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Increased Progesterone Receptor Concentrations in Bladder Lesions of Estrogen-treated Syrian Hamsters[1]

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ABSTRACT

During studies of renal tumorigenesis induced by estrogen in Syrian hamsters, we have observed that about 15 to 20% of animals develop bladder lesions with an increased wet weight of tissue from 0.2 g to 0.4 to 1.7 g. Histological examination of the lesions showed a spectrum of changes from inflammatory reactions to squamous metaplasia and intense hyperplasia of the transitional epithelium. The concentration of progesterone-binding sites was increased in the bladders with lesions. No specific progesterone-binding sites could be detected in the cytosol of bladders from hamsters not treated with estrogen. The affinity constant for the progesterone-binding sites in cytosol from bladders with lesions was 10^9 M^{-1}, the same as that reported for progesterone receptors in other target tissues for estrogen. The binding sites are specific for progesterone and are not competed for by 17β-estradiol, 5α-dihydrotestosterone, or aldosterone.

INTRODUCTION

Renal adenocarcinoma induced by estrogen is unique to the Syrian hamster and was described initially by Kirkman (5) and Matthews *et al.* (12). High-affinity receptors for estrogen (9) and for progesterone (10) have been demonstrated in primary renal adenocarcinoma tissue. The earliest biochemical change detected in the kidneys of estrogen-treated hamsters (7, 13) is an increased concentration of progesterone receptor (10); this change occurs several months prior to the transformation of normal tissue into adenocarcinoma. During our studies of renal tumorigenesis, we have observed that 15 to 20% of hamsters treated with estrogen for 7 to 10 months develop bladder lesions with increases of as much as 5-fold in wet weight of the evacuated bladder. Bladder lesions existed in some hamsters in the absence of visible renal tumors, suggesting that the bladder lesions were not a consequence of renal tumor metastasis. The induction of bladder carcinoma in the rat in response to prolonged estrogen treatment was described several decades ago (2). However, the induction of bladder tumors by estrogen in the hamster has not been previously described nor is the bladder a primary site of renal tumor metastasis (5). Since increased concentrations of progesterone receptors have been a sensitive probe for estrogen responsiveness in the preneoplastic hamster kidney, we have measured the concentration of progesterone-binding sites in bladders of estrogen-treated animals and in bladder lesions.

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MATERIALS AND METHODS

Chemicals and Reagents. [1,2,6,7-^{3}H]Progesterone (104 Ci/mmol), and Biofluor were obtained from New England Nuclear, Boston, Mass. Unlabeled progesterone, cortisol, Trizma base, Norit A, Dextran 80, dithiothreitol, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, Mo. Anhydrous glycerol was purchased from the J. T. Baker Chemical Co., Phillipsburg, N. J. Zephiran was obtained from Winthrop Laboratories, New York, N. Y.

Animals. Young, mature, castrated male Syrian hamsters (LVG:LAK, outbred strain, Charles River; Lakeview Hamster Colony, Newfield, N. J.) weighing 80 to 100 g were used. Pellets of diethylstilbestrol were administered as previously described (9). All animals were exsanguinated under ether anesthesia, and tissues were removed and immediately placed on ice.

Preparation of Cytosol. Bladder tissues were evacuated, weighed, rinsed with 0.9% sodium chloride solution, and homogenized with glass-glass conical grinders in 5 to 10 volumes of 0.01 M Tris-Cl, pH 7.5–1.5 mM disodium EDTA–1 mM dithiothreitol–0.1% bovine serum albumin containing 30% glycerol at 0°. Tissue homogenates were centrifuged for 60 min at 105,000 × *g*, and cytosol fractions were diluted to appropriate protein concentrations with the above buffer. Cortisol was added at a final concentration of 1 μM to prevent the binding of labeled progesterone to cortisol-binding globulin (6).

Assay of Progesterone Receptor. Cytosols were incubated with a saturating concentration (*i.e.*, 4×10^{-9} M) of tritiated progesterone; to correct for nonspecific binding, other samples of cytosol preparation containing labeled progesterone and a 100-fold excess of unlabeled progesterone were incubated. After incubation to equilibrium (2 hr) at 0°, unbound steroid was removed by treating with dextran-coated charcoal (16) for 5 min. The amount of specific radioactive progesterone bound to the cytosol receptor was measured and expressed as concentration of progesterone-binding sites for 1 mg protein per ml cytosol.

Scatchard Analysis. Cytosols were incubated with 10 to 40 $\times 10^{-10}$ M tritiated progesterone. To correct for nonspecific binding, other samples of the cytosol preparation containing labeled progesterone and a 100-fold excess of unlabeled progesterone were incubated. After incubation to equilibrium (24 hr) at 0°, unbound steroid was removed by charcoal treatment as cited above. The amount of radioactive progesterone bound was measured, and the data were expressed according to the method of Scatchard (15).

Radioactivity Measurement. Four ml of Biofluor were added to 0.5 ml of cytosol, and radioactivity was measured in a Packard Model 3002 scintillation spectrometer at 34% efficiency for tritium.

Miscellaneous. The protein content of the cytosol was measured by the procedure of Lowry *et al.* (11) using bovine serum albumin as a standard. Appropriate corrections were made for the serum albumin added to the homogenization buffer.

Histology. Bladder lesions for histological study were fixed in Bouin's solution and stained with hematoxylin and eosin.

Statistical Analysis. Differences in the variance of the concentration of progesterone-binding sites among groups of bladders were corrected with log transformation and analyzed with Student's *t* test procedure.

RESULTS

Concentration of Progesterone-binding Sites in Cytosols of Bladder Tissues from Estrogen-treated Hamsters. No specific progesterone-binding sites could be detected (at 8 mg protein per ml cytosol) in cytosols of bladders from hamsters not treated with estrogen. Even after 3 to 4 months of estrogen treatment, the concentration of binding sites was near the limit detectable in the assay (*i.e.*, 0.17×10^{-10} M) (Chart 1). By 7 to 10 months of estrogen treatment, the concentration of binding sites in most of the bladders of normal size, *i.e.*, 0.19 ± 0.05 (S.E.; $n = 8$) g remained at a concentration equivalent to that in bladders of animals after 3 to 4 months of estrogen treatment. In one of 6 bladders assayed for progesterone binding sites and weighing 0.29 g, the cytosol concentration of binding sites was 4-fold greater than the concentration in the other cytosols (Chart 1). Of the 27 animals examined after 7 to 10 months of estrogen treatment, 9 hamsters had bladders weighing 0.4 to 1.7 g. The mean weight for the enlarged bladders was 0.89 ± 0.18 ($n = 9$) g. Eight of the bladders with lesions were assayed for progesterone receptor. The concentration of progesterone-binding sites ranged from 8.67 to 61.56 $\times 10^{-11}$ M when expressed for 1 mg protein per ml cytosol (Chart 1). The difference in mean concentration of progesterone-binding sites between the total group of bladders with lesions and the group of bladders from animals after 3 to 4 months of estrogen treatment was statistically significant ($p < 0.01$).

Measurement of Binding Constant for Progesterone in Cytosol of Bladder with Lesion. To determine if the K_a for the progesterone-binding sites in the bladder was of a high affinity and similar to that previously reported for the receptors in the hamster uterus (6) and renal adenocarcinoma (10), Scatchard analysis (15) was used for the measurement of the binding constant. The affinity constant determined from the slope in Chart 2 was 1.5×10^{9} M^{-1}, a value nearly identical to that for the receptor in other target tissues for estrogen (6, 10). The concentration of progesterone-binding sites determined from the abscissa intercept in Chart 2 was 1.66×10^{-10} M when expressed for 1 mg protein per ml cytosol.

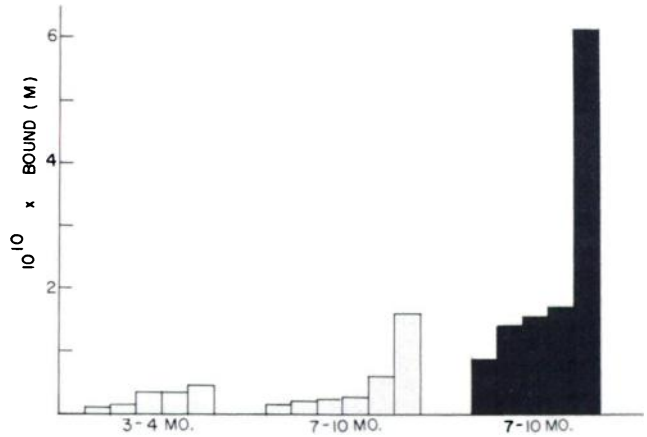

Chart 1. Concentration of progesterone-binding sites when expressed for 1 mg protein per ml cytosol of individual bladder tissues from animals after 3 to 10 months of estrogen treatment. ■, values for bladders with wet weights of 0.4 to 1.3 g; □, values for bladders of less than 0.3 g.

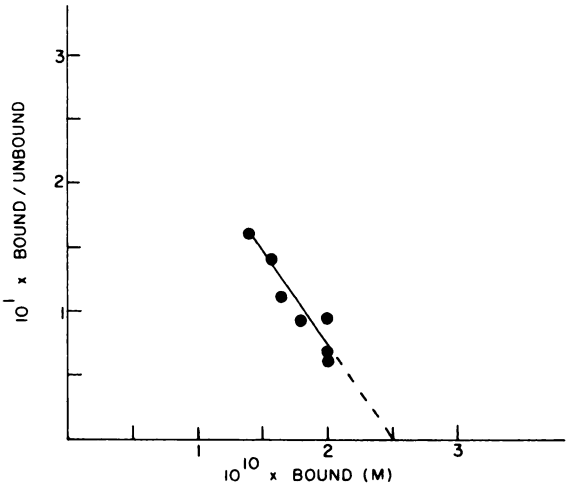

Chart 2. Scatchard plot of progesterone binding in cytosol from an enlarged bladder weighing 1.2 g. Scatchard analysis was performed at 1.5 mg protein per ml cytosol.

Table 1

Competitive binding of corticoids, androgens, and estrogens to the progesterone-binding sites in the cytosols of bladder tumors

Cytosol preparations of bladder tumors of the hamster were incubated with 4×10^{-9} M [^{3}H]progesterone plus a 100-fold excess, 4×10^{-7} M, of the steroid being tested as a possible inhibitor of progesterone binding. After incubation to equilibrium for 2 hr at 0°, unbound steroid was removed by treating with dextran-coated charcoal for 5 min. All of the experiments were carried out in the presence of 10^{-6} M cortisol to prevent binding of the [^{3}H]progesterone to cortisol-binding globulin. The amount of [^{3}H]progesterone bound to the cytosol receptor was measured and expressed as concentration of progesterone-binding sites per mg protein per ml cytosol. Each value is the mean of 3 experiments.

Steroid	[^{3}H]Progesterone bound ($\times 10^{-10}$ M/mg protein)
Cortisol	1.9
d-Aldosterone	1.6
5α-Dihydrotestosterone	1.5
Testosterone	1.2
17β-Estradiol	1.5
Estrone	1.7
Ethanol	1.1

Competition Studies. To determine the steroid specificity of these binding sites in the bladder, cytosols were incubated with a saturating concentration of [^{3}H]progesterone (4×10^{-9} M) plus a 100-fold excess of either 17β-estradiol, estrone, testosterone, 5α-dihydrotestosterone, or aldosterone (Table 1). After incubation to equilibrium at 0° (2 hr), unbound steroid was removed by treating with dextran-coated charcoal for 5

min. The amount of radioactive progesterone bound to the cytosol-binding sites was measured. None of the steroids tested decreased the binding of [^{3}H]progesterone to the cytosol binding sites. All of these experiments were carried out in the presence of 10^{-6} M cortisol to prevent the binding of labeled progesterone to cortisol-binding globulin (6).

Histological Observations in Bladder Lesions. Examination of histological sections from the bladder lesions revealed a spectrum of changes among animals from predominantly inflammatory reactions to squamous metaplasia and hyperplasia of the transitional epithelium. The inflammatory changes were characterized by the presence of calculi in the bladder lumen, polymorphonuclear cells, and interstitial fibrosis. These changes are similar to those reported just prior to the adenocarcinoma formation in renal tissue of estrogen-treated hamsters (17). Other lesions were characterized by intense hyperplasia of the transitional epithelium as well as by squamous metaplasia (Fig. 1). These changes were more severe and extensive in the larger bladders.

DISCUSSION

Although bladder carcinoma induced by estrogen has been described in the rat (2), the condition has not been demonstrated to occur in the hamster; neither is the bladder considered a primary site of metastasis of renal adenocarcinoma induced by estrogen in Syrian hamsters (5). We have observed that about 20% of castrated male Syrian hamsters treated for 7 to 10 months with estrogen developed bladder lesions with tissues increasing in weight from 0.2 g to 0.4 to 1.7 g. The bladder lesions contained high-affinity progesterone-binding sites with a binding constant of 10^9 M^{-1}, a value similar to that of progesterone receptors in target tissues for estrogen, *e.g.*, uterus, renal adenocarcinoma (6, 10). The highest concentration of progesterone-binding sites found in a bladder lesion was one-half of that reported for renal adenocarcinoma tissue (10) or uterine tissue at estrus (6) of the hamster. An increased concentration of progesterone-binding sites also was observed in one bladder within the normal size range and after estrogen treatment for 7 to 10 months. Whether this animal is more prone to develop a bladder lesion at a later time is unknown. It does indicate that differences do occur among animals in the responsiveness of the bladder to estrogen. No progesterone receptors could be detected in bladders from hamsters not treated with estrogen.

Histological examination of the bladders with lesions revealed morphological changes ranging from inflammatory reactions with interstitial fibrosis and leukocytic infiltration to squamous metaplasia and hyperplasia of the transitional epithelium. The inflammatory characteristics have been described for the hamster kidney just prior to induction of renal adenocarcinoma by estrogen (17) and for spontaneously forming tumors of the urinary bladder of rats (1). Squamous keratinization is not observed during renal tumorigenesis, although it is seen in certain types of rodent neoplasia of the bladder (14).

The occurrence of bladder lesions in some hamsters in the absence of visible renal adenocarcinoma suggested that the condition was not merely a consequence of kidney dysfunction or renal changes. The increased concentration of progesterone-binding sites observed in the bladders of estrogen-treated hamsters may precede neoplastic changes as has been observed in the hamster kidney. However, the incidence of bladder lesions is lower than the incidence of renal adenocarcinoma. There is also a lower incidence of increased progesterone concentrations in the bladders of animals treated for 3 to 10 months with estrogen than in the kidney. These observations suggest an important difference in estrogen responsiveness of the 2 tissues. It may be that a factor(s) influencing both the induction of tumors as well as increased progesterone receptor concentrations is lower or absent in the bladder relative to the kidney. One possible candidate for the factor(s) is the relative concentration of estrogen receptor in the 2 tissues. A low concentration of estrogen receptor has been found in the kidneys of untreated hamsters and is proposed to be important in renal tumorigenesis (8). Alternatively, the immunological surveillance of abnormal changes may be more efficient in the bladder than in the kidney (3, 4).

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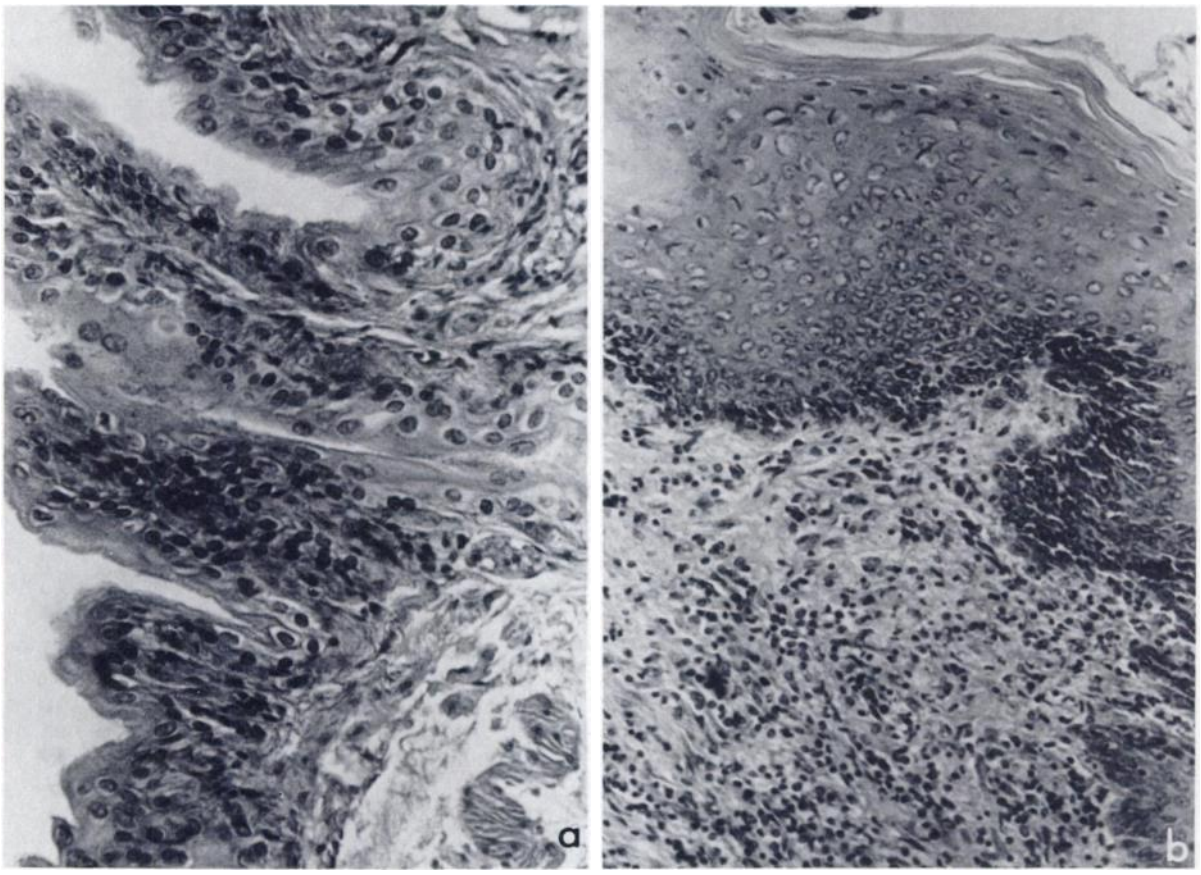

Fig. 1. Bladder lesions from a hamster after 10 months of estrogen treatment. The transitional epithelium exhibits squamous metaplasia with keratinization as well as intense hyperplasia. The transitional epithelium of a normal bladder of the hamster is shown for comparison (*a*). H & E, × 200.