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Hormonal Regulation of Nuclear Type II Estrogen Binding Sites in the Dorsolateral Prostate of Noble Rats

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We previously demonstrated that simultaneous treatment of Noble (NBL) rats with estradiol (E_2) and testosterone (T) for 16 weeks induces a proliferative response selectively in the dorsolateral prostates (DLP) of all treated animals [1, 2]. The unique sensitivity of rat DLP to the conjoint androgen–estrogen-induced mitogenic action may be attributable to the presence of a moderate affinity, high capacity, nuclear estrogen binder (type II sites) found exclusively in this prostatic lobe [2, 3]. Little is known about whether prostatic type II site levels are under hormonal regulation. The aim of this study is to determine whether testicular steroids play a role in regulating the basal and/or induced levels of type II site expression in rat DLP. In the first experiment, rats were castrated and immediately treated with 5α-dihydrotestosterone (DHT) and/or E_2 for 6 weeks to determine whether these steroids, separately or jointly, could sustain DLP type II site levels in castrates. Treatments of castrated rats with DHT and DHT + E_2 were found to be effective in maintaining DLP type II site levels and gland wet weights at values close to those found in intact untreated controls, while treatments with E_2 failed to maintain these parameters at levels observed in intact animals. In the second experiment, intact rats were treated with an androgen (T or DHT) or E_2, alone or in combination, for 16 weeks to ascertain which hormonal regimen could induce a higher level of type II site expression in the DLP. Treatments of rats with an androgen (T or DHT) or E_2 alone did not change DLP type II site levels even though T treatment caused a slight increase in gland weight, while E_2 treatment induced prostatic atrophy. Contrary to single hormone treatments, combined T + E_2 and DHT + E_2 treatments were effective in inducing a doubling of type II sites and increases in wet weight of the DLPs. These data indicate that testicular androgen is the primary factor responsible for maintaining a basal level of type II site expression in rat DLP, while conjoint androgenic–estrogenic action is needed for the induction of a higher level of type II site expression in the tissue.

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INTRODUCTION

A large number of studies have shown that estrogens have both inhibitory and stimulatory effects on prostatic growth [4]. Treatment of man or laboratory animals with estrogens alone inevitably leads to atrophy of the prostate, primarily due to suppression of pituitary gonadotropin secretion, which, in turn, reduces testicular androgen output [5]. In contrast, treatment of intact or castrated experimental animals with estrogen in combination with an androgen often induces weight gain in the gland [1–3, 5–13]. In the Noble (NBL) rat, we observed a marked proliferative response selectively in the dorsolateral prostates (DLPs) of rats treated simultaneously with testosterone (T) and estradiol-17β (E_2) for 16 weeks. The DLP proliferative response was characterized by a 7-fold increase in mitotic index in the ductal/acinar epithelium [1, 11], a 200% increase in total cell number [14], and an 80–100% gain in gland wet weight [2]. Although a mild increase in wet weight was also observed in the ventral prostates (VPs) of the T + E_2-treated animals, both epithelial mitotic index [1] and total cell number [14] remained unchanged in this prostatic lobe. Hence, unlike the proliferation response observed in the DLP, the mild increase in wet weight of the VP may be due principally to cell hypertrophy or increase in secretory activity.

The unique sensitivity of rat DLP to mitogenic action of the combined androgen–estrogen regimen

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was not explainable on the basis of changes in androgen receptor or estrogen receptor (classical) contents in the DLP. These parameters remained unchanged in the tissue following exposure of rats to T + E_2 [1, 2]. Instead, it might be attributable to the presence of a moderate affinity, high capacity, nuclear estrogen binder (type II sites) which was found exclusively in the DLPs of Sprague–Dawley and NBL rats [2, 3]. The biochemical properties and ligand binding specificity of prostatic type II sites were shown to be similar to those described for type II estrogen binding sites in the rat uterus [2, 3, 15–18]. In the uterus, treatment of ovariectomized rats with estrogen induced delayed and prolonged elevation of uterine type II sites, which closely paralleled long-term growth of this organ [15–17]. Thus it has been postulated that type II sites serve as cellular mediators of long term estrogen action in this female tissue. Treatment of NBL rats with T and E_2 induced a progressive increase in type II site levels to approximately 2-fold of control values. The elevation was attended by similar changes in wet weight, DNA content, and cell number in the DLP [2, 3, 14]. We have inferred from findings in the uterus that prostatic type II sites may play a role in conferring hormone susceptibility to the DLP as well as in mediating the synergistic action of T and E_2 in prostatic growth stimulation [2, 3]. The aim of the current study was to ascertain the effects of testicular steroids on nuclear type II site levels in the DLP. Our data demonstrated that while androgen is the primary factor needed for maintaining basal type II site expression in the DLPs, the combined action of an androgen and an estrogen is needed to induce an elevated level of expression in this tissue.

MATERIALS AND METHODS

Experimental approaches

Effects of testicular steroids in maintaining basal type II site expression in rat DLP. We previously demonstrated that untreated intact rats expressed approx. 1.6–2.0 pmol/mg DNA type II estrogen binding sites in their DLP nuclei [2, 3, 18]. This experiment was conducted to determine whether testicular steroids were involved in maintaining this basal level of type II site expression. Sexually mature Sprague–Dawley (S-D) rats, each weighing 250–280 g, were orchiectomized bilaterally and divided into 3 groups. The first group (8 rats) was surgically implanted with two 2 cm Silastic™ capsules (No. 602-205, 1.0 mm i.d. × 2.2 mm o.d. Dow-Corning Corporation, Corning, NY) filled with 5α-dihydrotestosterone (DHT, Sigma, St Louis, MO). The second group (10 rats) was implanted with two 2 cm DHT-filled capsules and one 1 cm capsule filled with E_2 (Sigma). The third group (8 rats) was implanted with one 1 cm E_2-filled capsule. The control group consisted of untreated intact animals (9 rats). Animals were treated for 6 weeks and at the end of the treatment period terminated for biochemical studies. Details of surgical procedures were as previously described [1, 11].

Effects of androgens and estrogens on type II site levels in the DLPs of intact rats. Sexually mature Noble rats, each weighing 250 g, were divided into 6 groups. One group of 12 animals was kept as untreated controls. The second group of 10 animals was implanted with two 2 cm T-filled capsules and one 1 cm E_2-filled capsule. The third group of 4 rats was implanted with two 2 cm DHT-filled capsules and one 1 cm E_2-filled capsule. The fourth group of 8 rats was implanted with two 2 cm T-filled capsules. The fifth group of 4 rats was implanted with two 2 cm DHT-filled capsules and the sixth group of 4 rats was implanted with one 1 cm E_2-filled capsule. All rats were treated for 16 weeks and details of surgical procedures were as described previously [1, 11].

Steroids, buffers and solutions

Unlabelled steroids were purchased from Sigma and [2,4,6,7-^{3}H(N)]E_2(^{3}H-E_2, 90–100 Ci/mmol) was purchased from Research Products International (Mount Prospect, IL). The TEG buffer contained 10 mM Tris–HCl, 1.5 mM Na_2EDTA (Sigma), and 10% glycerol (EM Science, Cherry Hill, NJ) (pH 7.4). Nuclei wash buffer contained 10 mM Tris–HCl (Sigma), 0.25 M sucrose (Sigma), and 5 mM $MgCl_2$ (Fisher, Pittsburgh, PA) (pH 7.5). Culture medium (Gibco, Grand Island, NY) was Eagle's minimal essential medium with Earle's salts, minus bicarbonate and glutamine, and with 0.25% bovine serum albumin (Sigma) (pH 7.5). Scintillation fluid was Bio-Safe NA from Research Products International Corp.

Preparation of crude nuclei and assay of type II sites

The protocols for these procedures were similar to those published previously [2, 3]. Briefly, DLPs were excised, weighed, minced and washed once in culture medium and once in TEG buffer. They were homogenized individually (or pooled if tissue weight was less than 0.25 g) in 5 vol (per gram tissue wet weight) of TEG buffer with a Tissumizer (Tekmar, Cincinnati, OH) using eight 7-s bursts at a rheostat setting of 8 with 30 s of cooling between bursts. The tissue homogenate was centrifuged at 800 *g* for 20 min. The supernatant was discarded, and the nuclear pellet was resuspended in 10 vol of nuclei wash buffer, filtered once through double-layered cheesecloth, and washed twice with 10 vol of buffer by resuspension and centrifugation at 800 *g* for 15 min. The washed nuclear pellet was resuspended in 5 vol (for original weight of tissue) of TEG buffer with a Teflon–glass homogenizer. An aliquot was taken for DNA determination, and the rest of the sample was used immediately in an ^{3}H-E_2 binding assay. Assay conditions optimized for ^{3}H-E_2 binding to prostatic type II sites were as previously described [2, 3].

^{3}H-E_2 binding in each sample was analyzed by saturation analysis. Aliquots of a sample were incubated with ^{3}H-E_2 over a range of 0.1 to 40 nM. The incubation was carried out for 30 min at 35°C in the presence or absence of a 300-fold molar excess of diethylstilbestrol (DES). At the end of the incubation period hydroxylapatite (HAP, Bio-Rad, Richmond, CA) suspension (30% HAP in TEG buffer) was added to each incubate, and the bound and free steroids were separated according to the procedure described by Ho *et al.* [19], except that the HAP pellet was washed with TEG buffer instead of a phosphate buffer. Specifically bound ^{3}H-E_2 at each ^{3}H-E_2 concentration was calculated by subtracting the nonspecifically bound (radioactivity determined in the presence of DES) from the total bound (radioactivity determined in the absence of DES). DNA contents of the nuclear suspensions were determined by the diphenylamine procedure [20]. The amount of saturable, specifically bound ^{3}H-E_2 radioactivity was expressed as pmol/mg DNA in the sample.

Statistical methods

Data points are group mean values and n indicates the number of nuclei preparations used to obtain a group mean value. One-way analysis of variance was used to analyze whether there was a significant difference between the group mean of a treatment group and that of the untreated intact controls. Tukey-B procedure was used to compare the individual group means.

RESULTS

Hormones involved in maintaining the basal level of type II site expression in rat DLP

We previously demonstrated that rat DLP constitutively expressed a basal level of type II estrogen binding sites. In intact S-D rats the levels of type II sites were estimated to be around 1.6 pmol/mg nuclei DNA in the DLP [3]. The purpose of this experiment was to determine whether testicular steroids were involved in maintaining basal expression of type II sites in rat DLP. In a preliminary study (unpublished data) we observed rapid declines in DLP type II site levels to close to non-detectable levels (<0.5 pmol/mg DNA) following bilateral orchiectomy of rats. Since the tissue underwent rapid regression at the same time, which severely limited tissue availability, we decided to use a different approach in this study. Rats were castrated and immediately treated with one of the following hormonal regimens: (a) treated with the non-aromatizable androgen, DHT, alone; (b) treated with the estrogen, E_2, alone; or (c) treated with DHT and E_2 simultaneously for 6 weeks. It has been reported that immediate hormone replacement therapies, even with E_2, prevent complete regression of the prostate [21]. We then asked the question of whether (and which) hormonal replacement therapies could maintain nuclear type II site levels in the DLPs of castrated animals. DHT was used as the androgen because it cannot be aromatized to estrogen and because it is generally regarded as the active androgen for prostate growth. Our data showed that treatment of castrated rats with E_2 alone was not effective in sustaining type II site levels in the DLPs of orchiectomized rats (Fig. 1). Levels of type II sites in the DLPs of E_2-treated castrates declined to 0.53 pmol/mg DNA, which approximated 33% of intact control value. On the other hand, treatment of castrated rats with DHT was rather effective in sustaining type II site levels, which remained at 88% of intact control value or at 1.4 pmol/mg DNA. Similarly, treatment of castrated rats simultaneously with DHT and E_2 was effective in sustaining type II site levels at 84%,

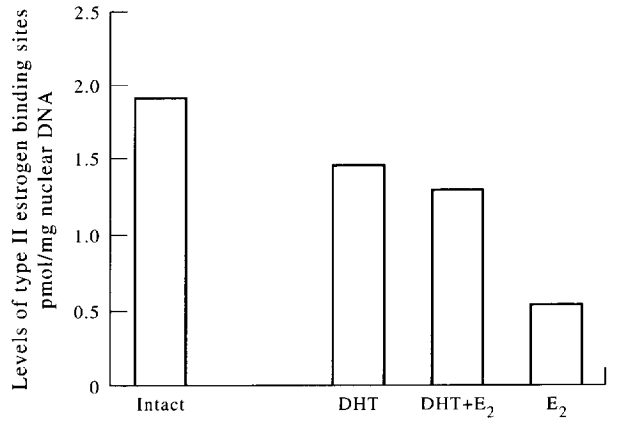

Fig. 1. Levels of type II estrogen binding sites in DLP nuclei preparations obtained from untreated intact rats (intact) and from castrated rats treated with DHT, E_2 and DHT + E_2 for 6 weeks. Histograms are mean values of levels obtained from two separate nuclei preparations. DLPs from 2–4 animals were pooled for each nuclei preparation. Levels were expressed as pmole ^{3}H-E_2 per mg nuclei DNA.

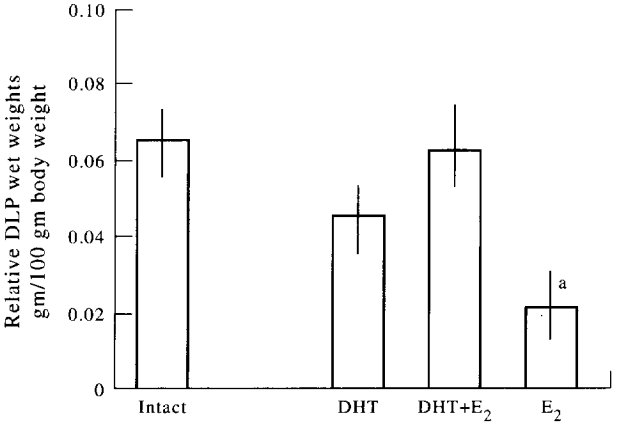

Fig. 2. Relative organ weights of DLPs excised from untreated intact rats (intact) and from castrated rats treated with DHT, E_2 and DHT + E_2 for 6 weeks. DLP wet weight expressed as a percentage of body weight. Histograms are group mean ± SD of values found in 8–10 animals. (See Experimental Approaches for the number of animals used in each group.) a = significant difference ($P < 0.05$) between the mean of the treated group and that of the intact untreated group.

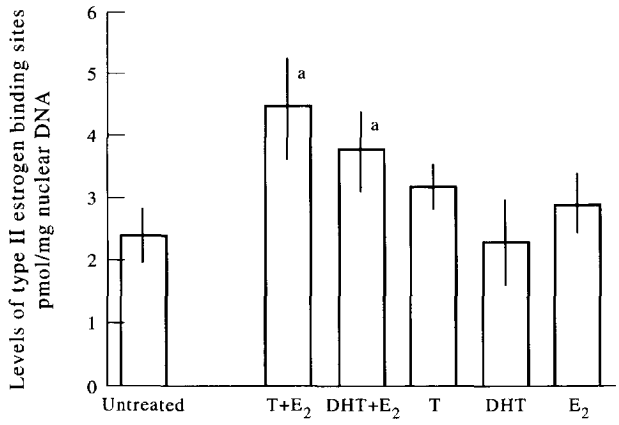

Fig. 3. Levels of type II estrogen binding sites in DLP nuclei preparations obtained from untreated intact rats and from intact rats treated with T + E_2, DHT + E_2, T, DHT, and E_2 for 16 weeks. Nuclei preparations were obtained from DLPs of individual animals. Histograms are mean values ± SD of levels obtained from 4–9 rats. Numbers of animals used in groups were described in Experimental Approaches. a = significant difference ($P < 0.05$) between the mean of a treatment group and that of the untreated control.

of intact control value or at 1.34 pmol/mg DNA. The combined hormonal treatment, however, did not maintain type II site levels at a value higher than that achieved with DHT alone. Together, these findings indicate that androgen is the primary testicular factor responsible for maintaining basal levels of type II site expression in the DLP.

Treatments of castrated rats with a single hormone, DHT or E_2, were effective in maintaining DLP wet weights at 71 and 34% of intact control values, respectively (Fig. 2). Treatment of castrated rats with DHT and E_2 simultaneously was effective in maintaining DLP wet weight at the pre-castration value (100%).

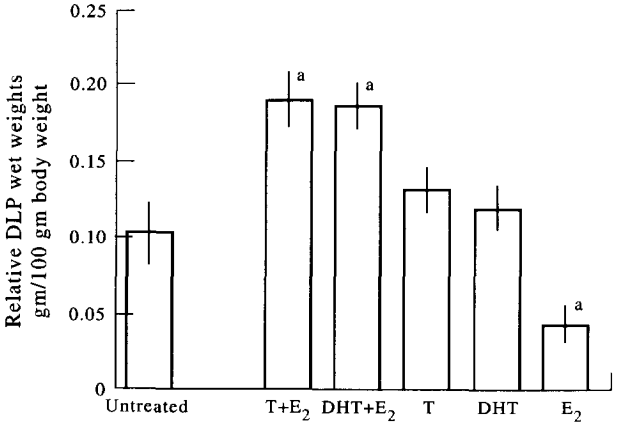

Fig. 4. Relative organ weight was gland wet weight expressed as a percentage of body weight. Histograms are means ± SDs of values found in 4–9 rats. Numbers of animals used in groups were described in Experimental Approaches. a = significant difference ($P < 0.05$) between the mean of the treated group and that of the untreated group.

Hormones involved in elevating type II site levels above basal values in rat DLP

We reported the induction of a doubling of nuclear type II sites in DLPs of intact NBL rats treated simultaneously with T and E_2 for 16 weeks [1, 2]. In this experiment we asked the question of whether separate or joint androgenic and estrogenic actions are required for the induction of augmented levels of type II site expression in rat DLP. Data from this study (Fig. 3) indicated treatment of intact rats with an androgen (DHT or T) or an estrogen (E_2) alone was not effective in elevating type II site levels in rat DLP. In contrast, combined administration of an androgen (DHT or T) with E_2 was shown to be effective in elevating type II site levels to values (4.3 ± 0.56 pmol/mg DNA) close to 2-fold of the values found in untreated controls.

Data on DLP wet weights (Fig. 4) indicated the combined androgen–estrogen treatments were effective in increasing DLP wet weights by approx. 100% above untreated control values. On the contrary, treatment of intact rats with T only marginally raised DLP wet weight while treatment of rats with DHT did not alter DLP wet weight. Finally, treatment of rats with E_2 caused significant regression of the gland.

DISCUSSION

These results demonstrate for the first time that testicular androgen is the primary factor responsible for maintaining basal level of type II site expression in rat DLP while combined androgenic–estrogenic action is needed for the induction of higher levels of expression in the tissue. Prostatic type II sites have been implicated in the regulation of tissue susceptibility and responsiveness to androgen–estrogen-induced growth of the DLP [1–3]. Present data lend further credence to this hypothesis.

Nuclear type II estrogen binding sites were first described in the rat uterus [15–17] and subsequently found in other normal and tumor tissues [22–26]. Although the functions of nuclear type II sites remain unclear, there is evidence to suggest that they are cellular mediators of long-term estrogen actions. For example, estrogen administration to ovariectomized female rats induces a transient increase in nuclear classical estrogen receptors, but a much more prolonged elevation of nuclear type II sites that parallels the time course of true uterine growth [15]. Likewise, in the avian liver, primary stimulation with estrogen induces long-term (months) expression of nuclear type II estrogen binding sites which may be the mediator of the "memory" effects of estrogen in the liver [22]. In NBL rats, following simultaneous treatment with T and E_2, levels of type II sites in the DLPs rose progressively and reached a level approx. 2-fold of the values found in untreated animals. The elevation of

type II sites in rat DLP was accompanied by gains in gland wet weight [2], increases in total cell number [14], augmented epithelial mitotic activity [1], overexpression of ras-proto-oncogene [27], and activation of the EGF/TGFα/EGF receptor paracrine system (unpublished data). Taken together these data argue for a causative relationship between type II sites and the dual hormone-induced growth of the DLP.

Data from the current study demonstrate a clear synergism between androgen and estrogen in the induction of elevated levels of type II sites and prostatic growth in rat DLP. A combined androgenic–estrogenic action is needed to induce these changes in the DLP. Levels of type II sites in the DLPs of long-term T, DHT, and E_2 treated rats remained unchanged from those observed in untreated controls. Androgen-treatment of rats also failed to induce any wet weight gain in the DLP while E_2-treatment caused gland atrophy likely via inhibition of the pituitary–testicular axis. These findings strengthen the suggestion that type II site elevation is an important factor in DLP growth. There are conflicting reports in the literature regarding the response of the rat prostate to various androgen–estrogen actions [8–10, 12, 28–30]. Different investigators have reported increased, decreased, or unchanged prostate weight after treatment with various androgen–estrogen combination regimens. Nevertheless, the focus of these reports was primarily on the VP, and little information is available on the response of the DLP to these hormonal regimens. In the NBL rats, T + E_2 consistently induced a profound growth response in the DLP while androgen-treatment alone failed to elicit a growth response in this tissue. Our demonstration of an absence of type II site elevation in the DLPs of androgen-treated rats offers an explanation to the apparent lack of response of rat DLP to androgen stimulation.

Little is known about the factors that are involved in the maintenance of basal level of type II site expression in a tissue. Low but persistent levels of nuclear type II sites have been reported in the uteri of mature ovariectomized female rats [17], in avian livers months after estrogen withdrawal [22], in a human hepatoma cell line grown in absence of estrogen [26] and in human leiomyomas during the luteal phase [24]. Thus it becomes apparent from these studies that basal levels of type II site expression are independent of estrogen. Administration of estrogen, however, dramatically elevates the levels of type II sites in these tissues or cell lines. In rat DLP, levels of type II sites decline following castration (our unpublished data). Nonetheless, we here demonstrate that androgens, rather than estrogens, are effective in sustaining type II site expression in the DLPs of castrated animals. Hence, it could be concluded that, in intact rats, testicular androgens are the principal factor responsible for maintaining basal levels of type II site expression in the DLPs. In this manner, these hormones are able to maintain DLP's unique susceptibility to sex hormone-induced growth.

In conclusion, our data indicate testicular androgens as the hormonal factor responsible for the maintenance of type II sites in rat DLP which may confer unique hormonal susceptibility to this prostatic lobe. Additionally, combined androgenic–estrogenic action is needed to induce an elevation of DLP type II sites, which may play a crucial role in mediating growth induced by the dual hormone action.

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