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AN ESTROGEN-STIMULATED 1,25-DIHYDROXYVITAMIN D_3 RECEPTOR IN RAT UTERUS

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Summary

Sucrose density gradient analysis was utilized to determine whether 1,25-dihydroxyvitamin D_3 receptors are present in the rat uterus. A distinct 3.6S [^{3}H]1,25-dihydroxyvitamin D_3 binding component was observed in chromatin extracts of estrogen-primed, ovariectomized rat uteri. Binding to this putative 1,25-dihydroxyvitamin D_3 receptor was inhibited by excess 1,25-dihydroxyvitamin D_3, but not by 25-hydroxyvitamin D_3, estradiol-17β, promegestone, or cortisol. Low levels of the receptor seemed to be present in the unprimed uterus. Estrogen injection significantly increased the number of 1,25-dihydroxyvitamin D_3 receptors and progesterone co-administration reduced, but did not abolish, this effect.

Intracellular receptors for 1,25-dihydroxyvitamin D_3, an active metabolite of vitamin D_3, have recently been described in several reproductive tissues. These non-classical target tissues include chick egg shell gland(1), mammary gland (2,3), and placenta (4,5). The presence of the receptors in these tissues prompted us to examine whether 1,25$(OH)_2D_3$ receptors were also present in the uterus. These studies were facilitated by the recent observation that 1,25$(OH)_2D_3$ receptors are predominantly associated with nuclei/chromatin after tissue homogenization in hypotonic buffers (6,7), whereas the interfering vitamin D binding globulin (DBP[1]) remains in the cytosol (6). The results indicate that 1,25$(OH)_2D_3$ receptors can be defined in chromatin extracts of the ovariectomized rat uterus, especially after estrogen priming.

1. Abbreviations used: 1,25$(OH)_2D_3$ = 1,25-dihydroxyvitamin D_3; 25$(OH)D_3$ = 25-hydroxyvitamin D_3; DBP = vitamin D binding globulin; TEDMo buffer = 10 mM Tris, 1.5 mM EDTA, 1.0 mM dithiothreitol, 20 mM Na_2MoO_4, pH 7.4; KTEDMo = TEDMo + 0.3 M KCl; HAP = 50% suspension of hydroxylapatite (BioRad's Biogel HTP); promegestone = R5020, a high affinity progesterone analog (New England Nuclear Corp.).

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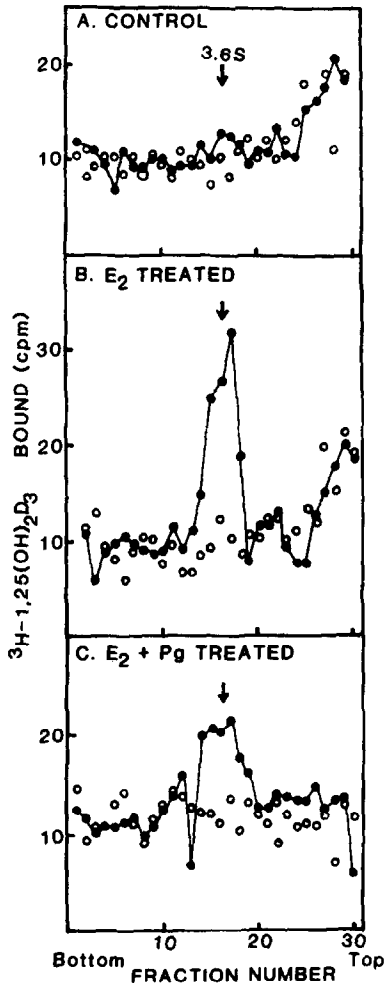

Figure 1. Sucrose density gradient analysis of [^{3}H]1,25(OH)$_2$D$_3$ binding in KCl-extracts of rat uterine chromatin. Uteri from ovariectomized (A), estradiol-17β (1.0 μg x two days) injected (B), or estradiol-17β plus progesterone (1.0 μg and 250 mg, respectively, x two days) injected (C) rats were homogenized in TEDMo buffer containing Trasylol. The crude chromatin preparation was resuspended in KTEDMo + Trasylol and incubated (4°, 1h) with 1.0 nM [^{3}H]1,25(OH)$_2$D$_3$ and 50 nM 25(OH)D$_3$ in the absence (●) or presence (o) of 1.0 μM unlabeled 1,25(OH)$_2$D$_3$ prior to centrifugation and layering (200 μl) onto 5-20% sucrose gradients prepared in KTEDMo + Trasylol. The arrows indicate the location of ^{14}C-ovalbumen (3.6S) in parallel gradients.

Methods

Overiectomized female rats (175-200 g) obtained from Hormone Assay, Chicago, Illinois, were provided normal lab chow and water ad lib. They were either untreated or were injected with estradiol-17β (1 μg) or estradiol-17β + progesterone (250-500 mg) at 24 and 48 h prior to sacrifice by decapitation. Uteri were excised, trimmed of fat, and placed into ice cold 0.9% NaCl. They were homogenized (250 mg/ml) in TEDMo[1] + 500 KIU/ml Trasylol (Mobay Chemical Co.) and centrifuged (6000 rpm, 10 min) to remove cytosol and the accompany-

ing DBP[1]. The crude nuclear pellet was washed three times with TED + 0.5% Triton X-100 prior to resuspension of this crude chromatin preparation in KTEDMo[1] + 500 KIU/ml Trasylol (half original buffer vol). Aliquots (300 µl) of the resuspension were incubated (4°C, 1h) with 1.0 nM [^{3}H]1,25(OH)$_2$D$_3$ (120 Ci/mmol, Amersham Searle) + 50 nM unlabeled 25(OH)$_2$D$_3$ ± 1 µM 1,25(OH)$_2$D$_3$. Then they were centrifuged (104,000 x g, 1h) to recover the KCl-extracted receptors. Aliquots (200 µl) were layered onto cold 5-20% sucrose gradients prepared in KTEDMo + Trasylol and centrifuged (Beckman SW56 rotor) at 104,000 x g for 21h. ^{14}C-ovalbumen (3.6S) was added to parallel gradients for standardization. The gradients were fractionated (4 drops) into assay tubes on ice, followed by the addition of 150 µl HAP[1], two washes with TED-Triton and the addition of 1.0 ml ethanol to extract radioactivity for scintillation counting.

RESULTS

Typical sucrose density gradient patterns of [^{3}H]1,25(OH)$_2$D$_3$ binding to the KCl-extracts of uterine chromatin are shown in Fig. 1. There was a consistent, albeit small, peak of [^{3}H]1,25(OH)$_2$D$_3$ binding in the 3-4S region of gradients prepared from estrogen-primed uteri (Fig. 1B). The elution patterns were more variable in uteri of the uninjected (Fig. 1A) and estrogen plus progesterone injected (Fig. 1C) groups. The identity of this [^{3}H]1,25(OH)$_2$D$_3$ binding component as a 1,25(OH)$_2$D$_3$ receptor was evident from its sedimentation coefficient, its inhibition by excess 1,25(OH)$_2$D$_3$, and the failure of 50 nM 25(OH)$_2$D$_3$ to compete for binding (Figs. 1 and 2A). Unfortunately, the very low levels (100-150 total cpm maximum) of the receptor in these preparations precluded Scatchard analysis. However, the identity of the 1,25(OH)$_2$D$_3$ receptor was further established by the lack of competition by high concentrations (1.0 µM) of estradiol-17β, promegestone[1], or cortisol (Fig. 2B).

A summary of the relative amounts of specific [^{3}H]1,25(OH)$_2$D$_3$ binding after different hormonal treatments (Fig. 3) indicated that estrogen-priming significantly ($P<0.001$) increased uterine 1,25(OH)$_2$D$_3$ receptor levels over those of the ovariectomized controls; and co-administration of progesterone (250-500 mg) reduced, but did not abolish, the estrogenic effect in these animals.

DISCUSSION

These data clearly demonstrate the presence of an estrogen-stimulated 1,25(OH)$_2$D$_3$ receptor in the rat uterus. The apparent difference between this

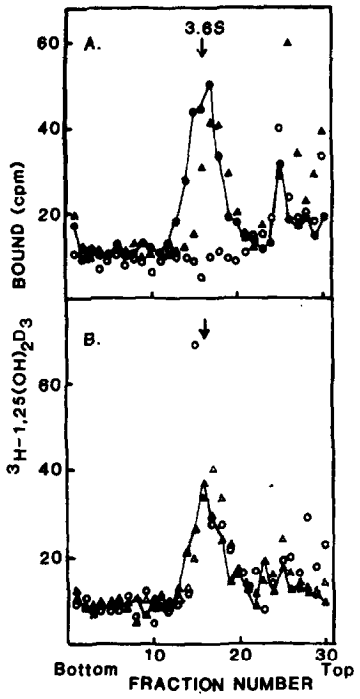

Figure 2. Specificity of [^{3}H]1,25(OH)$_2$D$_3$ binding in KCl-extracts of uterine chromatin from estrogen-primed rats. The chromatin preparation was incubated with 1.0 nM [^{3}H]1,25(OH)$_2$D$_3$ alone (A,●-) or with 1.0 nM [^{3}H]1,25(OH)$_2$D$_3$ and 50 nM 25(OH)D$_3$ (A, ▲) in the presence of 1 μM levels of 1,25(OH)$_2$D$_3$ (A, o), estradiol-17β (B, ▲), promegestone (B, Δ), or cortisol (B, o). In each graph only one data set is drawn out (⟷) for clarity.

report and the contrasting results of Colston et al. in the mouse uterus(2) may relate to the tissue concentrations used, as well as the chromatin extraction procedure and estrogen-priming regimen employed herein. This uterine receptor component is similar to 1,25(OH)$_2$D$_3$ receptors of other tissues(8) in sedimentation coefficient (Fig. 1) and steroid specificity (Figs. 1 and 2). Although Scatchard analysis was not feasible, the uterine 1,25(OH)$_2$D$_3$ receptor probably also has a similar affinity due to the 1,25(OH)$_2$D$_3$ inhibition of the binding of only 1.0 nM [^{3}H]1,25(OH)$_2$D$_3$.

This report adds the uterus to a growing list of nonclassical vitamin D target tissues, and raises important questions about the physiological and biochemical roles of 1,25(OH)$_2$D$_3$ in these tissues. Whether these 1,25(OH)$_2$D$_3$ receptors mediate classic effects of the vitamin D endocrine system(8) or

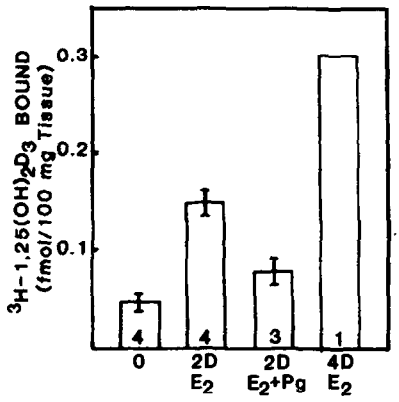

Figure 3. Effect of hormonal treatment on 1,25(OH)$_2$D$_3$ receptor levels in KCl-extracts of rat uterine chromatin. The amount of specific [^{3}H]1,25(OH)$_2$D$_3$ binding in the 3-4S region of sucrose gradients was calculated from the difference between gradients run in the absence (total binding) and presence (nonspecific) of excess 1,25(OH)$_2$D$_3$ (number of observations shown in the bars). Ovariectomized rats were untreated (0) or were injected with 1.0 µg estradiol-17β for two days (2D E$_2$) or four days (4D E$_2$) or with 1.0 µg estradiol-17β plus 250-500 mg progesterone for two days (2D E$_2$ + Pg).

whether they participate in more discreet, perhaps unknown, cellular functions awaits further study.

Although the uterine 1,25(OH)$_2$D$_3$ receptor is definitely stimulated by estrogen (Fig. 3), from these experiments it does not seem to be totally absent in the unprimed uterus. However, absolute confirmation of the latter observation will require the development of more sensitive assays than are presently available for these receptors. The estrogen stimulation of 1,25(OH)$_2$D$_3$ receptors in the uterus is particularly interesting, because we were previously unable to show a similar effect in the chick intestinal mucosa(9), in spite of well-documented effects of estrogen on the vitamin D endocrine system(10,11). This tissue difference suggests that the mode of control of 1,25(OH)$_2$D$_3$ receptor levels (and perhaps of other hormone receptors) is a property of the target tissue rather than an inherent property of the receptor species. While this concept is not necessarily surprising, a complete understanding of its physiological and biochemical ramifications will be of particular importance in defining the complex interplay between these different endocrine systems.

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