Permissive Role of the Pituitary in the Induction and Growth of Estrogen-dependent Renal Tumors

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ABSTRACT

Prolonged administration of estrogen to hamsters by implanted pellets induces not only renal adenocarcinomas but also enlarges pituitaries with hyperplastic and neoplastic changes, especially in the pars intermedia. The pituitaries of the diethylstilbestrol-implanted animals weigh 90 to 150 mg; those of control animals without diethylstilbestrol pellets weigh 7 to 12 mg. The enlarged pituitaries have $9.7 \times 10^{-10}$ M progesterone receptors compared to $0.75 \times 10^{-10}$ M in the controls. Castrated male hamsters were hypophysectomized, implanted with diethylstilbestrol pellets, fed laboratory chow ad libitum, and given 5% glucose in water to drink. New pellets were implanted every 3 months, and the animals survived for 12 to 15 months. At autopsy, none of the animals had a tumor. Sixty-two of 65 control castrated males with the same schedule of pellet implantation developed tumors. Hypophysectomized castrated males implanted with diethylstilbestrol pellets were given daily injections of 1 mg each of follicle-stimulating hormone, luteinizing hormone, and prolactin; or with 0.9% NaCl solution. These animals survived for 12 to 15 months, but none developed kidney tumors. Other castrated males were hypophysectomized and implanted with diethylstilbestrol pellets, and 2 months later tumor tissues were transplanted under the kidney capsule. Eighty days later, no tumors were evident in the kidneys of these animals. Control castrated males were implanted with diethylstilbestrol pellets, and 2 months later tumor tissue was transplanted under the kidney capsule. Between 60 and 85 days later, 13 of the 15 controls had developed renal tumors. The concentrations of follicle-stimulating hormone, luteinizing hormone, and prolactin were measured by radioimmunoassays. The concentrations of circulating follicle-stimulating hormone and luteinizing hormone in animals with diethylstilbestrol implants decreased with time, and, by 7 months, were similar to those in hypophysectomized animals. The concentration of prolactin in animals with diethylstilbestrol pellets increased with time and reached twice the value in the control animals without diethylstilbestrol pellets. These studies suggest that some factor secreted by the pituitary may be involved as a promoter or a cocarcinogen in the estrogen induction of kidney tumors.

INTRODUCTION

The estrogen-induced and -dependent tumor of the hamster kidney provides an intriguing model of tumorigenesis in general and of chemical or hormonal tumorigenesis in particular. The fact that prolonged estrogen treatment of the Syrian hamster results in the production of renal adenocarcinomas was first demonstrated by Matthews et al. (13). The renal tumors are malignant, require estrogen for maintenance and growth, and appear in 80% or more of all male hamsters that have been treated continuously with estradiol or DES for 250 days or more (10). Renal tumors can be induced in females by estrogen treatment only if the concentration of progesterone circulating in the blood is reduced by ovariectomy or by masculinization of the neonatal hamster with androgens. Males can be protected against induction of the tumor by estrogen if they are treated simultaneously with progesterone. The tumors are not only estrogen induced but also estrogen dependent and can be transplanted into other hamsters only if the host has been treated with estrogen. The renal tumors are metastatic, and new foci of tumor growth appear as metastases transported into the abdominal cavity or through the lymphatic and circulatory systems to organs such as the lung and lymph nodes.

The induction of pituitary tumors by long-term estrogen treatment has been studied in rats (14), mice (2), and hamsters (18). In the rat, tumors arise in both anterior (14) and intermediate (15) lobes of the pituitary. In the hamster, estrogen-induced pituitary tumors have been reported to arise in the anterior (7, 16), the intermediate (16, 18), or both lobes (11) of the pituitary. It now appears that the intermediate lobe tumor is more commonly observed, and it may be of primary importance in relation to the renal neoplasm (6). The intermediate lobe tumor induced by estrogen treatment enlarges 10-fold or more and shows cellular hyperplasia, neoplasia, and abundant mitotic figures. It invades both anterior and posterior lobes of the pituitary as well as the hypothalamus (18) and the third ventricle. It has been termed an adenoma-like hyperplasia of the pars intermedia.

That the pituitary may play a role in renal tumorigenesis in estrogen-treated hamsters was first considered when it was observed that female hamsters who had been neonatally masculinized with androgens develop renal tumors after long-term estrogen treatment, whereas normal females do not (9). The inhibition of the cyclic release of gonadotropins and the resulting lack of production of progesterone were initially considered as the probable mechanism for tumor induction in masculinized females, but a more direct role of the pituitary has been postulated (5). Kirkman (8) had reported that kidney tumors can be induced by estrogen in hypophysectomized hamsters. This claim has been criticized (5) because of the lack of documentation of the data. Complete hypophysectomy of these animals was not demonstrated.

The percentage of prolactin-secreting cells in the anterior lobe and the number of Type 1 light cells of the intermediate lobe are decreased by hypophysectomy (18). These cells are involved in the regulation of the cyclic release of gonadotropins (15) and the synthesis of growth hormone (16). The intermediate lobe tumor is an adenoma-like hyperplasia of the pars intermedia.

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lobe, believed to be the source of MSH, both increase in response to long-term estrogen treatment (17). Estrogen administration causes increased levels of prolactin in the serum, and prolactin has been implicated in mammary tumorigenesis in the rat and human (3, 4, 19). The drug 2-bromo-α-ergocryptine methanesulfonate (CB154), which inhibits prolactin secretion, has been shown to reduce the incidence of renal tumors in the hamster when given concurrently with DES (5). However, the administration of prolactin failed to induce renal tumors and, when given with DES, prolactin did not influence the induction time or the severity of the neoplasia (6). The hyperplasia of the type 1 light cells in the intermediate lobe of the pituitary suggested that α-MSH might be a promoter or cocarcinogen of estrogen-induced renal adenocarcinoma of the hamster. Long-term administration of DES to hamsters increases the content of MSH in both the pituitary and the serum (6). CB154, which had previously been believed to be solely an inhibitor of prolactin secretion, has been shown to inhibit the secretion of MSH as well. However, when the DES pellets are removed, the kidney tumor regresses, but the pituitary remains enlarged, and the MSH levels remain elevated. These data indicate that, although MSH may be involved in the induction of renal adenocarcinoma in the hamsters, it is unlikely that MSH is responsible per se for the maintenance of the tumor. The studies reported in this paper were undertaken to determine whether estrogen treatment could induce kidney tumors in hypophysectomized hamsters and whether some factor secreted by the pituitary may be involved as a promoter or cocarcinogen in the induction of the kidney tumors.

MATERIALS AND METHODS

**Chemicals and Reagents.** [1,2,6,7-3H]Progesterone (104 Ci/mmol) and Biofluor were obtained from New England Nuclear, Boston, Mass. Unlabeled progesterone, Noril A, dextran BS, dithiothreitol, and bovine serum albumin were obtained from Sigma Chemical Company, St. Louis, MO. FSH, LH, and prolactin were obtained from the National Pituitary Agency, NIH. DES was obtained from Sigma and was made into pellets by Copley Pharmaceutical Inc., Boston, Mass.

**Animals and Tumor Induction.** Young, mature, castrated, and hypophysectomized male Syrian hamsters (LAK/LG, outbred strain; Charles River Laboratories, Lakeview Hamster Colony, Newfield, N. J.) weighing 81 to 90 g were used. The hamsters were hypophysectomized by Charles River Laboratories 2 weeks before delivery. All the hamsters were housed in an environmentally controlled room with a 14-h light-10-h dark cycle and a temperature of 23–25°C. The intact hamsters were fed laboratory chow and tap water ad libitum. The hypophysectomized hamsters were supplied with 1% glucose solution instead of tap water. All hamsters were observed for 1 week before the DES pellets were implanted. Pellets of DES weighing 20 mg were implanted subcutaneously under ether anesthesia. Additional DES pellets were implanted every 3 months in each animal to maintain high estrogen levels. The hamsters were weighed every week. Renal tumors appeared 7 to 10 months after the first pellet was implanted; abdominal metastases were commonly observed. Animals were decapitated, tumor tissue and blood samples were collected, and the tissues were immediately washed twice in cold 0.15 M NaCl in 10 mM Tris-HCl-1.5 mM EDTA-1 mM dithiothreitol buffer, pH 7.4. They were then kept on ice until used.

**Tumor Transplantation.** Primary kidney tumor tissue from one animal was rinsed with sterile 0.9% sodium chloride solution to remove clotted blood and fatty tissue; the clean tumor tissue was minced into 1- to 2-mm fragments and stored on ice in sterile Petri dishes during the transplantation procedure. Host animals were anesthetized with Nembutal administered i.p. at a dosage of 7.5 mg/100 g body weight to hamsters that had received DES pellets 1 to 2 months earlier. The dorsal side of the animal in the flank area was shaved and swabbed with 0.13% Zephiran. The kidney was exposed by a 1.5-cm incision below the posterior rib, and a sterile gauze was placed beneath the kidney. A 25-gauge needle was used to puncture the renal capsule, and 5 to 6 tumor fragments (5 to 6 mg/fragment) were placed beneath each kidney capsule with microwireers. The total weight of tumor tissue transplanted per animal was 50 to 60 mg. The implanted fragments were pushed to separate sites on the exposed surface of the kidney. The kidney was then returned to its normal position, the muscle layer incision was closed with sutures, and the skin layer incisions were closed with metal clips. Kidney tissue from nontumor areas of the kidneys of tumor-bearing animals was transplanted into DES-implanted hamsters. None of the 15 control hamsters had developed tumors when sacrificed 85 days later.

**Preparation of Cytosol.** The tumor tissue was freed of necrotic or hemorrhagic areas, weighed, and homogenized with glass-glass conical grinders in 5 to 10 volumes of a buffer containing 10 mM Tris-HCl (pH 7.4), 1.5 mM sodium EDTA, 1 mM dithiothreitol, 0.1% bovine serum albumin, and 30% glycerol. 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 to a final concentration of 1 μM to prevent the binding of labeled progesterone to cortisol-binding globulin.

**Assay of Progesterone Receptor.** Cytosols were incubated with 4 × 10−9 M tritiated progesterone and, to correct for nonspecific binding, other samples of cytosol preparation were incubated with progesterone and a 100-fold excess of unlabelled progesterone. After incubation to equilibrium (2 hr) at 0°C, unbound steroid was removed by treatment with dextran-coated charcoal for 5 min. The amount of specific labeled progesterone bound to the cytosol receptor was measured and expressed as the concentration of progesterone-binding sites for 1 mg of protein per ml of cytosol. Binding site concentrations were assayed at 2 or more protein concentrations in the linear range and at a saturating concentration of progesterone (i.e., 4 × 10−11 M). The assay range under these conditions was less than 10% of the mean.

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

**Other Assays.** The protein content of the cytosol was measured by the dye-binding procedure of Bradford (1), using bovine serum albumin as standard.

RESULTS

Pellets containing 20 mg of DES were implanted subcutaneously in hypophysectomized or control castrated male hamsters. New pellets were implanted every 3 months until the animal died or was sacrificed. The body weights of the DES-implanted animals were less than those of control hamsters not given DES implants (Chart 1). The body weights of the DES-implanted hypophysectomized animals were even less. The weights of the pituitaries of the DES-implanted animals were slightly greater than those in control animals without DES pellets after 6 months of constant exposure to the estrogen. The pituitary weights subsequently increased rapidly (Chart 2). The weights of the control pituitaries ranged from 7 to 12 mg, whereas the pituitaries of animals bearing with DES implants for 12 months weighed 90 to 150 mg.

The number of progesterone-binding sites in the pituitaries, kidneys, and kidney tumors were estimated from Scatchard plots of progesterone binding. The number of progesterone receptors in the kidney increased significantly within 3 months of implanting the DES pellets, as we had shown previously.
Pituitary Role in Estrogen-induced Renal Tumors

Kidney tumors, 25 castrated hypophysectomized male hamsters were implanted with DES pellets, and 8 to 10 small pieces of kidney tumor were transplanted under the kidney capsule 2 months later. Eighty days later, no tumors were evident in the kidneys of these animals (Table 1). In the control series, castrated males were given implants of DES pellets, and tumor tissue was transplanted beneath the kidney capsule 2 months later. Between 60 and 85 days after the tumor tissue was transplanted, 13 of the 15 animals (87%) had developed kidney tumors.

Hypophysectomized castrated male hamsters were implanted with DES pellets and were given daily injections of 1 μg FSH and 1 μg LH in 0.9% NaCl solution. The second group of hypophysectomized castrated male hamsters implanted with DES pellets was given daily injections of 1 μg each of FSH, LH, and prolactin in 0.9% NaCl solution. The control group was treated with 0.9% NaCl solution alone. The animals survived for 12 to 15 months, but none developed renal tumors (Table 2).

The serum of hamsters collected by heart puncture or by exsanguination at autopsy was tested by radioimmunoassay (12). The number of progesterone receptors in the pituitary also increased within 3 months of implanting the DES pellets to $4 \times 10^{-10}$ M, compared to $1 \times 10^{-10}$ M in the control animals (Chart 3). The number of progesterone receptors in the pituitaries of DES-implanted hamsters continued to increase up to 12 months. The number of progesterone receptors in the kidney tumors was high when the tumors first appeared 8 to 9 months after DES implants and remained high thereafter (Chart 3). The number of progesterone receptors was also increased in the pituitaries of hamsters in which kidney tumors were transplanted under the kidney capsule 2 months after DES pellets were implanted (Chart 4).

To determine the possible role of the pituitary in the primary induction of the kidney tumor, 35 castrated hypophysectomized male hamsters were given implants of 20 mg DES pellets. The animals were fed laboratory chow and were given 1% glucose to drink. New pellets were implanted every 3 months, and the animals survived for 12 to 15 months. At autopsy, none of these animals had a kidney tumor. Sixty-five control castrated males were given the same schedule of estrogen pellet implantations. Sixty of these 65 animals (92%) had developed kidney tumors within 12 months (Table 1). To determine the possible role of the pituitary in the growth of transplanted kidney tumors, 25 castrated hypophysectomized male hamsters were implanted with DES pellets, and 8 to 10 small pieces of kidney tumor were transplanted under the kidney capsule 2 months later. Eighty days later, no tumors were evident in the kidneys of these animals (Table 1). In the control series, castrated males were given implants of DES pellets, and tumor tissue was transplanted beneath the kidney capsule 2 months later. Between 60 and 85 days after the tumor tissue was transplanted, 13 of the 15 animals (87%) had developed kidney tumors.

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Castrated hypophysectomized

**DISCUSSION**

The finding that none of the hypophysectomized animals developed kidney tumors whereas a large fraction of the control animals did develop kidney tumors strongly suggests that the pituitary may play a role in renal tumorigenesis in estrogen-treated hamsters. The role of the pituitary in the induction and maintenance of the hamster renal adenocarcinoma must be reexamined. Neither of the replacement regimens tried in these experiments, FSH plus LH or FSH plus LH plus prolactin, was successful in permitting the development of kidney tumors. Some other product of the pituitary, or perhaps FSH, LH, or prolactin in a different amount, dose schedule, or combination, may be required for renal tumorigenesis. The fact that the amounts of circulating FSH and LH are greatly reduced by prolonged estrogen treatment suggests that these are probably not involved in renal tumorigenesis. However, the amount of circulating prolactin is increased by prolonged estrogen treatment, and prolactin may be required for renal tumorigenesis. An increase in the percentage of prolactin-secreting cells in the anterior lobe of DES-treated hamsters and an increase in the numbers of type 1 light cells in the intermediate lobe (believed to be the source of α-MSH) in response to DES implants were reported by Saluja et al. (17). Estrogens have been shown to cause increased levels of prolactin in the serum, and prolactin has been implicated in mammary tumorigenesis in the rat and human (3, 4, 19). The drug 2-bromo-α-ergocryptine methanesulfonate (CB154) which inhibits prolactin secretion has been shown to reduce the incidence of renal tumors in hamsters when given concurrently with DES (5). However, prolactin treatment failed to induce renal tumors and when given with DES did not influence the induction or severity of the kidney tumor (6). Our current studies show that, when prolactin in combination with FSH and LH was given along with DES to hypophysectomized hamsters, no renal tumors were induced.

Hyperplasia of the type 1 light cells in the intermediate lobe of the pituitary suggested that α-MSH might be a promoter or procarcinogen in the estrogen-induced renal adenocarcinoma of the hamster. Long-term administration of DES to hamsters was shown to increase the content of MSH in the pituitary and in the serum (6). CB154, which had previously been believed to act solely as an inhibitor of prolactin secretion, has been shown to inhibit the secretion of MSH as well (6). However, when the administration of DES was withdrawn, the kidney tumor regressed but the pituitary remained enlarged and the MSH levels remained elevated. These data suggest that, if MSH is involved in the induction of renal adenocarcinomas in the hamster, it is unlikely that it is responsible per se for the maintenance of the tumor. The present studies demonstrate that renal tumors will not develop in hypophysectomized hamsters even though they have long-term estrogen implants.
Furthermore, transplants of kidney tumor placed under the renal capsule of the hypophysectomized hamster implanted with estrogen for the previous 2 months will not grow but will undergo regression and disappear. This suggests that the enlargement of the pituitary and its ultimate transformation to tumor tissue in hamsters with long-term DES implants may not simply be a parallel response of the pituitary to the estrogen. It may be that DES has a primary effect on the pituitary which then secretes some product that plays a key role in tumorigenesis in the kidney. As shown previously, the pituitary may also be involved in the mechanism by which progesterone protects the kidney against tumorigenesis. Does the binding of progesterone to the progesterone receptors in the pituitary result in the pituitary ceasing to secrete the factor necessary for tumor induction and growth? Or does the pituitary, in response to progesterone, secrete some factor which actively inhibits renal tumorigenesis?

In the course of our studies of transplanting tumors beneath the kidney capsule, we observed that the transplanted tumor would survive and grow only if the host had been exposed to high levels of estrogen from the implanted pellets for the preceding 8 weeks, but not if the host had estrogen pellets implanted for only 2 weeks. Under the latter conditions, the kidney tumor transplants regress and disappear. It is possible that a certain amount of time is required for the level of circulating estrogens in the estrogen-implanted animals to reach a critical level. Alternatively, the delay may reflect an effect of the estrogen on the number of estrogen receptors in the kidney or an effect of the estrogen on the pituitary that results in the production of some factor necessary for tumor growth. The fact that transplanted tumors regress and disappear from the kidneys of hypophysectomized estrogen-implanted animals favors the latter hypothesis.

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