Sex difference in the activity of cytosol 3 α -hydroxysteroid dehydrogenase in mouse submandibular gland

Nobuko Sato, Rie Baba and Minoru Ota

*Department of Biochemistry, Iwate Medical University School of Dentistry,
Morioka, Iwate 020*

[Accepted for publication: February 13, 1985]

Key words: 3 α -hydroxysteroid dehydrogenase / submandibular gland / mouse / sex difference

Introduction

3 α -hydroxysteroid dehydrogenase (HSD) involved in androgen metabolism is present in rodent submandibular glands^{1,2}. This enzyme converts 5 α -dihydrotestosterone (DHT) to 5 α -androstane-3 α ,17 β -diol and is one of the important factors which regulate the tissue concentration of androgens. We have recently found that, when ³H-DHT was incubated with cytosol prepared from mouse or rat submandibular gland, the binding level of ³H-DHT receptor formed decreased faster in females than in males after reaching the maximal level (unpublished data). This finding led us to determine whether the faster decrease of cytosol ³H-DHT receptor binding level in female mice submandibular gland is due to higher activity of 3 α -HSD for ³H-DHT. Here we describe a sex difference in the activity of 3 α -HSD in cytosol of mouse submandibular gland and also describe the effects of castration, testosterone or estradiol-17 β on the cytosol 3 α -HSD activity of the submandibular glands of male and female mice.

Materials and Methods

Animals and Treatments. Male and female mice of ddY strain, aged 8–10 weeks, were used in this experiment. In addition to intact animals of both sexes, male mice were castrated under ether anesthesia. In order to investigate the effects of sex steroids on the 3 α -HSD activity, estradiol-17 β benzoate

(100 μ g/100 g body weight as estradiol-17 β , Nutritional Biochemical Corp.) in propylene glycol was injected sc. daily into intact male mice for 1, 3, 5 or 10 days. The injection was given at 9 a.m.; the last injection being 48 hours before sacrifice. Testosterone propionate (500 μ g/100 g body weight as testosterone, Sigma) in propylene glycol was injected sc. into female mice in the same manner as for estradiol-17 β . Each control received only the vehicle. The animals were killed by cervical dislocation and the submandibular glands were dissected free of other tissues and minced with scissors. The mince was then homogenized in 5 vol of 0.1 M Krebs-Ringer phosphate buffer (pH 7.4) with a Potter-Elvehjem glass homogenizer cooled in ice. The homogenates were centrifuged at 160,000 $\times g$ for 30 min at 0–2°C in a Hitachi RP65T rotor and the supernatant fluid (cytosol) was obtained.

Assay of 3 α -HSD activity. The cytosol (0.4 mg protein) was added to a Krebs-Ringer phosphate buffer containing 40 nmoles of NADPH (Oriental Yeast Co.) and 112,000 dpm of the radioactive 5 α -DHT (specific activity: 50.6 Ci/mmol, New England Nuclear Corp., Boston, Mass. USA) mixed with a cold carrier to give a final concentration of 25 pmol. After incubation at 37°C for 30 min, the steroids were extracted twice with methylene dichloride, and then this solvent was dehydrated with anhydrous Na₂SO₄ and evaporated to dryness. The residue dissolved in ethanol was separated on thin layer chromatography (Silica Gel G, Analtech Inc.) in benzene:acetone (4:1,

v/v). The radioactivities in metabolites were measured by the method of Ota *et al.*³⁾ The activity of 3 α -HSD was expressed as a yield in pmol of 5 α -androstane-3 α , 17 β -diol produced from 5 α -DHT.

Protein Determination. The quantity of protein in each sample was determined by the method of Lowry *et al.*⁴⁾ Bovine serum albumin was used as a standard.

Results and Discussion

There was a sex difference in the 3 α -HSD activity of the cytosol of the submandibular gland, being significantly higher in females (26.4 pmol/mg protein) than in males (16.0 pmol/mg protein). In mouse submandibular gland, 4-androstenedione and testosterone are mainly metabolized to androsterone and 5 α -androstane-3 α , 17 β -diol, respectively¹⁾. A similar androgen metabolism was observed

in rat submandibular gland²⁾. Koshika⁵⁾ reported that the microsomal fraction of rat submandibular gland converts testosterone to 5 α -DHT and 5 α -androstane-3 α , 17 β -diol, and 5 α -DHT to 5 α -androstane-3 α , 17 β -diol. He also observed that 3 α -HSD is less localized in the cytosol than in the mitochondria and microsomes of rat submandibular gland, and the Km and Vmax of microsomal 3 α -HSD were similar in both sexes. Corpchot *et al.*⁶⁾ reported that the androgen level in rat prostate was not explained by the androgen level in the plasma, suggesting the importance of cellular enzymes and receptors in controlling the androgen level in the tissues.

The purpose of this study was not to investigate the androgen metabolism in the submandibular gland but to clarify the metabolism of 5 α -DHT, a native ligand for androgen receptor, by the cytosolic fraction of this gland which is commonly used as

Fig. 1 Effect of castration, androgen or estrogen on 3 α -HSD activity. A, Castration of male mice was performed 1, 3, 5 and 10 days before sacrifice. B, The male mice were injected with estradiol-17 β benzoate (estradiol-17 β 100 μ g/100 g body weight) for 1, 3, 5 and 10 days. C, The female mice were injected with testosterone propionate (testosterone 500 μ g/100 g body weight) for 1, 3, 5 and 10 days. The animals in B and C groups were sacrificed 48 h after the final injection. Submandibular cytosol prepared from mice described above was incubated with ³H-DHT for 30 min at 37°C and 3 α -HSD activity was assayed as described in "Materials and Methods". TP: testosterone propionate, EB: estradiol-17 β benzoate.

crude receptor, because it was observed that receptor-bound DHT was partially converted to 5 α -androstane-3 α , 17 β -diol, which exerts low affinity for androgen receptor, by 3 α -HSD in submandibular cytosol. These findings suggest that the use of native ligands is not recommended. However, if the native ligand is used, more attention should be paid to the study of cytosol androgen receptor.

As we found a sex difference in the activity of 3 α -HSD, the effects of endocrine manipulations were also studied. As indicated in Fig. 1A, castration of males increased the 3 α -HSD activity to the female level on the 10th day after operation. Fig. 1B and C show the 3 α -HSD activities in the submandibular gland cytosol in males receiving estradiol-17 β and in females injected with testosterone, respectively. The injection of estradiol-17 β to males increased the 3 α -HSD activity and elevated it above the female level after the three injections, whereas the injection of testosterone to females decreased this activity and lowered it to below the male level. These findings indicate that 3 α -HSD responds to both androgen and estrogen, and also that 3 α -HSD activity can be used as an indicator of sex characteristics in mice.

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