediates some of the effects of the hormone [4–7].

It has been demonstrated that the uterine estrogen receptor has a lower affinity for estriol than for estradiol *in vitro* [8–11] and the concentration of estriol required to half saturate the translocation of R to the nucleus *in vitro* is greater than that of estradiol [12]. In contrast to these observations, we have shown that estriol is at least as potent as estradiol with respect to the translocation of R to the nucleus under conditions *in vivo* [7–13]. These observations suggest that a factor (s) other than the interaction of E with R and the subsequent transfer of RE to the nucleus is involved in the enhancement of the translocation of R *in vivo*.

The ability of an estrogen to cause the translocation of R to the nucleus *in vivo* is dependent on the affinity of the receptor for the estrogen and the effective concentration of the estrogen in the plasma. The concentration of free estrogen in the plasma that is available for interaction with R depends on a number of factors which include the binding of the estrogen to plasma proteins and the half life of the hormone. In this report we have examined the effects of serum albumin on the ability of estradiol and estriol to cause translocation of the RE complex *in vitro*. The results of this study suggest that serum binding proteins play an important role in determining the relative potencies of estradiol and estriol with regards to the translocation of the receptor in the nucleus and the induction of uterine responses.

EXPERIMENTAL

Immature female rats (21–23-days-old) of the Purdue–Wistar strain were used in this study. 6,7-[^{3}H]-estradiol (New England Nuclear Corp; S.A. 46·7 Ci/mol) was examined for purity by thin layer chromatography. Estradiol or estriol, dissolved in 0·5 ml of 0·9% NaCl, 1% ethanol solution was injected subcutaneously for the *in vivo* studies. Animals were killed by cervical dislocation; the uteri were stripped of adhering fat and mesentery and placed in cold 0·9% NaCl. The quantity of the nuclear receptor–estrogen complex was determined by the [^{3}H]-estradiol

exchange assay [14]. The exchange assay consists of the incubation of washed nuclear fractions at 37°C for 1 h in the presence of near saturating levels of [^{3}H]-estradiol (13 nm) or [^{3}H]-estradiol (13 nm) plus diethylstilbestrol at 100 times the concentration of [^{3}H]-estradiol. After incubation the fractions are washed and the [^{3}H]-estradiol extracted. The quantity of specifically bound [^{3}H]-estradiol was determined by subtraction of non-specifically bound [^{3}H]-estradiol ([^{3}H]-estradiol bound in the presence of a 100-fold excess of diethylstilbestrol) from total [3H]-estradiol binding in the absence of diethylstilbestrol. The amount of [^{3}H]-estradiol specifically bound to the receptor in the uterine cytosol (800 *g* supernatant) was determined by the pellet binding assay using neutral alumina instead of glass beads [15].

Estradiol or estriol were prepared in ethanol and dried under nitrogen in 10 ml glass vials before use in the uterine incubation experiments. Each incubation vial contained two uteri in 2 ml of Eagle's Hela medium (Difco Inc.) in the presence or absence of 4% (w/v) bovine serum albumin (Sigma). The incubations were conducted at 37°C under air. After incubation, the quantity of nuclear receptor was determined as described above.

Table 1. The concentration of nuclear receptor–estrogen complex following an injection of estradiol or estriol

Time (h)	[^{3}H]-estradiol bound (pmol/uterus) Estradiol	Estriol
0	0·18 ± 0·012	0·18 ± 0·012
1/2	1·23 ± 0·13	1·67 ± 0·10
3	0·77 ± 0·04	0·75 ± 0·09
6	0·55 ± 0·03	0·29 ± 0·01

Rats were killed at various times after the injection of saline (0 h) or 1 μg of estradiol or estriol and the quantity of nuclear receptor–estrogen complex determined.

RESULTS

The concentration of RE in the nuclear fraction of the rat uterus following an injection of 1 μg of either estradiol or estriol is shown in Table 1. The amount of nuclear RE at 0·5 h following estriol injection is greater ($P < 0·05$) than that amount observed at 0·5 h after estradiol administration. Following injection of estradiol or estriol the concentrations of the nuclear RE complex are equivalent at 3 h. However, by 6 h the concentration of the nuclear receptor–estrogen complex which is elicited by estriol has declined to near the control level while that of estradiol remains well above the control.

Table 2 shows the effects of estradiol and estriol on [^{3}H]-estradiol binding to the rat uterine receptor. Uterine cytoplasmic or nuclear fractions were incubated with [^{3}H]-estradiol alone or [^{3}H]-estradiol plus either equimolar or 10-fold excess concentrations of estradiol or estriol. The quantity of [^{3}H]-estradiol bound to cytoplasmic and nuclear preparations was determined as described in Methods. At both concentrations, estradiol competes for both nuclear and cytoplasmatic sites to a much greater extent than does estriol.

Thus, although the ability of estriol to bind to the receptor *in vitro* is less than that of estradiol (Table 2), estriol is at least as potent as estradiol with regards to receptor translocation during the first 3 h after injection (Table 1). These observations suggest that a factor(s) other than the affinity of the receptor for estrogen is involved in the enhancement of nuclear receptor content by estrogen *in vivo*.

In the plasma, a large proportion of the circulating estrogens are probably bound to serum albumins [16, 17]. In order to evaluate the effects of serum albumin on receptor–estrogen interaction, intact uteri were incubated for 1 h at 37°C with increasing con-

Table 2. Effects of estradiol and estriol on [^{3}H]-estradiol binding

Concentration competitive extrogen	Cytosol (pmol/mg protein)	Nuclear (pmol/mg protein)
None	7·59 ± 0·22	6·59 ± 0·27
Estradiol (5 nM)	4·23 ± 0·13	2·81 ± 0·14
Estradiol (50 nM)	1·09 ± 0·08	0·06 ± 0·02
Estriol (5 nM)	6·24 ± 0·12	6·28 ± 0·19
Estriol (50 nM)	2·09 ± 0·21	4·13 ± 0·49

Cytosol: Non-labeled estradiol or estriol was mixed with [^{3}H]-estradiol (5 nM) at the indicated concentrations and incubated with the uterine soluble fraction. The quantity of bound [^{3}H]-estradiol was determined by the pellet binding assay [15].

Nuclear: Rats were injected with 2·5 μg of estradiol and their uteri removed one hour later. Non-labeled estradiol or estriol was mixed with [^{3}H]-estradiol (13 nM) and incubated with the uterine nuclear fraction. The quantity of [^{3}H]-estradiol bound was determined by the [^{3}H]-estradiol exchange assay [14].

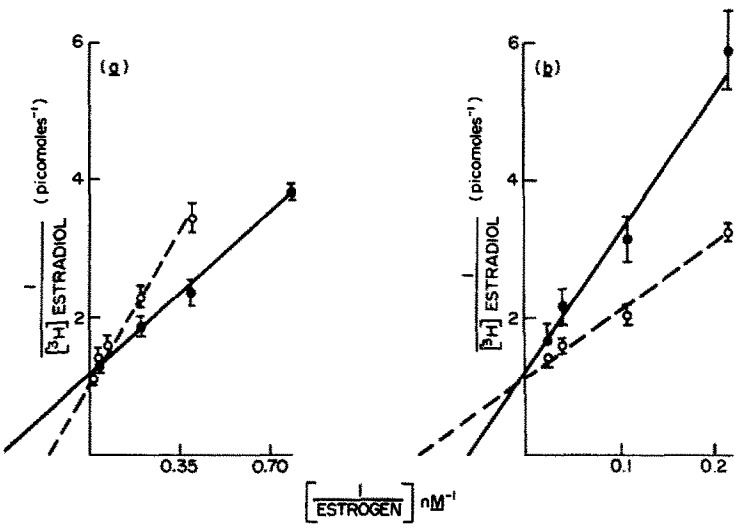

Fig. 1. Effects of serum albumin on the nuclear receptor–estrogen complex induced by estradiol or estriol. Intact uteri were placed in 2 ml of Eagle's medium that contained various concentrations of either estradiol ●——● or estriol O----O. The incubations were carried out in the absence (a) or in the presence (b) of 4% bovine serum albumin for 1 h at 37°C. The quantity of nuclear receptor–estrogen complex was determined by the [^{3}H]-estradiol exchange assay as described in Methods. The control levels of nuclear receptor, obtained by incubation of uteri without estrogen in the presence or absence of albumin, were subtracted from the experimental determinations.

centrations of estradiol or estriol in the presence or absence of bovine serum albumin (SA). After the incubation the quantity of RE in the nuclear fraction was determined by the [^{3}H]-estradiol exchange assay (Fig. 1). The control levels of nuclear RE, obtained by incubation of uteri without estrogen in the presence of absence of SA were subtracted from all experimental determinations. Table 3 shows the concentrations of estradiol and estriol required for half saturation of nuclear RE. The amount of nuclear RE at saturating concentrations of estradiol or estriol in the presence or absence of SA is approximately 0·9–1 pmol/uterus above control (Fig. 1). This value represents the maximal number of RE complexes which can be transferred to the nucleus *in vitro*. The concentration of estriol required to half saturate the translocation process in the absence of SA is three-fold greater than that of estradiol (Fig. 1 and Table 3, E_2 2 nM, E_3 6 nM). However, in the presence of SA, the concentration of estriol required to half saturate the translocation process is one half that of estradiol (Fig. 1 and Table 3, E_2 18 nM, E_3 9 nM). Figure 2 shows the effects of bovine serum albumin on the ability of estradiol and estriol to cause receptor translocation as a function of incubation time. Uteri were incubated with estradiol ($1{\cdot}8 \times 10^{-7}$ M) or estriol ($1{\cdot}8 \times 10^{-7}$ M) for 10 or 30 min in the presence or absence of SA. After incubation the amount of RE in the nuclear fraction was determined by the [^{3}H]-estradiol exchange method. Serum albumin has little effect on the levels of nuclear receptor–estriol complex at these shorter incubation times (Fig. 2, right). However, the amount of nuclear

Table 3. The effects of serum albumin on the concentrations of estradiol or estriol required for 50% saturation of the nuclear receptor–estrogen complex

Steroid	Concentration of estrogens required for 50% saturation of nuclear receptor (nM)	
	−BSA	+BSA
Estradiol	2	18
Estriol	6	9

The concentration of estrogens required for the 50% effect in the presence or absence of BSA (bovine serum albumin) were obtained from Fig. 1.

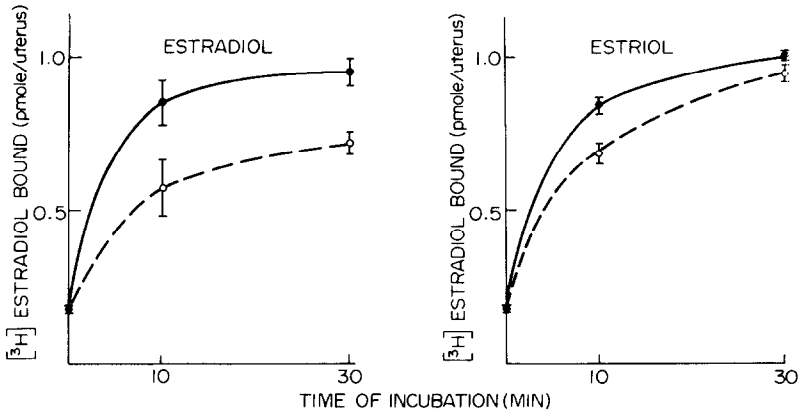

Fig. 2. Effects of bovine serum albumin on estradiol and estriol induced receptor transfer as a function of incubation time. Intact uteri were incubated with estradiol ($1{\cdot}8 \times 10^{-7}$ M) or estriol ($1{\cdot}8 \times 10^{-7}$ M) for 10 or 30 min in the presence (O ····O) or absence (●——●) of 4% bovine serum albumin. After the incubation the quantity of nuclear receptor–estrogen complex was determined.

receptor–estradiol complex in the absence of SA is greater than the quantity of the nuclear complex in the presence of SA at 10 and 30 min of incubation (Fig. 2, left). Further, in the presence of SA the quantity of RE elicited by estriol is greater than the amount elicited by the same concentration of estradiol. Thus, in the presence of SA (but not in its absence) estriol is more effective than estradiol in the formation of nuclear receptor–estrogen complex in the uterus.

DISCUSSION

Various investigators have attempted to define relationships between receptor estrogen binding as measured *in vitro* and the uterine responses elicited by the estrogen *in vivo* [9–11], and it is generally held that the affinity constant for a receptor–hormone interaction is a measure of its relative biological activity [11, 18, 19]. The results of this investigation suggest that such relationships should be viewed with caution. Measurements made *in vitro* in the absence of SA have shown that estradiol is more potent than estriol with respect to its affinity for the receptor [8–11 and Table 2] and the translocation of receptor to the nucleus [12 and Table 3]. In addition, the concentrations of estriol required for the induction of an estrogen specific protein *in vitro* in the absence of SA are greater than those of estradiol [20]. The results of this study show that during the first 3 h after estriol injection the quantity of the nuclear RE complex (Fig. 1) is greater than or equivalent to that induced by estradiol. Also the early uterotrophic responses stimulated by estriol and estradiol are equivalent [7]. Thus, the low activity of estriol for the stimulation of uterine responses as well as receptor binding and translocation to the nucleus *in vitro* is clearly not correlated with the marked potency of estriol for the uterine response and receptor translocation during the first 3 h of *in vivo* exposure. This descrepency is not due to metabolites of the estrogens since both estradiol and estriol are retained by the rat uterus *in vivo* as well as *in vitro* without undergoing significant metabolic transformation [20, 21].

The ability of an estrogen to cause receptor translocation and uterotrophic responses depends not only on the binding of the estrogen to its receptor but also on the amount of estrogen available for receptor binding. The quantity of estrogen that is available for receptor interaction *in vivo* depends on a number of factors which include the binding of the estrogen to plasma proteins and the rate of estrogen catabolism.

Estrogens are bound to serum proteins of high and low affinity depending on the age and endocrine status of the animal [22, 23]. However, since the animals used in this study were 21–22 days old the most important serum binding protein was probably serum albumin [16, 17]. The affinity of human serum albumin for estradiol has been shown to be several-fold greater than the affinity of the protein for estriol [17]. Thus, at equivalent plasma levels of total estrogen, the concentration of unbound estriol to plasma exceeds that of unbound estradiol [17]. In addition, the quantity of steroid that is not bound to plasma proteins is that amount of the hormone which is available for tissue interaction [24]. These observations suggest a mechanism to explain the marked potency of estriol for the enhancement of uterine responses and receptor transfer during the first 3 h after injection. That is, one would expect the concentration of estriol available for

interaction with the receptor to be greater than that of estradiol shortly after injection of equivalent doses of both estrogens. This suggestion is supported by Figs. 1, 2 and Table 3 which show that in the presence of serum albumin, estriol is more effective than estradiol for promoting the translocation of receptor to the nucleus. Further, the addition of SA has little effect on the ability of estriol to increase the levels of nuclear RE whereas the concentration of estradiol required to elicit 50% of maximal nuclear RE level is approximately 10-fold greater in the presence of the protein. Thus, *in vitro* incubation of uteri with estradiol or estriol in the presence of SA simulates the early (0·5 h) effects of the estrogen *in vivo* with regard to the relative potencies of the hormones for the induction of receptor translocation.

If the potency of estriol, as compared to estradiol shortly after injection is a result of its weak interaction with plasma proteins, one would expect that estriol would enter target and nontarget tissues at a faster rate and to a greater extent than estradiol. Jensen and coworkers have demonstrated that the initial accumulation of [^{3}H]-estriol is greater than that of [^{3}H]-estradiol in liver, muscle, uterus and vagina [21]. These observations probably account for the short blood and tissue half life of estriol as compared to estradiol [21] since a larger quantity of estriol is exposed to tissue mechanisms responsible for its degradation and excretion. In addition, this short half life of estriol in both target and nontarget tissues of the rat is paralleled by a short half life of the receptor estriol complex (Table 1). It has been shown that there is a correlation between the relative amounts of various estrogens in the blood, the quantity of the estrogens in the uterus [21], and the levels of receptor–estrogen complex in the uterine nuclear fraction [7]. Thus, the labile nature of the nuclear receptor–estriol complex compared to the receptor–estradiol complex (Table 1) may be due to a shorter blood half life of estriol as compared to estradiol. Alternatively, the rapid decline in the concentration of the nuclear receptor–estriol complex after 3 h may reflect physiochemical differences in the receptor–estriol complex when compared to the receptor–estradiol complex. Investigations are currently in progress to examine these possibilities.

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