Hormonally Induced Tumors of the Reproductive System of Parabiosed Male Rats

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ABSTRACT

Parabiosis of intact male rats to castrated males or oophorectomized females for a period of approximately 20 months resulted in three interstitial cell tumors of the testes. When unilateral nephrectomy was added to the parabiotic procedure in ten pairs, eight interstitial cell tumors of the testis and four adenocarcinomas of the prostate occurred in the target male parabionts. These changes were preceded by elevations in luteinizing and follicle-stimulating hormone levels in the serum of the castrates and high levels of testosterone and, to a lesser degree, of androstenedione in the target partners developing the tumors.

INTRODUCTION

When an intact female rat is parabiosed to a castrate male or female, increased gonadotropins generated in the castrate presumably cross over to stimulate excessively the ovaries of the intact female and thereby indirectly affect the target organs of the ovarian steroids (18). We have reported previously that parabioses of this type result in the development of carcinomas of the breast in the intact female partner (16). These observations raised the question of whether tumors could be induced in the male reproductive system or its accessories by the analogous maneuver of parabiosing an intact male rat to a castrated male or oophorectomized female. Although earlier experiments in which this was done led to the correct deduction that hormonally induced changes occurred in the intact or target partners, these experiments were not of sufficient duration to reveal their tumorigenic potential (6, 10).

In 1932, Lower and Hicken (9), utilizing parabiosis in males, produced prostatic enlargment which they attributed to androgenic action. The present study was designed to test the possibility that an increase in natural gonadotropins and androgens acting over a prolonged period could result in neoplasms of target organs of male rats parabiosed to castrated males or oophorectomized females. The current availability of radioimmunoassays has made feasible the determination of gonadotropin and steroid serum levels in parabionts resulting from this procedure.

MATERIALS AND METHODS

Parabioses of intact males with male or female partners were performed under Nembutal-ether anesthesia according to a modified Bunster-Meyer technique at 29 to 43 days of age (17). Castration or oophorectomy, without hysterectomy, of one member of the pair was done at approximately 72 days of age. Pathogen-free NEDH rats were used.

Since total exsanguination was necessary to secure sufficient serum for a large battery of hormone assays and because it was desirable to ascertain early hormone levels, 2 experimental groups were established. Group I consisted of 18 pairs which were killed at approximately 140 days postcastration. Sera from 17 of these were assayed for hormone content. Group II originally consisted of 24 pairs which were allowed to live until they developed tumors or appeared moribund (387 to 750 days postcastration). Four pairs died early in the experiment, leaving 20 pairs of which 15 were assayed. The pairs in Groups I and II were divided into 4 subgroups according to the composition of the parabiosed pairs as follows: A, intact male parabiosed to a castrate male; B, intact male parabiosed to an oophorectomized female; C, intact male parabiosed to a castrate male, each partner unilaterally nephrectomized; and D, intact male parabiosed to an oophorectomized female, each partner unilaterally nephrectomized. Six pairs of parabiosed control males (no castration, no nephrectomy) comprised Subgroup E. Complete serum assays were made on all controls except for the LH assays on one pair. Subgroups C and D were included because in previous experiments with parabiosed females unilateral nephrectomy of each partner increased the incidence of breast carcinomas, presumably by exaggeration of hormonal effects (4).

The rats were exsanguinated during ether anesthesia by aortic puncture, when blood was drawn for hormonal assay. Postmortem examinations were performed on all rats. The testes, seminal vesicles with coagulating glands, and ventral and dorsolateral lobes of the prostate were wet weighed individually, and average weights for each subgroup were calculated. Specimens of kidney, liver, adrenal, testis, ventral prostate, posterolateral prostate, coagulating gland, seminal vesicle, epididymis, breast, thyroid, parathyroid in some, thymus, bladder, and pituitary were taken for histological study. They were fixed in formalin or Zenker-formalin. Paraffin sections were routinely stained in hematoxylin and eosin, special stains being performed when indicated.

The blood for hormone analysis was allowed to clot in the refrigerator. It was then centrifuged; the serum was pipetted off and immediately frozen. Assays of the following hormones were performed on the sera: LH, FSH, prolactin, estrone,
estradiol, progesterone, testosterone, and androstenedione. Radioimmunoassays for the steroids were performed by the method of Abraham et al. (1, 2, 11), for FSH and LH by the method of Niswender et al. (13), and for prolactin by the method of Haug and Gautvik (8).

RESULTS

As seen in Table 1, 11 interstitial cell tumors of the testis and 4 adenocarcinomas of the prostate developed in the target partners of the 20 parabiosed pairs completing the long-term experiment. Sera from 15 of these were assayed for hormones. One interstitial cell tumor occurred in a target partner of Subgroup A and 2 in Subgroup B, which were not nephrectomized. The remaining 8 occurred in Subgroups C and D, in which a kidney was removed from each partner. No tumors were found in the short-term experiment, Group I.

Assays of serum levels for LH, FSH, testosterone, and androstenedione are shown in Charts 1 to 4. Assays for estradiol, estrone, progesterone, and prolactin showed considerable variability with no consistent pattern and were, therefore, excluded. As seen in Chart 1, the LH serum assays of all castrate and all but 2 target partners in Group I exceeded those of the maximum control value. It has been correctly deduced by others, in previous experiments with castration of one member of a parabiosed pair of rats, that gonadotropins, which rise in castrates because of interruption of the negative feedback by gonadal removal, cross over into the target partner (6). In Group II, 7 of 10 of the target partners with tumors had LH values above those of the maximum control. We cannot explain why 3 of our target partners with interstitial cell tumors failed to exhibit high LH levels. Perhaps the elevated levels of LH, as seen in the short-term Group I, are more representative of the LH levels present during most of the animal's life than the terminal values seen in the long-term group. Prolonged overstimulation of gonadotropins in the castrate may cause diminished pituitary secretion.

More consistent elevations of FSH are seen in both Groups I and II (Chart 2) than in the controls. This is particularly notable in Subgroups C and D where the incidence of interstitial cell tumors was highest.

As seen in Charts 3 and 4, the assays for testosterone and androstenedione in the target partners are above the controls, particularly in the long-term Subgroups C and D. It is unfortunate that sera from only 2 of the target partners with prostatic carcinoma were available for assay. These 2 showed very high androgen values. The unusually high testosterone values in Subgroups C and D are caused no doubt by interstitial cell hyperplasia and tumors. Two factors probably account for the failure of the high testosterone levels in the target partner to dampen gonadotropin secretion by the castrate: (a) the promptness with which steroids are conjugated in the liver; and (b) the drainage of peritoneal fluid into the portal system.

The interstitial cell tumors which developed in the testes of the target males varied in size from 1 mm to 30 x 17 x 17 mm, replacing an entire testis. The tumors were soft and yellow against a tan background and were frequently hemorrhagic.

Table 1

<table>
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<tr>
<th>Subgroup</th>
<th>No. of pairs</th>
<th>No. of pairs with assays</th>
<th>Interstitial cell tumors in target partners</th>
<th>Prostatic cancers in target partners</th>
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<tr>
<td>A</td>
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<td>E</td>
<td>6</td>
<td>6</td>
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*Details are given in text.

*Only one rat assayed.

Chart 1. LH assays (ng/ml). Seventeen pairs of rats in the short-term Group I were parabiosed as described in the text. The numbers of parabiont pairs in Group I were as follows: Subgroup A, 4 pairs; Subgroup B, 5 pairs; Subgroup C, 3 pairs; Subgroup D, 5 pairs; and Subgroup E, 5 control pairs. The 15 parabiont pairs in long-term Group II were distributed as follows: Subgroup A, 4 pairs; Subgroup B, 4 pairs; Subgroup C, 4 pairs; and Subgroup D, 3 pairs. Castrate partner of each pair (diagonally cross-hatched bar) precedes target partner (horizontally cross-hatched bar). In long-term groups, X indicates interstitial cell tumor in corresponding target partner.
Interstitial Cell Tumors in Parabiotic Rats

Chart 2. FSH assays (μg/ml) performed on the same pairs as in Chart 1. X, interstitial cell tumor incidence. Castrate assay precedes that of target partner of each pair.

Chart 3. Testosterone assays (pg/ml) on same pairs as in Charts 1 and 2. Open bar indicating target partner precedes solid bar indicating assay of castrate. Subgroups as in Chart 1. P, prostatic adenocarcinoma. In Charts 1 and 2, most of the elevated gonadotropin values occur in the castrates while in Charts 3 and 4 the elevated androgen values occur in the target partners.

Grossly they appeared localized, but microscopically they were not encapsulated, blunt fingerlike projections pushing outward between bordering atrophic testicular tubules. Elsewhere, the testicular tubules were not atrophic. The interstitial cell tumors were composed of spindle and polygonal cells with abundant finely and coarsely granular cytoplasm (Fig. 1). Their nuclei were large and round with sharp borders, abundant fine chromatin, and a prominent central chromatin body. Mitotic figures were easily found after a cursory scan of a few fields. Much more careful search was required to find Reinke’s crystalloid bodies, illustrated in Fig. 2. Diffuse and occasionally focal hyperplasia of the interstitial cells of the testes was noted in target partners with and without tumors, particularly in the unilateral-nephrectomy subgroups. The intertubular spaces often appeared edematous and contained thick-walled arterioles. The testes of target partners not involved with tumors showed a slight weight increase over controls.

The prostates and seminal vesicles of target partners were moderately enlarged and contained abundant thick, milky fluid. Prostatic tumors were not identified grossly, although they were suspected because of nodularity of some dorsolateral lobes. Microscopic foci of adenocarcinomas were found in the dorsolateral lobes of 4 target males near the prostatic urethra and entrance of the seminal vesicles. Two of them may have occurred at the seminal vesicle junction. All were in the unilaterally nephrectomized Subgroups C and D (Table 1). The
prostatic tumors were composed of distorted glands lying in coarsely fibrous or fibrohyaline stroma which they appeared to invade (Fig. 3). The glandular pattern was distinctly abnormal as were the epithelial cells which were crowded, misshapen, and hyperchromatic. Mitoses were occasionally encountered in their pleomorphic nuclei. Nerve sheath invasion was identified in 2 of 4 cancers (Fig. 4). Many of the glands in the prostates of target partners had papillary infolding to an abnormal degree, suggesting generalized hyperplasia.

The pituitary glands of the target partners showed no consistent changes, while those of the castrates contained varying numbers of castration cells. The reproductive systems of the castrates were atrophic. Sections of the remaining organs noted in the gross description were normal except for the random occurrence of pheochromocytomas in the adrenals, occasionally seen in our colony.

The kidneys remaining in the unilaterally nephrectomized target partners of the long-term subgroups were distinctly enlarged. Microscopically, they showed advanced pathology, characterized by extensive confluent patches in which glomeruli were occluded by fibrosis or hyalinization, and adjacent tubules were atrophic and dilated, sometimes to cystic proportions. Foci of lymphocytic infiltration were frequently encountered. Because of the severity and extent of this process, at least a moderate degree of functional impairment could reasonably be expected. In contrast, the kidneys of the castrate partners in the unilaterally nephrectomized subgroups and those in the nonnephrectomized subgroups appeared essentially normal except for the minor changes of senescence.

DISCUSSION

In their discussion of Leydig cell hyperplasias and tumors of the testes of humans and animals, Mostofi and Price (12) cite numerous references implicating chorionic and pituitary gonadotropins. The present studies show elevation of LH in many and of FSH in most target partners with interstitial cell tumors of the testis. The curious prevalence of these tumors in the unilaterally nephrectomized partners with advanced chronic nephritis could be explained by increased retention of gonadotropins already at marginally high serum levels. The fact that 3 of the partners with interstitial cell tumors and chronic nephritis failed to show an LH elevation may reflect the episodic nature of pituitary secretion.

There is support for the view that renal function is involved in gonadotropin excretion. Vilar et al. (15) have shown that fluorescent-labeled gonadotropins are concentrated in the renal convoluted tubules. The radioimmunographic studies of DeKretser et al. (5) support the concept of a secretory mechanism for the elimination of LH by the kidneys.

Relative to the etiology of experimental carcinoma of the prostate, Brendler (3) as recently as 1963 could cite no references implicating a hormonal etiology. In 1977, Noble (14) reported the production of prostatic adenocarcinomas in male rats by the s.c. implantation of testosterone pellets. The cancers reported by him were histologically and biologically malignant, since they metastasized and grew on transplantation. The prostatic adenocarcinomas in our series were small and localized. Their malignancy has not been tested biologically. They resemble the latent prostatic carcinomas described in humans by Franks (7) and others. The tumors in the present series occurred in the dorsal lobes of the gland where human prostatic carcinomas usually arise.

In summary, the present study shows that parabiosis of a male rat to a castrate male or oophorectomized female is associated with (a) the development of interstitial cell tumors of the testis in many of the target partners, particularly in those unilaterally nephrectomized, and (b) the development of a few adenocarcinomas of the prostate in the same partners.

The hormonal assays, although not conclusive, suggest a correlation between internally generated gonadotropins and the development of interstitial cell tumors of the testis and of serum androgens in the development of adenocarcinomas of the rat prostate.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Interstitial cell tumor of the testis; polygonal cells with finely and coarsely granular cytoplasm. Nuclei are round to oval and contain a prominent central chromatin mass. One distorted mitotic figure is seen. × 312.

Fig. 2. Interstitial cell tumor of the testis. Two intracellular Reinke crystalloids can be seen near the center of the photomicrograph (arrow). × 312.

Fig. 3. Adenocarcinoma of the prostate. Distorted glandular structures are lined with pleomorphic cells in irregular arrangement. × 125.

Fig. 4. Tangential section of nerve suggesting nerve sheath invasion by focus of adenocarcinoma. × 325.
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