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Suppression of testosterone and estradiol-17β-induced dysplasia in the dorsolateral prostate of Noble rats by bromocriptine

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We, and others, have previously described the histological changes that occur in the prostate gland of intact Noble (NBL) rats following prolonged hormonal treatment. Dysplasia, a pre-neoplastic lesion, develops specifically in the dorsolateral prostates (DLPs) of NBL rats treated for 16 weeks with a combined regimen of testosterone (T) and estradiol-17β (E_2) (T + E_2-treated rats). Concurrent with DLP dysplasia induction, the dual hormone regimen also elicits hyperprolactinemia, in addition to an elevation of nuclear type II estrogen binding sites (type II EBS), no alteration in estrogen receptors (ER), and marked epithelial cell proliferation in the dysplastic foci. The aim of this study was to investigate whether the dual hormone action is mediated via E_2-induced hyperprolactinemia. Bromocriptine (Br), at a dose of 4 mg/kg body wt per day, was used to suppress pituitary prolactin (PRL) release. Serum PRL levels were lowered from values of 341 ± 50 ng/ml in T + E_2-treated rats to 32 ± 10 ng/ml in Br co-treated animals. The latter values were comparable to those in untreated control rats. In addition, Br co-treatment effectively inhibited the evolution of dysplasia (six out of eight rats) and the often associated inflammation (five out of eight rats) in most animals. In contrast, Br co-treatment did not suppress the T + E_2-induced type II EBS elevation nor alter ER levels in the DLPs of these rats, when compared with T + E_2-treated rats. These data extend the many previous studies that have detailed marked influences of PRL on rat prostatic functions. However, the current study is the first to implicate PRL in prostatic dysplasia induction *in vivo.*

Introduction

Previous reports from this and other laboratories have described the Noble (NBL*) rat as an experimental model for hormonally induced prostatic dysplasia and cancer (1–6). Simultaneous life-time treatment of intact male NBL rats with testosterone and estradiol-17β (T + E_2) induced a 100% incidence of carcinoma in the dorsolateral (DLP), but not the ventral lobe (VP), of the rat prostate (6). Shorter-term (16 weeks) treatment with the combined hormonal regimen consistently generated prostatic dysplasia, a putative preneoplastic lesion, exclusively within the enlarged DLPs (4–6). Mitotic activity was increased in these glands, with mitoses restricted almost entirely to the epithelium of the dysplastic foci (4,5). Close histological similarities exist between this proliferative lesion in the rat gland and prostatic intraepithelial neoplasia (PIN), a presumed precursor of prostate adenocarcinoma in the human prostate (7,8). Since the separate long-term administration of either androgens (T or 5α-dihydrotestosterone, DHT) or E_2 did not induce dysplasia in the rat DLP, the combined androgen–estrogen action was deemed essential in the development of this proliferative, preneoplastic lesion (4,5,9).

Multiple estrogen binding sites have been described in the prostate glands of a number of mammalian species (10–15). These include the classical estrogen receptor (ER) which binds estrogen with high affinity, and a class of moderate affinity estrogen binding sites (EBS), termed type II EBS. Type II EBS were initially implicated in the long-term growth response of rat uterus to E_2 (16,17) and were more recently correlated with aberrant growth of many neoplasms and cancer cell lines (18–20). In the rat prostate, type II EBS are restricted to the DLP, and are expressed at much higher levels than those of ER in the normal gland (9,12,21,22). Treatment of NBL rats with T + E_2 for 16 weeks induces a gradual elevation of type II EBS in the DLP, which precedes the occurrence of dysplasia in this prostatic lobe (22). Concomitant with the appearance of dysplasia, a marked increase in mitotic activity is observed in the DLP epithelia. These observations led us to hypothesize that the type II EBS are involved in the development of dysplasia and/or abnormal proliferation in the rat DLP. In contrast, ER levels in the DLPs of untreated rats are low and remain unaltered throughout the entire period of T + E_2 treatment (22). Based on this finding we conclude that prostatic ER may not be directly involved in the development of DLP dysplasia.

The action of estrogen on the rat prostate may also be indirectly mediated via hyperprolactinemia. It is well-established that E_2 stimulates the secretion of PRL from the pituitary (23) and this peptide exerts marked influences on the prostate (24,25). The lateral lobe (LP) of the rat prostate is sensitive to PRL in terms of growth (26,27), zinc ion homeostasis (28), citrate production (25,29), pyruvate dehydrogenase E1-α (30) and ornithine decarboxylase synthesis (31), and induction of inflammation (32). Exposure of DLP organ cultures to PRL, alone or in conjunction with T, induced *de novo* DNA synthesis and morphological atypia (33). PRL also significantly increases the transcription of several lobe-specific proteins (RWB, probasin and prostatein) in the immature rat prostate in an androgen independent manner (34). We (4), and others (6), have observed pituitary hyperplasia and adenoma in rats treated chronically with T + E_2. In addition, Ofner and co-workers (35) have

***Abbreviations:** NBL, Noble; DLPs, dorsolateral prostates; T, testosterone; E_2, estradiol-17β; type II EBS, type II estrogen binding sites; Br, bromocriptine; PRL, pituitary prolactin; VP, ventral lobe; PIN, prostatic intraepithelial neoplasia; DHT, 5α-dihydrotestosterone; ER, estrogen receptor; LP, lateral lobe; D_2, dopamine receptor; DES, diethylstilbestrol; PCNA, proliferating cell nuclear antigen; H&E, hematoxylin and eosin; ARs, androgen receptors; IGF-1, insulin-like growth factor-1.

detected hyperprolactinemia in rats treated with the same regimen. Taken together, these observations raise the possibility that hyperprolactinemia may play a role in dysplasia induction in T + E_2-treated rats.

The goal of the current study was to investigate whether dysplasia induction in the DLP was mediated by E_2-induced hyperprolactinemia. Suppression of PRL release from the pituitary of T + E_2-treated rats was effected by co-administration of bromocriptine (Br), which is a dopamine receptor (D_2) agonist (36).

Materials and methods

Animals and treatments

Male NBL rats (4–5 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed, three to a cage, in stainless steel wire-mesh cages and allowed access to food and water *ad libitum*. Experimental treatments commenced at 11–12 weeks of age (280–300 g). Hormonal implants were prepared as previously described (4) using Silastic™ tubing (Dow Corning Corporation, Corning, NY; No. 602-205, 1.0 mm i.d.×2.2 mm o.d.) densely packed with steroid (Sigma, St Louis, MO). The length of the T and E_2 implants were 2 cm and 1 cm, respectively. Hormonal implants, two T and one E_2, were placed subcutaneously in the flank of the rats, under isoflurane (Ærrane®, Anaquest, WI) anesthesia. The implants were removed and replaced 8 weeks after the initiation of the experiment. Previously, this treatment protocol was shown to maintain normal levels of circulating T and a moderate elevation of plasma E_2 levels (4). All animal treatment protocols were previously approved by Tufts Institutional Animal Care and Usage Committee, and are in accordance with NIH animal usage guidelines.

Br was provided by Sandoz Research Institute (East Hanover, NJ) and incorporated into sustained release pellets by Innovative Research of America (Sarasota, Fl) to effect a daily release of 4 mg/kg body wt per day. This dose was shown to be effective in inhibiting T + E_2-induced inflammatory response in the LPs of Wistar rats (32). We used 60-day release pellets and these were changed once (after 2 months) during the 16-week protocol. The pellets were implanted sub-cutaneously under isoflurane anesthesia in the flank of the rat, at a site distant from the steroidal implants. Br-treated rats were continually monitored for aggressive behavior, and housed individually when necessary. The duration of treatments was 16 weeks. Treatment groups were as follows: age-matched untreated controls ($n = 5$); T + E_2-treated ($n = 7$); T + E_2 + Br-treated ($n = 8$).

Tissue processing

At the end of the 16-week treatment period, animals were briefly anesthetized with isoflurane followed by decapitation. Anesthesia generally did not last longer than 30 s. Although male rats can release PRL in response to the stress of a mild inhalant anesthesia (37), this brief exposure to isoflurane prior to blood collection is such that any increase in serum PRL would likely be minimal in our experimental males. Trunk blood was collected and the serum component retained for subsequent E_2 and PRL determinations. DLPs were immediately removed, weighed, and bisected, one half for [^{3}H]E_2 binding assay, and the other for subsequent pathological and immunohistochemical studies. The half DLP retained for [^{3}H]E_2 binding analysis was immediately placed on ice in Earles minimum essential medium (GIBCO, Grand Island, NY) plus 0.25% bovine serum albumin (pH 7.5) and then crude nuclei were processed as previously described (12).

Determination of [^{3}H]E_2 binding in DLP nuclei

Aliquots of crude nuclei preparations from individual half DLP glands, diluted with TEG (Tris–HCl 1.0 mM, EDTA 1.5 mM, glycerol 10%, pH 7.4; reagents supplied by Sigma, MO) buffer were subjected to saturation analysis by incubation with a broad range (1–40 nM) of [^{3}H]E_2 (2, 4, 6, 7-labeled, specific activity 87 Ci/mmol; Dupont, New England Nuclear, Boston, MA) concentrations, according to a previously described protocol (12). Incubations were performed at 30°C for 30 min in the presence or absence of a 300-fold molar excess of diethylstilbestrol (DES, Sigma). After the incubation period, receptor-bound and free radioligand were separated by hydroxylapatite (Bio-Rad, Richmond, CA) suspension. Specifically bound [^{3}H]E_2 at 1–3 nM was taken as an estimation of binding to the ER, and binding at 15–40 nM as binding to type II EBS.

Histological studies, proliferating cell nuclear antigen (PCNA) immunostaining, and establishment of a PCNA-labeling index

For dysplasia detection and immunohistochemical analyses, half of each DLP was fixed in 10% buffered formalin (Sigma) for ~1 h. When firm, the DLPs were parasagitally step-sectioned into slabs ~0.25 cm thick and labeled 1–3 according to their position from the urethral face to the periphery of the DLP. These slabs were then re-immersed in 10% buffered formalin for 24 h, and subsequently processed, embedded in paraffin with the urethral face up, sequentially sectioned 4–6 μm thick and mounted on Fisher Superfrost Plus® slides (Fisher Scientific, Pittsburgh, PA). Every fifth section was stained with hematoxylin and eosin (H&E). H&E stained sections were first examined histologically for the presence of dysplasia. Unstained sequential sections were then selected for PCNA immunostaining, as described previously (38,39).

Six DLP sections were chosen from each gland, of which two were from the region closest to the prostatic urethra, and two from the mid-portion, and the remaining two sections from the periphery of the DLP lobe. For enumeration of the PCNA-stained cells in sections, an outline of the shape of each immunostained section counted was drawn on paper using a ×4 objective. All structures in each section, including the dysplastic foci, were identified and entered on the drawing (the map). The map of each chosen section was randomly divided into four areas. Using a ×10 objective fitted with a grid, 500 cells were counted in each of the four areas, up to a total of 2000 cells counted per section. Only epithelial cells with strongly-positive PCNA stained nuclei were counted as positive. This counting process was repeated for each of the six sections from an individual DLP (12 000 cells counted per gland). In DLPs with dysplasia, the same counting procedure was used to generate separate labeling indices for non-dysplastic and dysplastic areas within these glands. PCNA-labeling index refers to the number of PCNA-positive epithelial cells divided by the total number of epithelial cells counted (positive and negative), and expressed as a percentage. The enumeration of PCNA stained cells was independently performed by two of us (J.Z., I.L.) in a double-blind manner. We and others have previously used PCNA to quantify cell proliferation in the rat and human prostate (38,39).

Radio-immunoassay technique for serum PRL and E_2

Serum concentrations of PRL were measured using the NIDDK rat PRL kit that was supplied by the National Hormone Pituitary program and Dr A.Parlow (director of the Pituitary Hormones and Antisera Center, Torrance, CA). This kit includes reference preparation NIDDK-rPRL-RP-3 and anti-PRL S-9. Serum samples were diluted with assay buffer and assayed in duplicate at volume equivalents of 0.1, 1, 10 and 50 μl serum. The assay sensitivities averaged 30–40 pg/tube; interassay and intra-assay coefficients of variation were 9% and 5%, respectively.

E_2 levels in serum were detected by a Serono Estradiol MAIA kit (purchased through Polymedco Inc, Portland, NY) using [^{125}I]E_2 with ether extracted serum samples. Intra-assay and inter-assay coefficients were 4.5% and 5.6%, respectively, and the detection limit was ~5 pg/ml.

Statistical analysis

Data were analyzed by one-way ANOVA with a *post hoc t*-test. Statistical significance ($P < 0.05$) is noted on figures by an asterisk (*).

Results

Serum hormone levels

Serum E_2 levels in T + E_2-treated (125 ± 55 pg/ml) and T + E_2 + Br-treated (136 ± 60 pg/ml) rats were significantly elevated compared with untreated controls (5.5 ± 1.4 pg/ml; Figure 1a). Br co-treatment had no effect on serum E_2 levels, since levels observed in T + E_2- and T + E_2 + Br-treated rats were similar.

Serum PRL levels are presented in Figure 1b. Treatment of rats with T + E_2 elevated PRL levels to 341 ± 50 ng/ml, compared with 22 ± 4 ng/ml in untreated control animals. Administration of Br to T + E_2-treated rats returned the PRL levels to untreated control values. Serum PRL levels in the six T + E_2 + Br-treated rats with no dysplasia in the DLPs were used to obtain the mean value for the group (32 ± 10 ng/ml), which is represented as a histogram bar in Figure 1b. Two of the animals treated with T + E_2 + Br developed dysplasia (see next section) and their individual serum PRL levels were 91 and 31 ng/ml. These two values are indicated as a triangle and a circle in Figure 1b.

Histological studies of dysplasia in rat DLP

As previously reported (4,5), when rats were treated with T + E_2 for 16 weeks, dysplasia was consistently present in the

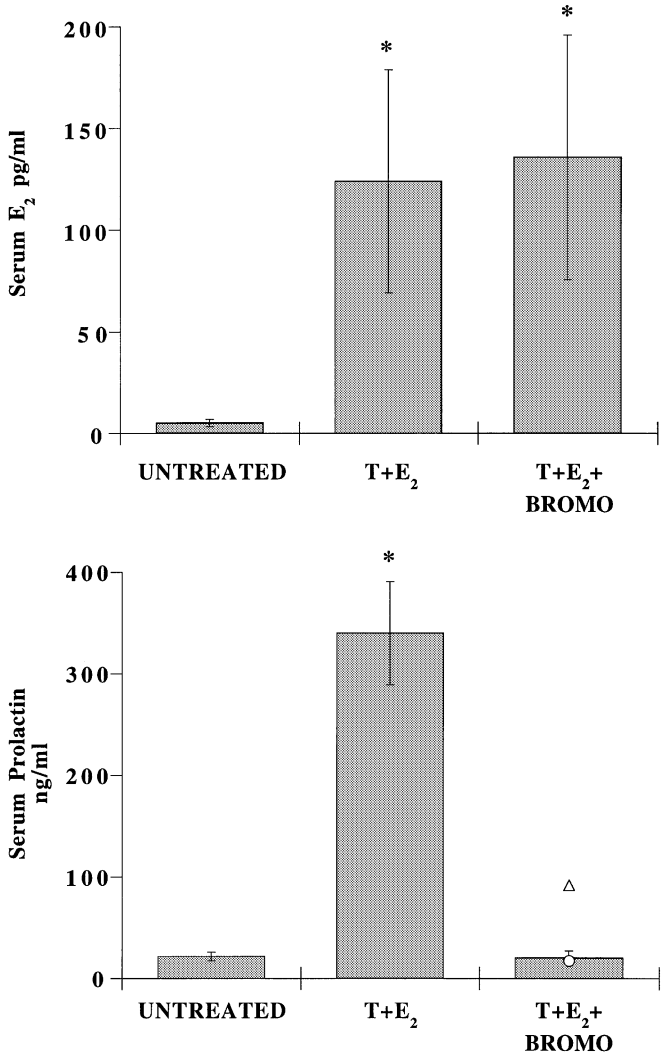

Fig. 1. (**a**) Serum E_2 levels. (**b**) Serum PRL levels. Trunk blood was collected from NBL rats [untreated (n = 5), treated with T + E_2 (n = 7), T + E_2 + Br (n = 8)] and analyzed by RIAs. Data are expressed as group means ± SEM (pg/ml). Two of the eight animals treated with T + E_2 + Br had dysplastic DLPs and the values for these rats have not been included in the calculation of group means in this figure. The individual serum PRL levels of these rats are depicted by a circle and a triangle. Statistical significance relative to untreated control ($P < 0.05$) is shown by *, n is the number of animals in each group.

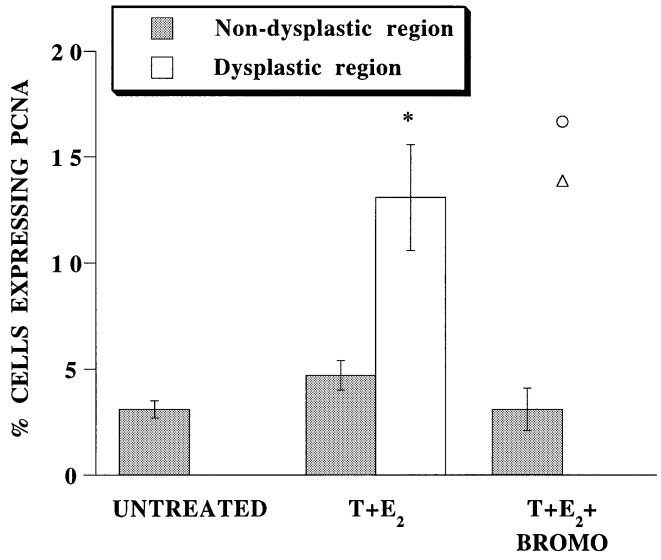

Fig. 2. PCNA labeling indices, measured as the percentages of PCNA-immunopositive cells, in normal epithelia of DLPs obtained from untreated control rats (n = 5), and in epithelia of non-dysplastic and dysplastic regions of DLPs from rats treated with T + E_2 (n = 7), and in the non-dysplastic and dysplastic regions of DLPs from rats treated with T + E_2 + Br (n = 8). Data are expressed as group means ± SEM. Two of the eight animals treated with T + E_2 + Br had dysplastic foci in their DLPs and the PCNA indices in these areas have been determined separately, and depicted by a circle and a triangle. Statistical significance relative to untreated control ($P < 0.05$) is shown by *, n is the number of animals in each group.

DLPs of all animals (seven of seven rats in this experiment). Dysplastic foci were largely found in the LP, but lesions were also present in the DP as well as in the periurethral ducts. Dysplasia was not observed in the DLPs of aged-matched untreated rats (zero out of five). The dysplastic lesions were often accompanied by inflammatory infiltrates. As was the case for dysplasia, the inflammatory lesions were mainly in the LP, with some also noted in the DP. Inflammatory foci were composed of neutrophils, which were most evident within acini and ducts, and interstitial accumulations of lymphocytes and plasma cells. In most instances the areas of inflammation were closely associated with dysplastic lesions. However, it was not uncommon to find dysplastic foci that had little or no obvious relationship to the inflammatory process.

Treatment of T + E_2-treated rats with Br inhibited DLP dysplasia development in six out of eight animals. In five of the six T + E_2 + Br-treated rats that did not develop DLP dysplasia, inflammatory infiltrates were completely absent in their DLPs. In the sixth animal of this group extensive inflammation was noted in the DLP, and its serum PRL level was not elevated (17.6 ng/ml). In the two T + E_2 + Br-treated rats that developed dysplasia, serum PRL levels were 91 and 31 ng/ml. In both cases inflammation, of the same cellular composition and extent as noted in rats treated with T + E_2 *per se*, was evident in the DLP. As serum samples were taken only at the end of the experimental period, it is possible that incomplete suppression of pituitary PRL release may have occurred during the course of the 16-week treatment regimen, and significantly contributed to the observed variability in response of different animals.

PCNA immunostaining

Figure 2 depicts the percentage of PCNA-positive cells in DLP epithelia of untreated, T + E_2-treated and T + E_2 + Br-treated rats. Since dysplasia is a focal lesion in the rat DLP, we have separately enumerated the PCNA-labeling indices in dysplastic and non-dysplastic epithelia in the DLPs of the T + E_2- and T + E_2 + Br-treated animals, and data are represented as histogram bars (Figure 2). In the DLPs of untreated control rats, all epithelia were normal, or non-dysplastic, hence only one histogram bar was used to depict the data from this group.

In the non-dysplastic regions of the DLPs there was no difference in the epithelial PCNA-labeling-indices from all three experimental groups (untreated = 3.1 ± 0.4%, T + E_2-treated = 4.7 ± 0.7%, T + E_2 + Br-treated = 3.1 ± 1%, respectively). The PCNA-labeling index was markedly elevated in the dysplastic foci of the DLPs of rats treated with T + E_2 (13.1 ± 0.9%), when compared with non-dysplastic regions of the DLP from the same rats, and also when compared with

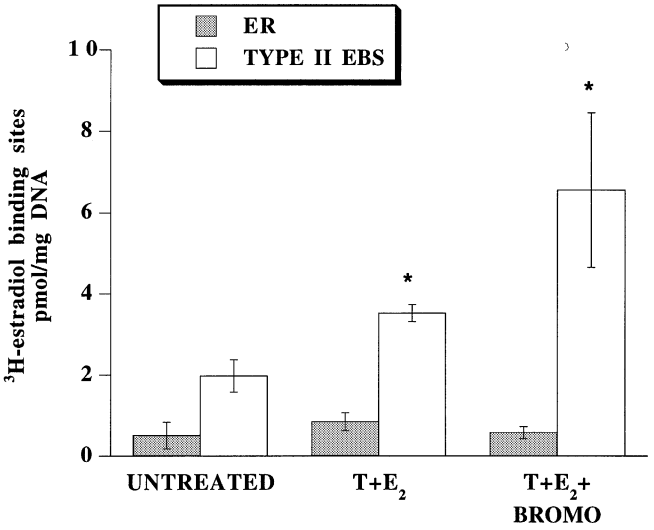

Fig. 3. Levels of [^{3}H]E_2 binding sites in the nuclei of DLPs from NBL rats treated with T + E_2 (n = 7), T + E_2 + Br (n = 8) or left untreated (n = 5). Binding to ER was determined as specific binding between 1–3 nM [^{3}H]E_2, and type II EBS binding was estimated as specific binding between 15–40 nM [^{3}H]E_2. [^{3}H]E_2 binding is expressed as pmol/mg DNA. Data are expressed as group means ± SEM. Statistical significance relative to untreated control ($P < 0.05$) is shown by *, n is the number of animals in each group.

the PCNA-labeling index in DLPs of untreated rats. In six out of eight T + E_2 + Br-treated rats only normal, non-dysplastic epithelia with low PCNA-labeling index (3.1 ± 1%) were noted in their DLP sections. The PCNA-labeling indices in the DLP dysplastic foci of the two T + E_2 + Br-treated rats that developed the lesion were high, with values of 16.7% and 13.9%, respectively. These values are represented as a circle and a triangle in Figure 2.

Levels of ER and type II estrogen binding sites (type II EBS) in the DLP nuclei

Figure 3 shows the effects of Br co-treatment with T + E_2 on nuclear ER and type II EBS in the DLP. DLPs from untreated rats expressed 1.98 ± 0.39 pmol/mg DNA type II EBS, and very low levels of ER (0.51 ± 0.33 pmol/mg DNA). A significant elevation in type II EBS to 3.5 ± 0.2 pmol/mg DNA was observed in rats treated with T + E_2. In contrast, the levels of ER in DLPs of T + E_2-treated rats remained unchanged (0.85 ± 0.2 pmol/mg), when compared with values from untreated animals. These data are in full agreement with our previous findings (22).

Treatment of rats with T + E_2 + Br did not significantly alter the T + E_2-induced elevation of nuclear type II EBS in the DLP, nor did this treatment regimen affect the levels of DLP ER. Individual serum PRL levels did not correlate with individual DLP type II EBS values (analyses not shown).

Discussion

In this study we investigated whether the action of T + E_2 in DLP dysplasia induction was mediated via protracted hyperprolactinemia, presumably caused by estrogen stimulation of hormone release from the pituitary. We have demonstrated that administration of Br, at a sustained daily dose of 4 mg/kg body wt, to T + E_2-treated rats effectively lowered circulating PRL levels to those observed in untreated rats. Concurrently, this treatment caused a general inhibition of dysplasia in the DLP. In addition, the sex-hormone induced type II EBS elevation, which had previously been shown to correlate with dysplasia induction (9,21,22), was not negated in the non-dysplastic DLPs from rats treated with T + E_2 + Br. Epithelial cell proliferation, measured as a PCNA-labeling index, was focally elevated in dysplastic foci, and remained low in neighboring non-dysplastic areas, irrespective of the treatment regimen.

PRL has been implicated as a cause of inflammation in the rat prostate (32), however the relationship, if any, of dysplasia to the presence of inflammatory cell infiltrates in the DLP remains undefined. In our study, many DLP dysplastic lesions in T + E_2-treated NBL rats were associated with profound inflammatory infiltrates. Tangbanleukal and Robinette (32) reported that treating Wistar rats with DHT and E_2 for 4 weeks induced florid inflammation, but no dysplasia, in the DLP. These workers demonstrated that the inflammatory process could be completely blocked by incorporating Br into the sex-hormone regimen. In addition, they showed that the inflammatory process was restored by administering ovine PRL to the Br-treated hormone-implanted rats. They concluded that the dual sex-hormone treatment caused PRL release from the pituitary, which was ultimately responsible for causing the inflammatory lesions. However, dysplasia was not induced in their study, presumably due to either the shorter duration of treatment (4 versus 16 weeks), choice of DHT rather than T, and/or the different strain of rat used.

While we cannot totally discount the possible contribution of the inflammatory process to the genesis of dysplasia in the T + E_2-treated rats, results from the current study, and previous work by others (33) suggests that direct effects of PRL and the sex-hormones are the major inducers of the lesion. In this regard, Nevalainen *et al*. (33) have shown that proliferative changes, which closely approximate dysplasia, were induced in organ cultures of DLP following the supplementation of explants with PRL, or combinations of androgens and estrogens. In addition, the finding of inflammation and no dysplasia in the DLP of one rat treated with T + E_2 + Br, who also had normal serum PRL suggests that the peptide, and not the inflammatory process, is the major factor in the pathogenesis of dysplasia. In further support of this, one of us (I.L.) has observed spontaneous inflammatory lesions in the DLP of untreated rats, of the same cellular composition and extent as found in the glands of T + E_2-treated animals, that were devoid of dysplastic foci.

Other reported roles of PRL in the rat prostate fall broadly into two categories; those related to differentiated functions (25,28–32,34,40,41), and those linked to growth. The trophic effects of PRL on rat prostate have only been demonstrated during pubertal development (34,42,43) and in regeneration experiments involving atrophic glands (26,27,44). The growth promoting actions of PRL are believed to be partially direct, as demonstrated by organ culture (33,45) and pituitary grafting (27) experiments, and in part dependent on the actions of other trophic mediators, such as androgens (ARs), insulin-like growth factor-1 (IGF-1) and IGF-1 receptors (40,42). It has been proposed that PRL action may be mediated via androgenic pathways. Several investigators have demonstrated that elevated serum PRL elicits a trophic effect on the LP by increasing the turnover of tissue DHT content (27,46,47). In addition, it has been reported that PRL elevates AR protein (40) and mRNA (42) levels in the rat prostate. Other investigations, however, demonstrated that PRL exerts androgen-independent

actions on the prostate (34,48). Thus, the precise nature of the synergism between androgens and PRL in the prostate remains undefined.

The existence of prolactin binding sites in the rat (49–52) and human (53–55) prostate is well established, although the lobe-specific distribution of these sites in the rat gland is not entirely clear. The PRL receptor is a membrane-spanning protein, and a member of the cytokine receptor growth factor family (56). This receptor is known to associate with either a tyrosine kinase (JAK-2; 57) or a serine/threonine kinase (RAF-1; 58) to turn on cellular proliferation in several cell- and organ-culture systems. Cell division *per se* is considered to be a risk factor in tumorigenesis, as sustained cellular proliferation may promote and fix DNA damage in addition to affecting DNA fidelity check-points and repair capabilities (59). In the current study we demonstrated augmented cellular proliferation to be focally localized to the dysplastic areas of affected DLPs. Demonstration of upregulation of PRL receptors and associated signal transduction pathways in the affected DLPs would further strengthen the notion that PRL signaling is of significance to dysplasia development and/or the proliferation response found in the DLPs of T + E_2-treated animals. Yet, it remains difficult to delineate whether enhancement of cell proliferation is a cause or consequence of the development of dysplasia in this model.

Previously we demonstrated temporal and hormone treatment-specific correlations between dysplasia induction and an elevation in moderate affinity, type II EBS in the DLPs from NBL rats (4,5,9,22), and have postulated that this estrogen-binding site may play a major role in the pathogenesis of DLP dysplasia. Our current results suggest that the high level of circulating serum E_2, supported by T and unaltered by Br administration, is responsible for maintaining the elevated levels of type II EBS in the DLPs of T + E_2 + Br-treated rats. However, we noted that in the general absence of dysplasia in the DLPs of T + E_2 + Br-treated rats, type II EBS remained elevated in this prostatic lobe. This finding leads us to re-assess the role of type II EBS in DLP dysplasia induction. It is possible that nuclear type II EBS are not involved in the induction of dysplasia, and the elevation of these binding sites in the DLPs of T + E_2-treated rats is an independent event. Alternatively, elevation in type II EBS may still be a contributing factor in DLP dysplasia, but the elevation has to act in a conjoint manner with hyperprolactinemia. Therefore, when hyperprolactinemia is inhibited by co-treatment with Br, dysplasia development is arrested in the majority of the treated rats. In future investigations, experimental means of inhibiting type II EBS alone, without affecting the T + E_2-induced hyperprolactinemia, would have to be employed to further clarify the role played by this binding site in dysplasia induction in the rat DLP.

In summary, this is the first report to demonstrate that sustained hyperprolactinemia plays a direct role in the induction of dysplasia in the DLPs of T + E_2-treated rats. Thus, in addition to the well-documented effects of PRL on rat prostate growth, inflammation and metabolism, we must now consider the possibility that this peptide is involved in dysplasia induction in the prostate.

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