NOTE *Theriogenology*

Induction of Primer Pheromone Production by Dihydrotestosterone in the Male Goat

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(Received 28 August 2000/Accepted 28 November 2000)

ABSTRACT. Castrated goats were treated with dihydrotestosterone (DHT) for four weeks. Skin samples were collected from the head and the rump regions before and after the DHT treatment. The primer pheromone activities of these samples were assessed neurophysiologically by recording electrophysiological manifestations of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator activity. Pheromone activity was detected in both the head and rump skin samples following the DHT treatment, although the development of sebaceous glands was limited to the head region. Taken together with our previous finding that testosterone treatment results in the appearance of primer pheromone activity in the skin sample of the head region but not of the rump region, these observations suggests that the regional difference of pheromone production would be ascribed to intrinsic expression levels of 5α-reductase, an enzyme converting testosterone to DHT.

KEY WORDS: caprine, dihydrotestosterone, pheromone.

J. Vet. Med. Sci. 63(3): 347–348, 2001

Dihydrotestoserone (DHT) is a target tissue-produced androgen, which is converted from testosterone by 5α-reductase. DHT is known to have higher affinity with androgen receptors (AR) than testosterone and to play a major role for the development of sebaceous glands and sebum production in the skin [13, 14]. The results of our previous study [16] imply the involvement of DHT in the production of primer pheromone in sebaceous glands of the male goat, which is responsible for the "male effect" [1,3] of the male goat. Specifically, four weeks treatment of castrated goats with testosterone resulted in the appearance of primer pheromone activity in the skin sample of the head region but not of the rump region. Much higher immunoreactivities to 5α-reductase and androgen receptor were expressed at the sebaceous glands of the head region than those of the rump region. Based on these findings we assumed that the regional difference of the responsiveness to testosterone was due to different enzymatic activities of 5α-reductase determining local DHT availability. In order to test this hypothesis, skin samples were collected from the head and the rump regions of castrated male goats which had been treated for 4 weeks with DHT. These samples were then assessed for pheromone activity and the development of sebaceous glands as an indicator of sebum production.

For the present study, three long-term (>1 year) castrated Shiba goats were obtained from the closed colony at the experimental station of the University of Tokyo. Each animal was implanted subcutaneously with six Silastic capsules (1 cm × 1 cm, Dow Corning, Midland, MI, U.S.A.) each of which contained 1 g of DHT (Wako Co., Osaka, Japan). Skin sampling was conducted on Day 0 (the day of DHT implantation) and on Day 28. For sampling, the goat was anesthetized, and a square (1 cm × 1 cm) of skin was excised from the head and the rump region using a scalpel as described previously [7, 16].

The bioassay for pheromone activity in skin sample was carried out as follows: Arrays of bilateral recording electrodes [10] were stereotaxically placed in the medial basal hypothalamus [8] of long-term (>2 years) ovariectomized female goats. Three goats, from which clear multiple unit activity (MUA) volleys had been consistently recorded [8-11], were used for the bioassay of pheromone activity according to the procedure described elsewhere [6, 7]. In brief, the estradiol-primed goats were loosely tied to the stanchion in a Faraday cage during the MUA recording. For bioassay, half of each skin sample was extracted with diethyl ether [7]. The gauze containing the sample extract was placed 2 to 3 cm in front of the goat's nose to allow olfactory, visual, and tactile investigation. When a following MUA volley appeared within 3 min of the sample application, the sample was assessed to be pheromone-positive [6, 7].

The remaining halves of the skin samples were fixed in 10% paraformaldehyde, stained with Sudan black B, and histologically examined. The lengths of the major axes of 50 sebaceous glands were measured for each animal [7, 16], and the mean values (± standard errors) were calculated. Analysis of variance (ANOVA) was used for detection of significant differences, and the post hoc analysis was performed using Fisher's Protected Least Significant Difference (PLSD) test.

The presence of pheromone activity in the skin samples and the size of sebaceous glands on Day 0 and Day 28 are shown in Table 1. On Day 0, neither the head nor the rump samples showed pheromone activity, and the size of the sebaceous gland was small. On Day 28, all the samples from both the head and the rump regions were pheromone-positive. The sebaceous glands of the head region, but not that of the rump region, developed markedly, and the length of major axes of the sebaceous glands on Day 28 was significantly longer than other samples ($F_{1,3}=1559.53$, $p<0.01$).

Table 1. Pheromone activity and size of sebaceous glands in head and rump skin samples collected from castrated male goats treated with DHT implantation

	HEAD		RUMP	
	Day 0	Day 28	Day 0	Day 28
Pheromone positive[a)]	0/3	3/3	0/3	3/3
Sebaceous gland size (μm)[b)]	161.12 ± 5.78	1200.11 ± 25.42*	128.07 ± 4.08	150.72 ± 4.01

a) The number of pheromone positive goats / total number of goats.
b) Sebaceous gland size is expressed as the mean (± SD) length of the major axis (μm) .
*P<0.01 compared to head samples on Day 0 or Rump samples on Day 0 and 28.

These results indicate that the primer pheromone is produced, if sufficient amounts of DHT are provided, even in the skin of the rump region where the testosterone replacement was unable to induce pheromone production in castrated goats [16]. On the other hand, the DHT treatment resulted in the development of sebaceous glands only in the head but not the rump region. This suggests that pheromone production is not necessarily associated with the development of the sebaceous glands, and that there may not be a causative relation between these two testosterone-induced biological phenomena. Therefore, the absence of pheromone activity in the rump region following testosterone implantation [16] can be explained as a consequence of different levels of the expression of 5α-reductase.

5α-reductase is known to exist in two isoforms, i. e., it has been shown in humans that type 1 is located in the skin and is considered to be responsible for most of the 5α-reductase activities of the sebaceous glands because of its dense localization to sebocytes [2, 4, 15], whereas type 2 is located primarily in the prostate gland and the skin of the genitals [2, 4]. The existence of these two subtypes of 5α-reductase has also been confirmed in other species such as rats and monkeys [5, 12]. However, the 5α-reductase of ruminant species is yet to be characterized in terms of either molecular isomers or their topographical localization. To elucidate the mechanism of pheromone production in goat skin, the nature of 5α-reductase should be clarified thoroughly. In conclusion, the results of the present study suggest that the production of primer pheromone is mediated by DHT that is converted from testosterone by 5α-reductase, whose activity differs regionally, leading to region-specific pheromone production in the male goat.

ACKNOWLEDGEMENTS. This study was supported by the “Research for the Future” Program, The Japan Society for the Promotion of Science (JSPS-RFTF 97L00904), and by Grants-in-aid, for Scientific research from the Ministry of Education, Science, Sports and Culture, and from CREST of the Japan Science and Technology Corporation.

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