Effects of TAP-144-SR Administration on the Prostates of Male and Female Mastomys

Akihiko OHTA, Yoshinori MIZOGUCHI and Yasuhiko KANOH

Department of Animal Physiology, Meiji University, 1-1-1 Higashimita, Tama-ku, Kawasaki 214, Japan

Abstract. Mastomys [Praomys(mastomys) coucha] of both sexes have morphologically functional prostates. To evaluate endocrine control of the prostate and other genital organs in mastomys, TAP-144-SR, a biodegradable sustained-release formulation of a potent Gonadotrophin-releaseing hormone (GnRH) agonist (leuprolide acetate), which is able to induce desensitization of the pituitary-gonadal axis, was used in this study. Three weeks after a single sc injection of TAP-144-SR (5 mg/kg), the mastomys were autopsied.

In males, administration of TAP-144-SR resulted in decreases in the weights of testes, prostates and seminal vesicles in association with a marked reduction in serum testosterone levels. In females, TAP-144-SR treatment caused remarkable ovarian and uterine regression and reductions in serum levels of estradiol-17β and progesterone. However, the weights of female prostates and serum levels of testosterone remained unchanged by TAP-144-SR. These results show that the structure and function of male accessory sex organs and the uterus are mainly controlled by the gonads, which are regulated by gonadotrophins, but that those of the female prostate are less influenced by gonadal control.

Key words: Mastomys, GnRH agonist, Desensitization, Prostate.

Hypophysectomized animals have been widely used to study endocrine control of reproduction. However, hypophysectomy eliminates not only luteinizing hormone (LH) and follicle stimulating hormone (FSH) but also all other pituitary hormones including hormones essential to maintain homeostasis. Therefore, hypophysectomized animals are usually subject to severe physiological damage which may nonspecifically affect reproductive function. Thus, it is occasionally difficult to obtain precise information on specific actions of LH and FSH by hypophysectomy and hormonal replacement administration.

Active immunization of animals against GnRH is advantageous for the study of endocrinological control of reproductive functions because this method causes deficiency of LH and FSH without disturbing secretion of other pituitary hormones [1, 2]. Although it is well known that GnRH and its agonists stimulate gonadal function, chronic administration of these agents results in strong inhibition of function [3-6]. These paradoxical inhibitory effects of GnRH agonists in man have been used for treatment of gonadal hormone-dependent diseases [7-10].

TAP-144-SR is an 1-month release injectable microcapsule of a GnRH agonist, D-Leu⁶-(Des-Gly¹⁰-NH₂)-GnRH ethylamide acetate (leuprolide acetate). A single injection of TAP-144-SR caused hypogonadism by drastic, sustained suppression of the pituitary and inhibited gonadal steroidogenesis in...
male and female rats [11-14] and man [10] for over 4 weeks. These effects suggest that TAP-144-SR treated animals can be used to study the pituitary-gonadal axis and reproductive function as well as in animals actively immunized against GnRH.

Mastomys have a morphologically functional prostate in both sexes. Studies of gonadectomy and hormone replacement showed that the mastomys exhibited sexual differences in the endocrine control of the prostate [15-17]. In the present study, we further investigated the endocrine control of the genital organs, including the prostate, in mastomys treated with TAP-144-SR.

Materials and Methods

Animals

Inbred, wild colored mastomys (MWC strain) were obtained from the National Institute of Health (Tokyo, Japan) and bred in our laboratory. Animals were kept under controlled temperature (22 ± 2°C) and light (14h of light, 0500-1900h) conditions and allowed food (Lab. MR breeder, Nihon Nosan Co. Ltd., Yokohama, Japan) and tap water ad libitum.

Treatment of animals with TAP-144-SR

The sustained-release formulation of leuprolide [D-Leu6-(Des-Gly10-NH2)-GnRH ethylamide] acetate (TAP-144-SR) was supplied by Takeda Chemical industries Ltd. Adult (3-5 months of age) mastomys were administered a single subcutaneous injection of TAP-144-SR (5 mg/kg). Mastomys in the control group were given only the suspension vehicle (an aqueous solution containing 5% mannitol, 4% polysorbate 80, and 0.5% sodium carboxymethyl cellulose). Three weeks after injection, blood samples were collected by heart puncture under light ether anesthesia, and then the animals were killed by cervical dislocation. Pituitary glands and genital organs were removed and weighed. The weights of prostates were determined after combining ventral and dorsal lobes in the male animals.

Histology

Testes, ovaries, uteri and prostatic glands were fixed in Bouin’s solution, embedded in paraffin, and sectioned at 10 μm. After staining with hematoxylin-eosin, they were examined under a light microscope.

Assay of steroids

Serum concentrations of testosterone, estradiol-17β and progesterone were determined by commercial double antibody enzyme immuno assay kits (Cayman Chemical Co. Ann Arbor, USA).

Statistical analysis

All data were presented as the mean ± standard error (SE). The statistical analysis was carried out by Student’s t-test. A P value of less than 0.05 was regarded as significant.

Results

Organ weights

The body and organ weights of control and TAP-144-SR treated males are summarized in Table 1. Body weight and the anterior pituitary weight of TAP-144-SR treated males were comparable to those of the control males. The weights of testes, prostates and seminal vesicles in the TAP-144-SR treated males were significantly lower than those in controls (P<0.05, P<0.05 and P<0.001, respectively). As shown Table 2, body weight and weight of the anterior pituitary of TAP-144-SR treated females were also comparable to those of control females. The weights of ovaries and uteri of TAP-144-SR treated females were significantly lower than those of controls (P<0.05 and P<0.05, respectively, Table 2). Although mean weight of prostates in the TAP-144-SR treated females was slightly lower than that in controls, the difference

<p>| Table 1. Body and organ weights of TAP-144-SR or vehicle treated male mastomys |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Body weight (g)</th>
<th>Anterior pituitary (mg)</th>
<th>Testes (mg)</th>
<th>Prostate (mg)</th>
<th>Seminal vesicle (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>57.2 ± 4.0</td>
<td>2.3 ± 0.3</td>
<td>898.4 ± 25.2</td>
<td>175.4 ± 16.0</td>
</tr>
<tr>
<td>TAP-144-SR</td>
<td>6</td>
<td>54.9 ± 3.6</td>
<td>2.1 ± 0.2</td>
<td>821.3 ± 21.3*</td>
<td>117.7 ± 22.9*</td>
</tr>
</tbody>
</table>

Mean ± SEM, *: P<0.05, **: P<0.001 (Significantly different from the control by t-test).
was not statistically significant (Table 2).

**Histology**

As shown in Fig. 1A, in the ovary of controls, there were several corpora lutea and various sizes of follicles. In contrast, the ovary of the TAP-144-SR treated animals consisted of small preantral follicles and interstitial tissue with no corpora lutea.

**Table 2. Body and organ weights of TAP-144-SR or vehicle treated female mastomys**

<table>
<thead>
<tr>
<th></th>
<th>No. of animals</th>
<th>Body weight (g)</th>
<th>Anterior pituitary (mg)</th>
<th>Ovaries (mg)</th>
<th>Prostate (mg)</th>
<th>Uterus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>38.6± 0.6</td>
<td>1.9± 0.1</td>
<td>20.9± 0.7</td>
<td>62.5± 2.6</td>
<td>216.0± 75.4</td>
</tr>
<tr>
<td>TAP-144-SR</td>
<td>6</td>
<td>39.4± 0.6</td>
<td>1.7± 0.1</td>
<td>17.2± 0.8*</td>
<td>57.8± 4.1</td>
<td>67.1± 5.2*</td>
</tr>
</tbody>
</table>

Mean ± SEM, *: P<0.05 (Significantly different from the control by t-test).

**Fig. 1.** Effects of TAP-144-SR on morphology of the ovary and uterus. Control (1A, ×20) and TAP-144-SR treated ovaries (1B, ×20). Uteri of the control (1C, ×20; 1E, ×200) and the TAP-144-SR treated (1D ×20; 1F, ×200).
or growing antral follicles (Fig. 1B). This observation shows that ovulation did not occur in these animals for a long time after TAP-144-SR administration. The uteri of control animals had a thick stromal layer of endometrium and high columnar epithelial cells (Fig. 1C and E). In the uteri of TAP-144-SR animals, the cavity and stromal layer of endometrium were shrunken, and epithelial cells became cuboidal (Fig. 1D and F).

Testes of TAP-144-SR treated males (Fig. 2C) and controls (Fig. 2A) underwent spermatogenesis. However, the leydig cells of the TAP-144-SR animals (Fig. 2D) were smaller than those of the controls (Fig. 2B).

The prostates of male controls had many large cavities that were enclosed by the epithelium, which had cuboidal cells, and the cavities were filled with a secretion stained by eosin (Fig. 3A). In the prostate glands of the control females, the epithelium, which consisted of tall, columnar cells, formed complex folds (Fig. 3B). In the prostates of TAP-144-SR treated males (Fig. 3C), the cavities were relatively small in comparison with those of controls, and the structure of epithelium was somewhat similar to that of control females. No remarkable histological changes were observed in the TAP-144-SR treated female prostates (Fig. 3D).

**Steroids**

Serum levels of sex steroids are summarized in Table 3. TAP-144-SR administration resulted in a remarkable decrease in serum levels of testosterone in male mastomys (P<0.01). In the females, however, testosterone remained unchanged by TAP-144-SR administration. Serum levels of estradiol-17β and progesterone were significantly decreased by TAP-144-SR administration in the females (P<0.001 and P<0.05, respectively).

**Discussion**

The mechanism(s) and paradoxical effects of GnRH agonists, such as a TAP-144-SR, on gonadal functions are rather controversial. It has been proposed that those agonists induce pituitary de-
sensitization and that the pituitary is the primary site for gonadal inhibition in primates [10, 18]. Desensitization of gonads to gonadotrophins is also regarded as a potential mechanism for the inhibitory effect of GnRH agonists in rat [4–6]. Additionally, GnRH analogues could have a direct inhibitory effect on gonads in rats [19, 20]. Because LH and FSH assay systems for mastomys have not yet been established, the primary site of desensitization by TAP-144-SR could not be determined.

The present study showed that TAP-144-SR administration caused gonadal atrophy and a marked reduction of serum testosterone in males and of estradiol-17β and progesterone in females. The lack of growing antral follicles and corpora lutea in the ovary of TAP-144-SR treated females indicates that long, successive GnRH stimulation causes strong suppression of ovarian function. In males, Leydig cells showed atrophic changes, but spermatogenesis was not impaired by TAP-144-SR administration. Spermatogenesis was arrested by

---

**Fig. 3.** Effects of TAP-144-SR on morphology of the prostate. Male control (3A), female control (3B), male TAP-144-SR treated testes (3C) and female TAP-144-SR treated prostates (3D). ×50.

**Table 3.** Serum testosterone, estradiol-17β and progesterone concentrations of TAP-144-SR or vehicle treated mastomys

<table>
<thead>
<tr>
<th></th>
<th>Testosterone (pg)</th>
<th>Progesterone (pg)</th>
<th>Estradiol-17β (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4149.1 ± 1200.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TAP-144-SR</td>
<td>507.1 ± 153.4**</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>102.8 ± 13.4</td>
<td>7153 ± 1439</td>
<td>72.0 ± 4.9</td>
</tr>
<tr>
<td>TAP-144-SR</td>
<td>149.1 ± 65.8</td>
<td>2400 ± 580*</td>
<td>45.0 ± 3.0***</td>
</tr>
</tbody>
</table>

Mean ± SEM, *: P<0.05, **: P<0.01, ***: P<0.001 (Significantly different from the control by t-test).
hypophysectomy of mastomys (unpublished data). Although the possibility can not be excluded that suppression of gonadotrophin and/or testicular androgen release by TAP-144-SR is not enough to arrest spermatogenesis, it remains another possibility that pituitary hormones other than LH and FSH are involved in spermatogenesis. The study of active immunization against GnRH in rats suggested that prolactin is involved in the process of spermatogenesis [2].

Weights of seminal vesicles and prostates in males were decreased in association with a remarkable reduction of serum testosterone after TAP-144-SR administration. These data are in agreement with observations after castration [15, 17], which indicated that the male accessory sex organs are mainly controlled by testicular androgen.

The uterus also was markedly regressed by TAP-144-SR administration. Similar regression of mastomys uteri was induced by ovariectomy and restored by estradiol administration [15]. Thus, it is conceivable that uterine regression was the result of decreasing ovarian estrogen following TAP-144-SR administration.

In contrast to the uterus, the female prostate showed no remarkable change after TAP-144-SR administration. This result indicate that the prostate of the female mastomys is less influenced by ovarian steroids. The prostate is a male sex accessory and a typical androgen target organ [21] as mentioned above. The prostate of female mastomys also responded to androgens [15–17].

Adrenalectomy results in strong regression of the female prostate but does not influence the male prostate [16]. The adrenal gland is a major source of androgens in mammalian females [22]. Therefore, the adrenal of mastomys may be capable of secreting testosterone [15]. It should be noted that the serum concentration of testosterone in the female mastomys was not decreased by TAP-144-SR administration.

In general, most adrenal androgens have weak androgenic Activity [22]. It was reported that the enzymes required for converting these steroids to an active androgen are present in prostatic tissue in man [23]. As shown in the present study, the serum testosterone concentration of female mastomys was much lower than that of males. However, in the prostatic tissue of both males and females, high concentrations of testosterone and dihydrotestosterone were found [15]. It is highly probable that the prostate of females incorporates testosterone and other adrenal androgens and converts these steroids to an active androgen. Thus, it is conceivable that the prostate of female mastomys is primarily maintained by testosterone and/or other androgens that were secreted from the adrenal.

Human males are unique because they secrete relatively large amounts of adrenal androgen. Thus, the female mastomys may be a useful animal model for evaluating the roles of adrenal androgens in the growth of benign prostatic hyperplasia and prostatic cancer.

The present study demonstrated that administration of TAP-144-SR can induce “pharmacological castration” in mastomys. It is noteworthy that sexual differences in prostatic gonadal dependency was detected by administration of TAP-144-SR. These results indicate that the agent selectively inhibits the gonadal axis without disturbing the adrenal axis. Thus treating animals with TAP-144-SR makes a useful model for clarifying the control mechanisms of gonadotrophins and gonadal steroids on reproductive functions.

Acknowledgment

The authors are grateful to Takeda Chemical Ind. Ltd. for contributing TAP-144-SR.

References


3. Auclair C, Kelly PA, Labrie F, Coy DH, Schally AV. Inhibition of testicular LH/FSH receptor level by treatment with a potent LHRH agonist or hCG.
EFFECTS OF A GNRH AGONIST IN MASTOMYS


