NOTE

Effects of Gonadal Hormones on Hyaluronic Acid Content in Male Mouse Skin

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Synopsis

Male mice were subcutaneously injected with several gonadal hormones daily for 5 days in order to study their effects on hyaluronic acid (HA) content in the skin.

Increases in both total hexosamine and HA contents were in the following order: estradiol-17 β (10 μ g) > estradiol-17 α (100 μ g) > estradiol-17 α (10 μ g) > testosterone (100 μ g) > control ($p < 0.001$). No significant differences were noted between 5 α -dihydrotestosterone (100 μ g) and control. Testosterone (100 μ g) or 5 α -dihydrotestosterone (100 μ g) injected with estradiol-17 β (10 μ g) blocked the increase in HA content produced by estradiol-17 β alone.

Thus, it can be suggested that an increase in HA is not specific for estradiol-17 β and that the action of testosterone or 5 α -dihydrotestosterone is antagonistic to that of estradiol-17 β in the male mouse skin.

Estradiol-17 β (E₂-17 β) has been found to cause a marked increase in HA content in the male mouse skin (Sobel *et al.*, 1965), but the mechanism has not been unraveled in contrast to that of other actions of estrogen. In the present work, as part of our continued interest in the mechanism of the skin HA increase by E₂-17 β (Kanke *et al.*, 1977), we have attempted to study the effects of estrogen and androgen, alone and in combination, on the HA content in the male mouse.

Materials and Methods

Estradiol-17 α (β isomer-free) was purchased from Sigma Chemical Co. Ltd. (St. Louis, Mo., U.S.A.). Testosterone propionate was obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Estradiol-17 β benzoate and 5 α -dihydrotestosterone were generous gifts from Teikoku Hormone Co. Ltd. (Tokyo, Japan). Hyaluronidase (pure grade) from *Streptomyces hyalurolyticus* was obtained from Seikagaku Kogyo Co. Ltd. (Tokyo, Japan). Pronase E was purchased from Kaken Co. Ltd. (Tokyo, Japan).

Male mice of the dd/Y strain (20-25 g) were fed with standard laboratory pellets and tap water *ad libitum* throughout the experiment. The animals were divided into 8 groups and received the hormone tested subcutaneously in 0.1 ml sesame oil once daily for 5 days. Group 1 was the control group and was given the vehicle only. Groups 2, 3, 4, 5 and 6 received 10 μ g or E₂-17 β , 10 μ g and 100 μ g of E₂-17 α , 100 μ g of T and 100 μ g of DHT respectively. Groups 7 and 8 received T (100 μ g) and DHT (100 μ g) respectively together with E₂-17 β (10 μ g). All the mice were sacrificed 24 hr after the last injection and the back skin was removed and then the adhering tissue was cleaned off. The skin was minced, dehydrated, and

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Abbreviations used are: DHT, 5 α -dihydrotestosterone; E₂-17 α , estradiol-17 α ; E₂-17 β , estradiol-17 β ; GAG, glycosaminoglycan; HA, hyaluronic acid; T, testosterone.

defatted with several changes of acetone followed by CHCl_3 -MeOH (1:1, v/v) mixture. Approx. 100 mg of the fat-free dried sample was mixed with 10 ml of 0.05 M Tris-0.01 M CaCl_2 buffer, pH 7.9, containing 10 mg of Pronase E. The sample was then incubated at 47°C for 24 hr and another 10 mg of the enzyme was added after adjustment of pH, and the incubation was continued for another 24 hr. The digest was added with trichloroacetic acid to 10% concentration and the precipitated protein was centrifuged off. The supernatant was dialyzed against distilled water and evaporated *in vacuo*. The total hexosamine was determined by a modification of the Elson-Morgan procedure (Gatt and Berman, 1966). In order to determine HA, the crude GAG fraction was precipitated by adding cetylpyridinium chloride. The precipitate was dissolved in 4M NaCl and reprecipi-

tated with ethanol twice. HA in GAG obtained here was measured using *Streptomyces* hyaluronidase according to the method of Hatae and Makita (1975). Briefly, 25 μl of the aqueous crude GAG solution (up to 50 μg of HA) and 50 μl of the enzyme (2 TRU) in 50 mM sodium acetate buffer, pH 5.0, were incubated at 66°C for 150 min; the reaction mixtures were measured for N-acetyl glucosamine by Morgan-Elson reaction (Reissig *et al.*, 1955).

Results and Discussion

The concentration of hexosamine in the pronase digests (total hexosamine), expressed

Table 1. Effects of gonadal hormones on total hexosamine content in male mouse skina

Group	Treatment ^b	No. of Animals	Hexosamine Content	% of Control
1	Control	7	1.85±0.07	100
2	E ₂ -17 β (10 μg)	7	6.01±0.97 ^c	324
3	E ₂ -17 α (10 μg)	5	2.62±0.34 ^c	141
4	E ₂ -17 α (100 μg)	6	3.64±0.47 ^c	196
5	T (100 μg)	7	2.45±0.41 ^c	133
6	DHT (100 μg)	7	1.92±0.31	104
7	E ₂ -17 β (10 μg)+T (100 μg)	8	5.12±0.76 ^{c,e}	277
8	E ₂ -17 β (10 μg)+DHT (100 μg)	8	5.08±0.76 ^{c,d}	274

a Values are expressed as $\mu\text{mole}/100\text{mg}$ dry tissue and represents the mean±S.D.

b Animals were treated daily for 5 days with various doses of hormones in parentheses. E₂-17 α : estradiol-17 α , E₂-17 β : estradiol-17 β , T: testosterone, DHT: 5 α -dihydrotestosterone.

c Significantly different from control (group 1), $p < 0.001$.

d Significantly different from E₂-17 β (group 2), $0.01 > p > 0.001$.

e Significantly different from E₂-17 β (group 2), $0.05 > p > 0.01$.

Table 2. Effect of gonadal hormones on hyaluronic acid content in male mouse skina

Group	Treatment ^b	No. of Animals	Hyaluronic Acid Content	% of Control
1	Control	7	199.5± 17.5	100
2	E ₂ -17 β (10 μg)	7	1847.4±281.3 ^c	926
3	E ₂ -17 α (10 μg)	5	559.3± 55.2 ^c	280
4	E ₂ -17 α (100 μg)	6	974.7±248.4 ^c	489
5	T (100 μg)	7	475.3±108.9 ^c	238
6	DHT (100 μg)	7	221.0± 78.9	111
7	E ₂ -17 β (10 μg)+T (100 μg)	8	1387.8±307.6 ^{c,e}	696
8	E ₂ -17 β (10 μg)+DHT (100 μg)	8	1296.5±290.1 ^{c,d}	650

a Values are expressed as $\mu\text{g}/100\text{mg}$ dry tissue and represents the mean±S.D.

b Animals were treated daily for 5 days with various doses of hormones in parentheses. E₂-17 α : estradiol-17 α , E₂-17 β : estradiol-17 β , T: testosterone, DHT: 5 α -dihydrotestosterone.

c Significantly different from control (group 1), $p < 0.001$.

d Significantly different from E₂-17 β (group 2), $0.01 > p > 0.001$.

e Significantly different from E₂-17 β (group 2), $0.05 > p > 0.01$.

as μ mole of hexosamine per 100 mg of dry tissue, is shown in Table 1. The total hexosamine contents of the hormone-treated groups except for the DHT-injected group were significantly higher than that of the control group.

It was further investigated whether these changes in the total hexosamine content depended on HA content. As shown in Table 2, the response to various gonadal hormones was more marked in HA (expressed as μ g of HA per 100 mg of dry tissue) than in the total hexosamine.

Administration of $E_2-17\beta$ caused a large increment in both total hexosamine and HA contents in the male mouse skin, confirming earlier results (Grosman *et al.*, 1971).

Also the injection of $E_2-17\alpha$ (10 and 100 μ g) significantly increased in both total hexosamine and HA contents as $E_2-17\beta$ did. This result is very interesting, because $E_2-17\alpha$ is not thought to have biological activity in uterus (Jensen *et al.*, 1966).

T produced a similar but less marked change in HA content compared with that by $E_2-17\beta$. This demonstrates that T did not exhibit the species specificity in HA-increasing action, since the same effect by T was already observed in the rabbit skin (Davidson and Small, 1963) and rat skin (Kofoed *et al.*, 1970) in contrast to the result of $E_2-17\beta$.

On the contrary, DHT showed only insignificant changes in this system, though DHT is known as the most potent androgen in many tissues. This is of interest in connection with the findings of other investigators which indicated the nuclear retention of T, not DHT, in the mouse and rat kidney (Bullock *et al.*, 1971; Ritzén *et al.*, 1972).

T or DHT injected with $E_2-17\beta$ blocked the increase in both total hexosamine and HA contents produced by $E_2-17\beta$ alone in the male mouse skin. These data suggest that T and DHT are antagonistic to $E_2-17\beta$ in this tissue.

The mechanism and mode of action of

these gonadal hormones including $E_2-17\beta$ involved in the HA increment are not known at present. Although Sasaki and Ketkar (1968) previously demonstrated that both $E_2-17\beta$ and T increased UDP-glucose dehydrogenase and L-glutemine-D-fructose-6-phosphate aminotransferase activities in the male mouse skin, the specific enzyme for HA metabolism such as HA synthetase and hyaluronidase (under the influence of various gonadal hormones) should be investigated directly. It might be possible that the HA increment is caused by the general metabolic disturbances through the administration of $E_2-17\beta$ rather than by the direct action of $E_2-17\beta$, because we were unable to demonstrate the presence of a cytoplasmic receptor of $E_2-17\beta$ in the same tissue (Kanke *et al.*, 1977).

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