Induction of Prostatic Carcinomas and Lower Urinary Tract Neoplasms by Combined Treatment of Intact and Castrated Rats with Testosterone Propionate and N-Nitrosobis(2-oxopropyl)amine

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ABSTRACT

The effect of exogenous testosterone on prostatic carcinogenicity of N-nitrosobis(2-oxopropyl)amine (BOP) in intact and castrated rats was examined in Wistar-derived MRC rats. Daily administration of BOP (either s.c. or i.g.) for 3 days, at a dose of 20 mg/kg b.w. at the heights of prostatic cell proliferation induced by testosterone, led to development of a large number of prostatic tumors, the incidence of which, however, was dependent on the duration of testosterone administration. Testosterone given for life following BOP administration induced prostatic cancer in over 60% of rats, regardless of whether BOP was given orally or s.c., or whether the rats were orchiectomized or not, whereas tumor incidence was significantly lower in rats treated with testosterone for only a short period of time. One (3%) orchiectomized rat, which received testosterone only during BOP treatment, and four (15%) of rats treated with testosterone only for life also developed carcinomas. Histologically, a large number of BOP + testosterone-induced prostatic tumors were adenocarcinomas of various histological patterns and arose primarily from the dorsal lobe, whereas the great majority of squamous cell carcinomas were found in the ventral lobe. Simultaneously induced tumors were papillomas and carcinomas of the urinary bladder and urethra. Testosterone appeared to enhance the incidence of urinary bladder tumors, but not of the urethral tumors, whereas orchiectomy inhibited urachal carcinogenesis, and, to much lesser extent, urinary bladder tumor development. Rats treated weekly for 20 weeks with BOP (10 mg/kg/week i.g.) did not develop any prostatic tumors and all rats died of rectal cancer. Of rats treated similarly with BOP and with testosterone pellets for life resulting after the last injection of BOP, 17% of rats developed prostatic cancer, all of the squamous cell type. Simultaneous testosterone and BOP treatment for 20 weeks followed by testosterone pellets for life resulted in a 39% tumor incidence (three adenocarcinomas, one anaplastic carcinoma, and five squamous cell carcinomas). The overall results suggest that testosterone plays an important role in the initiation of prostatic carcinogenesis, whereas the promotional phase is governed by the interaction of testosterone with other factors.

INTRODUCTION

Prostatic cancer was induced in 33% of Wistar-derived MRC rats by weekly intragastric administration of BOP3 (1). However, tumors were induced simultaneously in a variety of tissues, particularly in the colo-rectum, thyroid, nasal cavity, lungs, and liver. Another disadvantage of this model was that most of the induced prostatic tumors were of a squamous cell type, although all of the hyperplastic and preneoplastic lesions were of a glandular type. This change in morphological expression of the neoplasms could be due to an induced hormonal imbalance produced by the carcinogen, e.g., a reduced testosterone level with an absolute or relative increase in estrogen. Since exogenous testosterone has been shown to induce prostatic cancer per se (2) and make prostatic tissue target for carcinogenic action of N-nitroso-N-methylurea (3), we examined the effect of exogenous testosterone on prostatic carcinogenesis by BOP. In a short-term experiment, we first investigated the response of the prostatic epithelium in intact and castrated rats to exogenous testosterone by examining DNA synthesis, to define a correlation between the rate of cell replication and the carcinogenic effect of BOP treatment. Comparison was also made between the effects of BOP given s.c. and orally, because our previous study (1) indicated that BOP administered orally (i.g.) was more effective in inducing prostatic tumors than BOP given s.c.

MATERIALS AND METHODS

Animals

Male Wistar-derived MRC rats with an initial body weight of 340 g at the age of 8 weeks were kept in plastic cages on granular corn cob bedding (Bed-O-Cobs, Maumee, OH) under standard laboratory conditions (21°C ± 2°C, 40% ± 5% relative humidity, 12 h light/12 h dark cycle). They were given Wayne pelleted diet (Wayne, Chicago, IL) and water ad libitum. They were observed twice daily and weighed biweekly. Moribund rats were euthanized by CO2, as were those who were still alive at the end of the carcinogenesis experiment (72 weeks). Rats were assigned to groups according to a random table.

BOP

BOP was synthesized by a previously described method (4), dissolved in physiological saline immediately before its use, and injected s.c. or by gavage.

Testosterone

Testosterone propionate (Sigma Chemical Co., St. Louis, MO) was given either as a s.c. injection of 100 mg/kg b.w. or as a pellet in silastic membrane, which was prepared as described in (5). Each pellet was composed of a silastic tubing of 20-mm length, 2-mm internal, and 3.2-mm outer diameter, and contained 35-45 mg testosterone propionate. The ends of the tubing were closed with medical adhesive. Pellets were handpacked and every fifth pellet was weighed. Pellets were inserted s.c. in the intrascapular region under ether anesthesia. The 10-mm incision was closed with a metal clip, which was removed 3 days later. Because of the tendency of these rats to fight, they were caged individually. Pellets were replaced every 6 weeks for life.

Orchiectomy

Bilateral orchiectomy was performed under nembutal anesthesia (50 mg/kg b.w.). After surgery, rats were caged individually until wound healing was complete.

Autoradiographic Study

Tritiated thymidine with a specific activity of 25 Ci/mmol was injected s.c. at a dose of 1.0 μCi/g 2 h after the 12-h dark cycle and 1 h before the rats were killed. To evaluate labeling index (LI), different areas of the prostate were evaluated under 1000× magnification by...
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Short-Term Experiments

To determine the rate of cell proliferation, LI of prostatic glandular cells were examined in intact and castrated MRC rats. Two types of castration were used, chemical castration (group A) and surgical castration (bilateral orchietomy; group B) as described above. For chemical castration, 50 mg/kg b.w. CYA (Schering Co., Berlin, Germany) were given s.c. to rats daily for 21 days as reported (3). CYA was dissolved in 20% benzyl benzoate in corn oil. Both types of castration were performed in 8-week-old rats. Three weeks later, these rats, and an equivalent number of animals (16 rats) that had also been orchietomized 3 weeks earlier, received daily s.c. injections of testosterone for 5 days. From each of these groups, three rats were killed 2, 3, 4, and 5 days after testosterone treatment and their prostate glands were examined by autoradiography (see above). There were similar numbers of testosterone-treated (testosterone control; group C), orchietomized (group D), and vehicle-treated control (group E) rats for each interval. There was no CYA-treated control group. At necropsy, the testes, seminal vesicles with attached coagulating glands, prostate, and adrenal glands of all rats were weighed.

Experimental Design. Twelve groups of male rats, each composed of 30 rats, were treated as shown in Table 1. Testosterone (100 mg/kg b.w.) was given daily s.c. for 5 days. BOP injection was started after the second testosterone dose, i.e., rats received both testosterone and BOP daily for 3 consecutive days. In groups 2, 3, 6, and 8–10, testosterone pellets were inserted one day after the last testosterone (and/or BOP) injection. Rats receiving testosterone pellets were caged individually for life. Moribund rats were euthanized with CO2. The experiment was terminated when the rats were 72 weeks of age.

Histology. At autopsy, all organs were examined for gross abnormalities. Prostatic glands with the urinary bladder and urethra attached were fixed in toto in buffered formalin for 24 h. Vertical sections were prepared through the midline of the preparation, which provided two equal specimens, containing the left or right portions of the urinary bladder, prostate and urethra. These were processed for histology by conventional methods, cut into step sections (six sections from each of the two parts, each 1-mm apart), and stained with hematoxylin and eosin. Additional sections were prepared as required for immunohistochemistry, to demonstrate testosterone and estrogen by a peroxidase-antiperoxidase method. The antibodies for testosterone and estrogen were obtained from Ortho Diagnostic System, Raritan, NJ.

RESULTS

Short-Term Experiment. The treatment schedule and the weights of the adrenals, testes, prostate, and seminal vesicles, as well as the average body weights of rats treated with testosterone or CYA are summarized in Table 2. Atrophy of the prostate, adrenal glands, and seminal vesicles occurred in CYA-treated (group A) and orchietomized rats (group B), compared with those in the controls (group E). However, the differences were not statistically significant, except for seminal vesicles, which showed a significant reduction in weight following CYA-treatment (group A) or orchietomy (groups B and D; Table 2) at similar rates (P < 0.0001).

The trophic effect of testosterone on the prostate and seminal vesicles was similar in both orchietomized and CYA-treated rats, and showed a positive dose-response relationship, i.e., the longer the treatment, the greater the weight increase, which, however, did not reach statistically significant levels. This was also true for the prostate of intact and testosterone-treated rats.

The patterns of LI are given in Table 3. Testosterone, when given following surgical or chemical castration, increased the rate of LI in the prostate gland 6- to 60-fold. However, LI varied in the different prostatic regions, in part, according to the type of castration. Coagulating gland and dorsal prostate were most responsive to testosterone-induced cell proliferation than ventral and lateral prostate. Moreover, testosterone was more effective in stimulating the cell proliferation (P < 0.0001) in CYA-treated (group A) or orchietomized rats (group B) than in intact rats (group C). On the other hand, testosterone in intact rats had a greater effect on LI in dorsal prostate (P < 0.006) than in coagulating gland (P < 0.03) or in ventral prostate (P < 0.04), whereas it had no significant action in lateral prostate. The latter tissue was also less responsive to the testosterone action in CYA-treated (group A) or intact rats (group C), whereas in orchietomized rats (group B) testosterone increased the LI significantly (P < 0.001).

The peak of LI in each prostatic region was achieved after two testosterone injections and declined slightly thereafter. Testosterone also increased the rate of these parameters in intact rats about 8-fold above the control value (P < 0.03).

Carcinogenesis Experiment. The carcinogenesis experiment...
Table 2 Weights of tissues in rats, expressed in g and as percentage body weight, after CYA and orchiectomy with or without testosterone

<table>
<thead>
<tr>
<th>Group Subgroups</th>
<th>Treatment</th>
<th>Adrenals</th>
<th>% b.w.</th>
<th>Testes</th>
<th>% b.w.</th>
<th>Prostate</th>
<th>% b.w.</th>
<th>Seminal vesicles</th>
<th>% b.w.</th>
<th>Average body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CYA, T 2x</td>
<td>0.020 ± 0.011 (0.006)</td>
<td>2.316 ± 0.291 (0.694)</td>
<td>0.205 ± 0.097 (0.060)</td>
<td>0.191 ± 0.052 (0.060)</td>
<td>337 ± 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>CYA, T 2x</td>
<td>0.020 ± 0.011 (0.008)</td>
<td>2.505 ± 0.185 (0.834)</td>
<td>0.384 ± 0.047 (0.128)</td>
<td>0.417 ± 0.131 (0.139)</td>
<td>301 ± 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>CYA, T 4x</td>
<td>0.026 ± 0.013 (0.009)</td>
<td>2.298 ± 0.201 (0.761)</td>
<td>0.332 ± 0.043 (0.110)</td>
<td>0.409 ± 0.067 (0.135)</td>
<td>302 ± 23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>CYA, T 5x</td>
<td>0.026 ± 0.011 (0.009)</td>
<td>2.236 ± 0.161 (0.756)</td>
<td>0.489 ± 0.048 (0.165)</td>
<td>0.505 ± 0.052 (0.171)</td>
<td>386 ± 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>CYA, T 6x</td>
<td>0.094 ± 0.018 (0.026)</td>
<td>1.015 ± 0.015 (0.046)</td>
<td>0.192 ± 0.035 (0.056)</td>
<td>356 ± 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.0001 compared with group C or E.
* P < 0.0001 compared with group D.
* P < 0.0001 compared with group E.

Table 3 Labeling index in different parts of the prostate following orchiectomy and/or testosterone or CYA treatment

<table>
<thead>
<tr>
<th>Group Subgroups</th>
<th>Treatment</th>
<th>Coagulating gland</th>
<th>Dorsal prostate</th>
<th>Ventral prostate</th>
<th>Lateral prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CYA, T 2x</td>
<td>44.2 ± 3.9</td>
<td>33.2 ± 2.8</td>
<td>7.2 ± 2.3</td>
<td>6.0 ± 3.3</td>
</tr>
<tr>
<td>B</td>
<td>CYA, T 2x</td>
<td>34.5 ± 5.2</td>
<td>9.7 ± 0.6</td>
<td>9.7 ± 3.0</td>
<td>9.0 ± 4.0</td>
</tr>
<tr>
<td>C</td>
<td>CYA, T 4x</td>
<td>22.3 ± 1.6</td>
<td>10.2 ± 1.4</td>
<td>11.7 ± 1.8</td>
<td>10.2 ± 0.8</td>
</tr>
<tr>
<td>D</td>
<td>CYA, T 5x</td>
<td>22.6 ± 2.3</td>
<td>9.3 ± 3.2</td>
<td>9.5 ± 3.2</td>
<td>7.2 ± 2.2</td>
</tr>
</tbody>
</table>

* NS, not significant.

was designed on the basis of the above-mentioned findings. It was decided to perform surgical castration and to administer BOP at the peak of cell replication, i.e., 2, 3, and 4 days following testosterone injection.

The patterns of induced prostatic lesions are summarized in Table 4. Rats treated with BOP i.g. for 20 weeks (group 1) died earlier than those in the other groups, as a consequence of colorectal cancer. At the end of the experiment (72 weeks), 13% of rats in group 2, 11% each of groups 3 and 5, 17% of group 6, 31% of group 7, 39% of group 9, 14% of group 10, and 30% of group 12 were still alive. Blood was taken from these rats for determination of testosterone.

There were no complications, including scarring of the tissue, associated with the repeated insertion of pellets. In a preliminary study, using 10 male rats of the same age, periodic determination of testosterone levels in the rats with testosterone pellets showed a nearly constant serum testosterone level of around 40 ng/ml, which lasted for 6 weeks and declined thereafter. Therefore, pellets were replaced every 6 weeks to maintain the serum testosterone level.

Although the weight gain was initially slower in all rats with testosterone pellets, no differences in the body weights were found among the groups at the end of the experiment.

At autopsy, rats with testosterone pellets had enlarged prostates, which exceeded normal size by 3- to 5-fold. The seminal vesicles were also enlarged and voluminous. In BOP-treated rats having testosterone pellets, however, the prostate had an irregular and grainy texture and contained grayish white nodules up to 20 mm in diameter. Small mucous-filled cystic lesions were noticeable in many of these rats. The urinary bladder of most rats was distended. In castrated, non-testosterone-treated rats, the prostate and seminal vesicles were atrophic.

Histological examination of rats with testosterone pellets showed a hypercellular prostate with hyperplastic and vacuolized cells. Glandular distention was found in the ventral and dorsal prostate. Focal and multifocal sclerotic changes with atrophy and/or hyperplasia of the glandular epithelium were present in the lateral prostate in all rats treated with testosterone for life. The sclerotic areas commonly showed budding of small groups of cells from distorted glands. On several occasions dysplasia and atypia with cellular pleomorphism was encountered. However, these lesions were not considered malignant, although several early or invasive adenocarcinomas were found at the same location (lateral prostate). Focal or multifocal nodular and/or diffuse hyperplasia of the alveolar epithelium and polyposes or papillary polyps, primarily in the ventral prostate, occurred in many rats, particularly in those treated with testosterone pellets (groups 2, 3, 6, 8–10, and 12), regardless of whether or not rats were castrated. However, the rate of occurrence of these hyperplastic changes and benign tumors was low in rats that received testosterone pellets after 20 weeks of age (group 11). Squamous cell metaplasia, often of a multifocal nature, was seen primarily in the ventral prostate and rarely in the lateral or dorsal prostate. There were no differences in the frequency and type of prostatic lesions be-
with metastases to the lungs (in five rats) also occurred in carcinomas with massive infiltration of the pelvic cavity and in our previous study (1). Adenocarcinomas derived primarily from the dorsal and lateral prostate and only a very few derived from the ventral region. Most seemed to develop from the area between the dorsal part and the coagulating glands. Invasive carcinomas with massive infiltration of the pelvic cavity and with metastases to the lungs (in five rats) also occurred in a greater incidence in rats with testosterone pellets, with the highest incidence found in castrated rats given BOP i.g. (group 10).

The morphology of tumors, which in many rats were multiple (up to six tumors per tumor-bearing rat), varied greatly, even within the same tumor, and ranged between well-differentiated, small, glandular types of adenocarcinomas and poorly differentiated, sometimes sarcoma-like tumors, all of which, however, showed focal abortive glandular patterns (Figs. 1–9). Tubular, large cell, and clear cell carcinomas occurred either individually or as mixtures (Fig. 4–6). Poorly differentiated and anaplastic carcinomas showed a tendency to invade the blood vessels and perineural spaces (Fig. 9) and metastasized into distant tissues (paraaortic lymph nodes, lungs). Most of these invasive adenocarcinomas were found in groups 7 and 8. Pure squamous cell carcinomas were also seen as solid growths or were intermingled with adenocarcinomas.

Immunohistochemical examination demonstrated an intense reaction of all induced hyperplastic and neoplastic lesions, including the anaplastic types, with both anti-testosterone and anti-estrogen (Figs. 10–13). The reactivity of the normal prostate stroma was focal, weak and was seen more consistently in the stroma. In some tumors, there was a greater reactivity with estrogen than with testosterone. In this respect, there were no differences between the tumors induced in intact and castrated rats.

The types and the incidence of urinary bladder and urethral carcinomas are given in Table 5. Weekly BOP administration for 20 weeks was less effective in inducing urinary bladder neoplasms (groups 1 and 2) than was three injections of BOP (group 5). Testosterone had no apparent effect on bladder tumor induction. This may be due to the earlier death (by 10 weeks) of rats in groups 1 and 2. Lifetime administration of testosterone...
Fig. 1. A papillary polyp within a distended prostatic gland of the ventral prostate of a 42-week-old rat treated with BOP and testosterone (H & E; × 26).

Fig. 2. An early adenocarcinoma in the dorsolateral prostate. A 43-week-old rat treated with BOP and testosterone (H & E; × 130).

Fig. 3. A small invasive adenocarcinoma around the duct of coagulating gland. BOP and testosterone-treated rat, 46 weeks old (H & E; × 130).

Fig. 4. Prostatic adenocarcinoma composed of glandular structures lined by epithelium resembling that of the lateral prostate. 52-week-old rat treated with BOP and testosterone (H & E; × 130).

Fig. 5. A well-differentiated prostatic adenocarcinoma of the dorsal prostate. The lining epithelial cells are low cuboidal or flat. A 48-week-old rat treated with BOP and testosterone (H & E; × 130).

Fig. 6. Adenocarcinoma in the dorsal prostate of a 57-week-old rat treated with BOP and testosterone. Note pleomorphic character of the glandular epithelial cells and desmoplastic reaction (H & E; × 130).

Fig. 7. Poorly differentiated adenocarcinoma, which had invaded the dorsolateral lobe and the pelvic tissue. There are a few abortive glandular structures (upper and lower part) (H & E; × 130).

Fig. 8. Anaplastic part of an invasive, poorly differentiated adenocarcinoma. A 65-week-old rat treated with BOP and testosterone (H & E; × 130).
Fig. 10. A well-differentiated prostatic adenocarcinoma showing intense reaction to human anti-testosterone. (PAP technique; × 130).

Fig. 11. Same tumor in Fig. 10 processed with human anti-estrogen. Note that a larger number of cells are stained, compared with those in Fig. 10 (PAP; × 130).

Fig. 12. Poorly differentiated prostatic adenocarcinoma expressing cytoplasmic T (PAP; × 130).

Fig. 13. Same tumor in Fig. 12 showing intense reaction of virtually all tumor cells to anti-estrogen (PAP; × 130).
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**Table 5 Patterns of urinary bladder and urethral tumors in male rats treated with BOP**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. (%) of bladder</th>
<th>No. (%) of urethra</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BOP* 20x</td>
<td>24 (8)</td>
<td>9 (28)</td>
</tr>
<tr>
<td>2</td>
<td>BOP* 20x, T</td>
<td>23 (8)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>3</td>
<td>T, BOP 20x, T</td>
<td>28 (10)</td>
<td>16 (57)</td>
</tr>
<tr>
<td>4</td>
<td>BOP 3x</td>
<td>13 (23)</td>
<td>5 (38)</td>
</tr>
<tr>
<td>5</td>
<td>T, BOP 3x</td>
<td>27 (19)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>6</td>
<td>T, BOP 3x, T</td>
<td>24 (79)</td>
<td>16 (67)</td>
</tr>
<tr>
<td>7</td>
<td>Orch, T, BOP 3x</td>
<td>29 (10)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>8</td>
<td>Orch, T, BOP 3x, T</td>
<td>26 (23)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>9</td>
<td>T, BOP 3x, T</td>
<td>28 (21)</td>
<td>15 (54)</td>
</tr>
<tr>
<td>10</td>
<td>Orch, T, BOP 3x, T</td>
<td>28 (23)</td>
<td>9 (31)</td>
</tr>
<tr>
<td>11</td>
<td>T for 20 weeks</td>
<td>16 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>12</td>
<td>T for life</td>
<td>27 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* BOP was given i.g.
* P < 0.05 compared with group 2.
* P < 0.005 compared with group 7.
* P < 0.005 compared with group 10.
* P < 0.005 compared with group 5.

One (group 3) was more effective in inducing urinary bladder papillomas, but had no effect on the development of urethral papillomas. Castration was ineffective in urinary bladder carcinogenesis, whereas it inhibited urethral carcinogenesis in a significant fashion (P < 0.02; compare tumor incidences between groups 6 versus 8). Also, in castrated rats not receiving testosterone after BOP (group 7), the urethral tumor incidence was lower (P = 0.05) than in the group treated with testosterone for life (group 8). Since urethral tumor yield was significantly lower (P < 0.02) in castrated rats treated with BOP i.g. and then with testosterone for life (group 10) compared with the matched intact controls (group 9), testosterone did not seem to augment urethral carcinogenesis when BOP was given i.g. The urinary bladder tumor incidence in the latter two groups showed a similar pattern. As in our previous study (1), metaplastic changes in the urethra, particularly in a defined area between the opening of the ejaculatory and prostatic ducts, were found in all BOP-treated rats, irrespective of castration or testosterone-treatment.

**DISCUSSION**

The present study shows that the rate of testosterone-induced cell proliferation varies in different prostatic lobes and that the coagulating gland is the most responsive tissue. Prior castration, either chemically or surgically, renders the prostatic gland and coagulating gland more responsive to the action of testosterone. Moreover, the present experiments demonstrate that, in analogy to findings in other tissues, stimulation of cell replication makes the prostatic epithelium vulnerable to carcinogenic insults. It was remarkable that a higher tumor incidence was obtained by three doses of BOP, given at the peak of testosterone induced cellular proliferation, compared with the effect of 20 weekly BOP injections in nonstimulated glands (Table 4). It is also noteworthy that epithelial cell hyperplasia induced by five daily injections of testosterone persisted throughout the experiment (in average for about 59 weeks) and that testosterone alone, given for life, also induced a few prostatic carcinomas, as has also been shown previously by others (5, 6). The enhancing effect of exogenous testosterone in prostatic carcinogenesis has also been documented with N-nitroso-N-methylurea (3, 7).

The present study, nevertheless, implies that it is not the nature and/or the dose of carcinogens that is a prerequisite for prostatic tumor induction, but the cellular activity triggered by tissue specific stimulants. Development of a high tumor incidence by only three BOP injections and their selective induction in a greater number of rats demonstrates the unequivocal role of testosterone in prostatic carcinogenesis. It also appears that testosterone determines the pattern of tumor differentiation, as has been suggested to be the case in humans (8, 13), because long-term treatment with BOP alone resulted in the development of tumors mostly of squamous cell character (1), whereas in rats receiving testosterone also, a large proportion of carcinomas was of the glandular type, a common cancer type in human prostate.

Interestingly, almost all adenocarcinomas were found in the dorsal prostate, whereas, as in our previous study, most squamous cell carcinomas originated in the ventral prostate. For adenocarcinomas, the region between the dorsal lobe and the base of the coagulating gland was the predilected area, as has also been noted in NB rats following prolonged testosterone-treatment (2). Since the I:1 was not significantly different in the dorsal and ventral lobe of the prostate following testosterone, factors other than the rate of cell replication must be involved here. The existence of biochemical and functional differences in rats between the individual prostatic lobes and their varying sensitivity to hormones is known (9-11). In this context, the remarkable atrophic and sclerosing changes, which were limited to the lateral prostate in testosterone-treated rats, amplify the differing responses of the epithelial and mesenchymal component of various prostatic regions to hormones. Similar changes in the lateral prostate have been observed following prolonged treatment with pellets of testosterone-propionate in aged NB and Lobund-Wistar rats (2, 5). If one considers the atypical cell proliferation from the distorted glands in the sclerotic areas of the lateral prostate as precancerous, as has been suggested to be the case in men (12), the incidence of precancer and adenocarcinomas in the present experiment in rats treated with testosterone before and after BOP was much higher.

The important role of testosterone in the initiation of prostatic carcinogenesis is supported by the induction of carcinomas in the prostate of castrated rats, which were treated with testosterone only for 5 days (group 7). However, testosterone may also have a promoting effect on prostatic carcinogenesis, since its administration after BOP resulted in the development of a larger number of carcinomas. In fact, the tumor incidence in castrated and intact rats following lifetime treatment with testosterone was similarly high (group 8 versus group 6). Accordingly, a significantly (P < 0.005) fewer prostatic tumors were found in the intact rats receiving testosterone only during BOP administration (group 5) than in those treated with testosterone for life (group 6). This finding indicates that physiological doses of testosterone have no promotional activity and that large amounts of testosterone are required for such an effect, or that some yet unknown factors, in concert with testosterone, are involved in the growth of prostatic cancer.

Although the effect of hormones in prostatic carcinogenesis is still a subject of debate, the presented results, along with the published data, lead us to propose the following hypothesis for the present model: The initiation of prostatic cancer is governed by testosterone, whereas its promotion is maintained by an interaction between testosterone and other factors, possibly hormones. This view is supported by experience in human patients (8, 14-21). Further studies are required for clarification.
different segments of the urogenital tract of rats to hormonal manipulation. Testosterone appears to enhance urinary bladder carcinogenesis by BOP when it is given before and after repeated doses of BOP, but not when it is given only after BOP, indicating that testosterone is involved in tumor initiation in the urinary bladder but not in the urethra. On the other hand, orchietomy inhibits carcinogenesis of the urethra but not of the urinary bladder. This observation is in line with our previous studies showing that the female's urethra and urinary bladder epithelium are less sensitive and resistant, respectively, compared with the response of these tissues to BOP in male rats (22). Although we do not know the effect of testosterone on the rate of cell replication in the urinary bladder and urethra, this effect seems to be unrelated to cell proliferation. (This statement is based on the differing effects of castration and testosterone treatment on carcinogenesis of the prostate and the lower urinary tract). The resistance of female urothelial epithelium to BOP carcinogenesis indicates that not estrogen alone but factors such as the relative ratio of estrogen and testosterone (and/or other hormones, such as prolactin) are operating.

REFERENCES


Induction of Prostatic Carcinomas and Lower Urinary Tract Neoplasms by Combined Treatment of Intact and Castrated Rats with Testosterone Propionate and \( N \) -Nitrosobis(2-oxopropyl)amine

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