

Identification of sex-associated protein in the preputial gland of house rat (a new insight in rodent pest management)

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Our recent findings revealed that the preputial gland of male house rat contains 20 kDa protein, however, the role of androgen in the production of this protein is not known. Hence, the present study was carried out to evaluate the androgen dependency of 20 kDa protein in the preputial gland of house rat (*Rattus rattus*) and to compare its presence in female clitoral gland. Further, on castration the amount of glandular protein in male was significantly decreased to a certain extent, while testosterone treatment on castrated males showed an increasing trend. The electrophorogram of male house rat showed six different protein fractions with molecular weights of 90, 70, 60, 50, 35 and 20 kDa. However, the 70, 60, 50 and 35 kDa were absent in female. Among the different fractions, 90 and 20 kDa proteins were prominent. On castration, the 20 kDa protein was disappeared; while on testosterone treatment the protein reappeared.

Thus, the present study concludes that the 20 kDa protein is a testosterone dependent sex-associated protein. Since urinary protein is found to act as carrier for volatile substances in pheromonal communication. The present study suggests that the glandular protein may bind with the volatile compounds produced from preputial gland. Identification of this carrier protein in the preputial gland explores the possibility of developing pheromonal trap for rodent pest management (RPM).

Keywords: pheromone, preputial gland, *Rattus rattus*, carrier protein

Pheromonal communication plays an important role in mammalian reproduction and social behaviours like sexual attraction (1–3, 18–20,) territorial marking (5, 27), mother-young interactions (22), individual identification (26) and evocation of aggression (11). The excretory products, urine and faeces constitute important sources for pheromones in mammals. Apart from these, sweat, saliva and secretions of specialized integumentary

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glands also facilitate transmission of chemical messages (4, 5). Among the scent glands, the preputial gland of rodents produced chemical cues that are used in attraction and aversion for the opposite sex and it is sex, respectively (3, 5, 7, 19, 20).

The preputial glands appear as paired structures on either side of the penis in the male rat and homologue glands are associated with the clitoris in female. These glands have been suggested as a source of primer pheromone that helps in estrus synchronization (12) and acceleration of puberty in mice. The female partner prefers the odours of the male preputial gland to that of other tissues, while male rats are attracted to the female preputial odours (21, 24).

Kannan (16) identified sex-specific compounds from the preputial glands of house rat (*Rattus rattus*) namely, Decanoic acid and 3-Decyn-2-ol in males and 1-nitropentane, and *n*-hexyl-methyl ketone, in females. The behavioural analysis carried out in the laboratory revealed that decanoic acid acted as both sex attractant and 3-Decyn-2-ol as opposite sex attractant, whereas the compound, *n*-hexyl-methyl ketone was reported to be male attractant (16). Kannan (16) used these compounds in trapping the field rats and got partial success. The attainment of partial success is due to the volatility of these compounds, which evaporate and diffuse quickly into the environment. So there is a need for a slow release of sex-specific compounds in the field.

Recent reports revealed that both mice and rats secreted urinary pheromones and deposited these signals as scent marks throughout their home ranges. These chemical signals are held up tightly within the core of small sponge like proteins in urine. The molecular weights of these proteins are around 18–20 kDa and they play a crucial role in protecting these pheromones from being rapidly lost by evaporation or degradation. It thereby extends the ‘shelf-like’ characteristic of the scent marks releasing the pheromones slowly as the protein degrades (9, 15). Such proteins are already reported in other body fluids namely mouse urine (9), pig saliva (6), hamster vaginal secretion (29) and human sweat (30) which are known for pheromonal activity. The above literature clearly established that the protein present in the pheromonal source played a significant role as ligand carriers that are involved in chemo-communication. Our recent report revealed that the preputial gland of male house rat contained 20 kDa protein (4). Another report (14) indicated that a high level of androgens in males resulted in higher excretion of urinary proteins. However, there is no evidence of endocrine dependency of a glandular protein in house rat and of its relationship with the pheromonal communication. Hence, the present study was focused on androgen dependency of 18–20-kDa proteins in the preputial gland of house rat (*Rattus rattus*) and to compare the presence of similar protein in female rats.

Materials and Methods

Test animals

Adult male (220–235 g) and female house rat (175–200 g), *Rattus rattus*, were trapped in and around Bharathidasan University, Tiruchirappalli, South India and housed

separately in polypropylene cages (40×25×15 cm) with 2 cm of rice husk lining the bottom as bedding material. They were reared on pelleted food (Hindustan Lever Ltd., Bangalore) and water *ad libitum*.

Castration

Surgical removal of testes (bilateral gonadectomy) was performed as per the procedure of Kannan and Archunan (18) in twelve rats. Castrated male rats were maintained under normal conditions for about two weeks.

Hormonal replacement

Testosterone enanthate (Testoviron, Schering, Germany) was diluted in arachis oil and administered intramuscularly to gonadectomized rats of 0.1 mg/day/rat for two weeks. One ml of testoviron contained 250 mg of testosterone enanthate. In order to prepare 0.1 mg of testosterone enanthate, 1 ml of testosterone was made up to 25 ml by adding 24 ml of arachis oil. 0.1 ml was taken and administered intra-muscularly into the six castrated males.

Isolation of gland

Healthy normal adult male, castrated and castrated plus hormone treated males and intact female rats were taken as source. Animals were sacrificed by chloroform exposure and dissected out near the external genitalia to locate the preputial gland. After dissecting the preputial gland, it was carefully removed and weighed immediately, and placed in vials containing 400 µl of extraction buffer at –20 °C.

Sample preparation

Approximately 100 mg of the tissue was taken and homogenized in a glass homogenizer at 4 °C. The homogenate was centrifuged for 10 minutes at 10,000 rpm at 4 °C, then the supernatant was transferred to a clean-labeled Eppendorf tube and stored at –20 °C. Thus, the supernatant collected can be directly used for protein estimation (23) as well as for separation by electrophoresis (28).

Results

The amount of protein was higher in preputial glands than in clitoral gland in the house rat. Further, on castration the amount of glandular protein in male was significantly decreased and to a greater extent. The testosterone treated castrated males showed an increase in glandular protein concentration (Table I). The electrophorogram of male house rats showed six different protein fractions with molecular weight such as 90, 70, 60, 50, 35 and 20 kDa. Among these fractions, the 90 and 20 kDa appeared prominently (Fig. 1). The glandular extract of castrated male house rat showed less protein fractions

compared to that of normal males. Infact, the 70, 50, 35 and 20 kDa protein fractions were absent in the preputial gland (Fig. 2). Further, the intensity of the protein fraction was less in preputial gland of castrated males to that of normal males. In addition, 70, 60, and 35 kDa proteins are also absent in clitoral gland (Fig. 4). In testosterone treatment 90, 80, 60, 50, 35 and 20 kDa proteins were observed in the preputial gland of castrated males (Fig. 3). The intensity of protein fraction was higher in the preputial gland of hormone-treated animals.

Table I

Protein concentration in the preputial/clitoral glands of house rat

Sl. No	Samples	Protein concentration mg/ml
1.	Preputial gland	0.190 ± 0.034^a
2.	Preputial glandcastrated	0.068 ± 0.012^d
3.	Preputial castastrated + testosterone	0.113 ± 0.029^b
4.	Clitoral gland	0.063 ± 0.06^d

Values are expressed in Mean $\pm$ SE. Those means in the same vertical column that are not marked with the same superscript letters are significantly different at $\alpha=0.5$ level (Duncan's test).

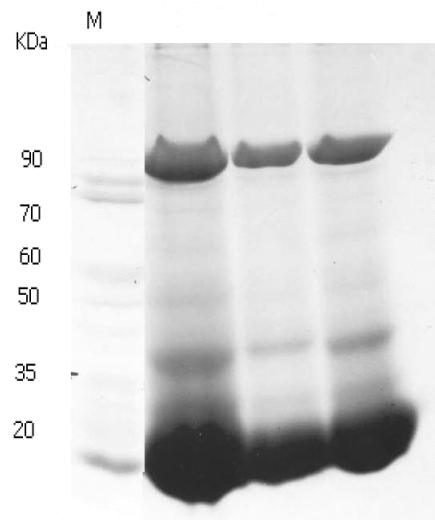


Fig. 1. SDS-PAGE profiles of preputial gland from male house rat Lane M indicate marker protein

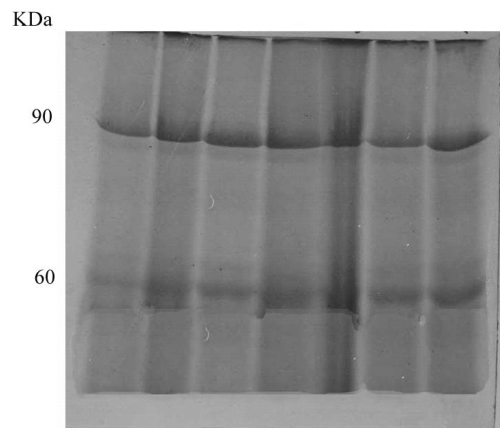


Fig. 2. SDS-PAGE profiles of preputial gland from castrated male house rat

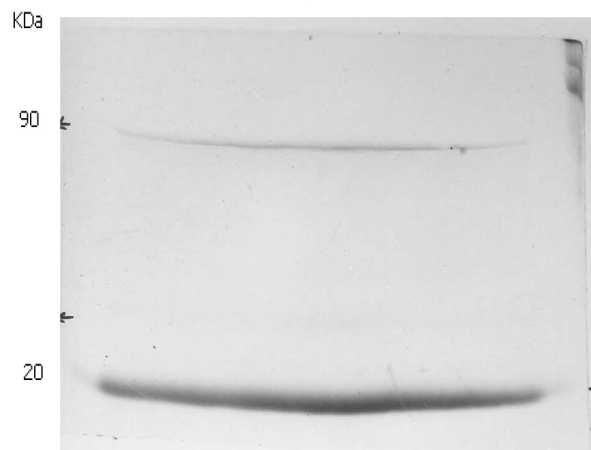


Fig. 3. SDS-PAGE profiles of preputial gland from castrated plus testosterone treated male house rats

The electrophorogram of the glandular proteins in female rat revealed only two different protein fractions (i.e.) 90 and 20 kDa. However, the 70, 60, 50 and 35 kDa proteins were absent in the female clitoral gland. The two different fractions, the 90 and 20 kDa proteins were prominent (Fig. 4).

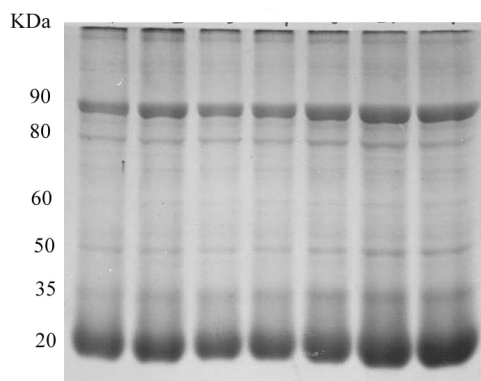


Fig. 4. SDS-PAGE profiles of clitoral gland from female house rat

Discussion

The present study revealed that the 20 kDa protein, observed to be present in the preputial gland was significantly decreased in intensity in castrated rats. Removal of testes depleted the testosterone level and caused significant changes in the protein profiles of the preputial glands. In fact, the intensity of the band is rather less when compared to that obtained in the normal male rat. Further on replacement of testosterone, the castrated rats showed higher intensity of 20 kDa protein thus suggesting that this protein is androgen dependent. The testosterone dependent sex-associated protein was clearly demonstrated in the urine of mice. Indeed, the testosterone induces the synthesis of Major Urinary Proteins (MUPs) in adult female mice (9). In addition, the estimation of protein in preputial glands showed a decrease in concentration in castrated rats compared to normal rats, while the testosterone treatment increased the protein concentration in the preputial gland. From these findings, it may be concluded that the normal synthesis and secretion of these proteins are androgen-dependent.

It has already been known for a long time that the rate of protein secretion in the submandibular gland of adult male hamsters is several times higher than in females and this difference in protein concentration becomes manifested at, or shortly after puberty (10). Similarly, in the present study the 70, 60, 35 kDa proteins were absent in the clitoral gland of house rat, while they were absent in male rats. It suggests that these proteins can be considered as sex-associated protein in house rat. Likewise, our previous work revealed that contrary to females male mammals excreted higher concentration of proteins suggesting the role of male hormones in the secretion of urinary proteins (2). Thus, the present finding is in agreement with the concept that

males depend on a higher concentration of proteins for the maintenance of reproductive and dominance status. The high levels of these proteins seen in the gland largely reflect a high rate of intracellular synthesis.

As the preputial gland plays a significant role in pheromonal activity the presently identified 20 kDa protein has close similarity with odour binding proteins. Alpha 2 μ -globulin, the principal urinary protein of the male rat, has extensive sequence of homology with many lipid binding proteins. The alpha 2 μ -globulin is found in the preputial gland, a holocrine secretory organ having pheromonal function (7). The present findings are also consistent with the earlier reports that carrier proteins having a molecular weight of 18–20-kDa were present in the urine of mouse (9), saliva of pig (6), vaginal fluid of hamster (29) and sweat of human (30). Interestingly, odour-binding proteins (OBPs) of vertebrates are involved in perception of odorants also having the same molecular weight around 20-kDa (25). Similarly, the protein identified in the present study may be the carrier of ligands and belonging to the lipocalin family. However, at present it is unable to predict the pheromonal effects of glandular proteins in rats.

Up to now most pheromonal characterization studies have been carried out through identifying the volatile fraction and less attention was paid carrier proteins. In our laboratory, the pheromonal compound(s) have been already characterized(s) in the preputial gland of house rats (16) and the identification of 20-kDa glandular protein in the present study is noteworthy. It is possible to further investigate the binding activity of these proteins with the identified pheromonal compounds (i.e. ligands) which provides possibility in developing pheromonal traps for rodent pest management.

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