Influences of Gonadal and Adrenal Androgens on the Side Glands of *Suncus Murinus*

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**Abstract.** In order to know the contribution of adrenal and gonadal androgens to the development of the side gland of *Suncus murinus*, we studied the effects of gonadectomy and adrenalectomy on gland thickness and the plasma levels of testosterone, androstenedione (Δ4-dione) and dehydroepiandrosterone (DHA). In males, castration decreased gland thickness to 71% of the control. The plasma levels of Δ4-dione and testosterone were also decreased from 4.16±0.50 and 0.65±0.10 ng/ml to 1.44±0.17 and 0.12±0.02 ng/ml, respectively. Adrenalectomy following castration caused no notable additional decrease in gland thickness, although the plasma levels of Δ4-dione and DHA were further decreased by this treatment. In females, ovariectomy affected neither gland thickness nor plasma androgen levels, except for a peculiar rise in the plasma concentration of Δ4-dione. In contrast, adrenalectomy in addition to ovariectomy decreased gland thickness to 63% of the control and the plasma concentrations of Δ4-dione and DHA from 1.43±0.26 and 0.43±0.05 ng/ml to 0.37±0.11 and 0.10±0.04 ng/ml, respectively. Therefore, testicular androgens are required for the male side gland to fully develop, whereas in the female adrenal androgens are important for the maintenance of sebaceous gland activity and Δ4-dione is quantitatively more important than DHA. One hour after the intraperitoneal administration of [3H]Δ4-dione, dihydrotestosterone was found to be the major androgen bound to nuclei of the side gland. Thus, the side gland can utilize Δ4-dione as a precursor of a more active androgen. The side gland seems to be a useful model for understanding the role of adrenal androgens in target tissues.

**Key words:** Adrenal androgen, Side gland, *Suncus murinus.*

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**IT IS WELL known that human sebaceous glands develop and secrete a large amount of sebum with the onset of puberty and stimulation of androgens [1]. In addition, it has been recognized that sebaceous gland activity is enhanced even in late childhood [2]. This change in sebum secretion in children considered to be a consequence of adrenarche. Besides, in females, in spite of much lower levels of plasma testosterone, hyperplasia of sebaceous glands occurs in adolescence to almost the same degree as in males. However, there is a paucity of information on the mechanism of action of adrenal androgens in the sebaceous gland. This is due partly to the lack of suitable animal models, since in most of the experimental animals used adrenal androgens remained scarce even in adulthood [3, 4].

*Suncus murinus*, which is an insectivorous mammal and inhabits the southern part of Japan, possesses a pair of side glands consisting of large sebaceous glands [5] (Fig. 1). The side gland is well developed in both sexes. This is in sharp contrast to other models, including the hamster flank organ which contains much smaller sebaceous glands in the female sex [6]. Therefore, the side gland provides another interesting model for the study of androgens in sebaceous glands. Our
Fig. 1. The side gland of Sumcus murinus. The arrow indicates the gland.

previous report demonstrated that there were no significant differences between the two sexes with respect to 5α-reductase activity, androgen receptor content, or the intranuclear concentrations of testosterone and dihydrotestosterone (DHT) in the side gland, but the serum testosterone level was 14 times higher in the male [7]. These data suggest that the female side glands utilize other weak androgens as precursors of testosterone.

In the present study with this animal model, we first performed gonadectomy and adrenalectomy in order to examine the effects of androgens secreted by gonads and adrenal glands. We also measured plasma androgen levels in the surgically treated animals. In addition, we studied whether suncus could metabolize androstenedione (Δ4-dione) to more active androgens and accumulate them in the side gland.

Materials and Methods

Experimental animals
Japanese house musk shrews (Sumcus murinus) were kindly supplied by Dr. Oda, Research Institute of Environmental Medicine, Nagoya University, Japan, and by the Central Institute of Experimental Animals, Kawasaki, Japan. The animals have been bred and maintained for 6 years in our animal center. They have been fed special chow supplied by the Central Institute of Experimental Animals, Kawasaki and given water ad libitum. Mature male and female suncus (12-20 weeks old) were used in the present study. Their head-and-body length was 100-130 mm. Females are a little smaller than males. Side glands, 6-9 mm in diameter and 0.2-0.3 g in weight, were excised under ether anesthesia (Fig. 1). Blood samples were obtained by cardiac puncture.

Measurement of the size of side glands
One of the paired side glands was excised immediately before gonadectomy under nembutal anesthesia, and used as a control. Adrenalectomy was performed on some animals 7 days after gonadectomy. The other side gland was excised 4 weeks after gonadectomy. Eight animals were used for each treatment. Each side gland was fixed in 10% formalin, sectioned at 8 μm, and stained with hematoxylin and eosin. The thickness (length from duct to the distal end of the gland) of side glands was measured microscopically on the scale attached to the eye-piece.

Effects of castration and adrenalectomy on the prostate
Prostates were surgically obtained from intact, castrated or castrated-adrenalectomized suncus and their wet weight was measured.

Measurement of steroid concentration in plasma
Steroids were extracted from plasma with 4-7 volumes of benzene-petroleum ether (1:10, v/v). The extracted steroids were spotted on a thin-layer chromatograph plate. The plate was developed in a chloroform-acetone solution (185:15, v/v), as described by Chakraborty et al. [8]. The spots corresponding to testosterone, Δ4-dione and DHA were extracted with ethyl acetate. The amount of each steroid was measured by radioimmunoassay with antisera to testosterone-11α-succinate-BSA [9], androst-4-ene-3, 17-dione-3-oxime-BSA [10] and dehydroepiandrosterone-11-succinate-BSA [11]. The sensitivities of these antibodies were 10 pg of testosterone/assay and 20 pg of Δ4-dione or DHA/assay.
Intracellular binding of steroid

The animals were gonadectomized and adrenalectomized 10 and 3 days before the experiment, respectively. The assay methods were described previously [12, 13]. Briefly, 1 h after the intraperitoneal injection of [3H]Δ4-dione (50 μCi) to gonadectomized-adrenalectomized suncus, side glands (500–600 mg) were excised and homogenized in 2 ml of 20 mM Tris-HCl, pH 7.4, containing 0.32M sucrose, 1 mM MgCl2, and 1 mM dithiothreitol (DTT). The homogenate was centrifuged at 600 g for 10 min, and subsequently at 18,000 g for 20 min. The resultant supernatant was used as a cytoplasmic fraction. The 600 g sediment was washed and extracted with 20 mM Tris-HCl, pH 7.4, containing 0.6 M KCl, 1 mM EDTA, and 1 mM DTT. This was used as a nuclear extract. The radioactivity of each fraction was separated into free and bound radioactivities on a Sephadex G-25 column. Steroids in the aqueous fractions were extracted and analyzed by thin-layer chromatography [13]. DHT and testosterone were recrystallized to constant specific activity.

Radioactive compounds and other chemicals

[1α, 2α,3H(N)]-Testosterone (49 Ci/mmol), [1, 2, 6, 7-3H(N)]-dehydroepiandrosterone (106 Ci/mmoll) and [1, 2, 6, 7-3H(N)]-androst-4-ene-3,17-dione (93 Ci/mmol) were purchased from New England Nuclear (Boston, MA, USA), and unlabeled steroids from Sigma (St. Louis, MO, USA). The antisera to testosterone-11α-succinate-BSA, androst-4-ene-3, 17-dione-3-oxime-BSA and dehydroepiandrosterone-11-succinate-BSA were obtained from Teikoku Zohki (Tokyo, Japan). Other chemicals were obtained from Nakarai Chemicals (Kyoto, Japan).

Results

Effects of gonadectomy and adrenalectomy on side gland thickness and prostate wet weight

The thickness of the side glands of male suncus was 1.4–1.9 mm. The female had somewhat smaller glands (about 80% in thickness) than the male. In males, the thickness of the side glands was decreased to 71% of the control by castration, but virtually no additional decrease was caused by adrenalectomy following gonadectomy (Fig. 2). In
females, it was not affected by ovariectomy, but was decreased to 63% of the control when adrenalectomy was also done.

The wet weight of the prostate was decreased to as low as 38.5% of the control by castration (p<0.001), but was hardly affected by the additional adrenalectomy (Fig. 3).

**Plasma levels of steroids**

Table 1 shows the concentrations of testosterone, DHA, and Δ^4^-dione in the plasma of normal, gonadectomized and gonadectomized-adrenalectomized animals. The testosterone levels in males were about six times as high as those in females. The plasma levels of Δ^4^-dione were also significantly higher in males than in females. The DHA levels were below 0.5 ng/ml in both sexes. Gonadectomy reduced plasma testosterone and Δ^4^-dione levels in males to 18.5 and 34.6% of the respective control values. No significant changes in the DHA level were observed in castrated males. In females, gonadectomy alone did not affect plasma DHA or testosterone levels, while it increased the concentrations of Δ^4^-dione by 80%. Adrenalectomy significantly decreased the concentrations of DHA and Δ^4^-dione in gonadectomized females.

**Androgen binding to the side gland**

Both the nuclear and cytoplasmic fractions of the side gland were separated into bound and free radioactivity by Sephadex G-25 (Fig. 4). The sharp peak corresponded to high molecular weight material in the void volume. The rest of the fractions following this macromolecular peak represented unbound steroids. Actually, DHT was the only steroid which could be recrystallized to constant specific activity. In the bound fraction of the nuclear extract, DHT accounted for 41% and 50% of the total radioactivity in the male and female, respectively. In the free fraction of nuclei, 42% and 59% of the total radioactivity coincided with DHT in the male and female, respectively. Testosterone accounted for less than 5% of the
Table 1. Plasma steroid levels in suncus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>DHA</td>
<td>Δ^4</td>
</tr>
<tr>
<td>Intact</td>
<td>0.37±0.06</td>
<td>4.16±0.50</td>
</tr>
<tr>
<td>Cx</td>
<td>0.27±0.03</td>
<td>1.44±0.17</td>
</tr>
<tr>
<td>Cx+Ax</td>
<td>0.08±0.02</td>
<td>0.12±0.08</td>
</tr>
<tr>
<td>Ox</td>
<td>0.43±0.05</td>
<td>1.43±0.26</td>
</tr>
<tr>
<td>Ox+Ax</td>
<td>0.29±0.05</td>
<td>2.61±0.48</td>
</tr>
<tr>
<td></td>
<td>0.10±0.04</td>
<td>0.37±0.11</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM (n=8–16/group).
DHA: Dehydroepiandrosterone, Δ^4: Androstenedione, T: Testosterone. Statistically significant difference as compared with the values for intact animals (a) and castrated and ovariectomized animals (b): *p<0.05, **p<0.01, ***p<0.001.
Cx: Castrated, Cx + Ax: Castrated-adrenalectomized, Ox: Ovariectomized, Ox + Ax: Ovariectomized-adrenalectomized.

Radioactivity in these fractions in either sex. The rest of the radioactivity corresponding to 5α-androstane-3α, 17β-diol, Δ^4-dione and androstenedione failed to result in constant specific activity by recrystallization. The radioactivity of the cytoplasmic fraction was less than 30% of that of the nuclear fraction. In the cytoplasmic fraction, DHT accounted for 28% and 18% of the bound radioactivity in the male and female, respectively, and testosterone for only 4.5% and 7.8%, respectively. Most of the free radioactivity of the cytoplasmic fraction resided at the origin of the chromatogram, and it was not further analyzed.

Discussion
The present data indicate that testicular androgens such as testosterone and Δ^4-dione are required for male side glands to fully develop. Although adrenal glands secrete an appreciable amount of Δ^4-dione (Table 1), adrenalectomy caused only a marginal decrease in the thickness of the side glands of castrated males. Similarly, adrenalectomy decreased prostate weight only slightly, although it was markedly decreased by castration. On the other hand, ovariectomy hardly affected side gland thickness. Besides, ovariectomy caused a peculiar increase in Δ^4-dione. For the moment we have no explanation for this phenomenon. Removal of adrenal glands following ovariectomy led to a significant (37%) reduction in the thickness of the side gland and a marked decrease in the plasma level of Δ^4-dione as well as DHA.

As mentioned previously, adrenal androgens are a physiologic source of stimulation for the human sebaceous gland, especially in newborns [14], preadolescent children [2] and adult females [15]. In fact, adrenal suppression in normal women [16] and bilateral adrenalectomy in previously ovariectomized women [1] significantly reduced sebum excretion. Therefore, in both man and suncus, adrenal androgens are important for the maintenance of female sebaceous gland activity, but the quantitatively important androgens are different: DHA in the former [17, 18] and Δ^4-dione in the latter. In castrated men, adrenal suppression further decreased sebum secretion [16], while adrenalectomy had little effect on the gland thickness of castrated suncus.

Since the plasma Δ^4-dione levels were high in suncus of either sex compared to other mammals including human beings (Table 1), we studied whether this particular species can utilize [3H]Δ^4-dione as a precursor of more active androgens in vivo. The results clearly demonstrated that Δ^4-dione could be transformed into DHT which was accumulated in the side gland, although the site of metabolic conversion has not been clarified. The absence of Δ^4-dione even in the free fraction may be due to rapid conversion of injected Δ^4-dione into polar metabolites, probably conjugated steroids, in the side gland, and minimum contamination of blood (the side gland contains few blood
vessels). Recently the hypothesis was proposed that \( \Delta^4 \)-dione and DHA exert their androgenic effects in the human prostate through their peripheral conversion into DHT [4, 19, 20]. Thus, the side gland is likely to help our understanding of the role of adrenal androgens in target tissues.

References