論文の内容の要旨

応用動物科学専攻
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Possible mechanism for the detection of the male effect pheromone in female goats

(ヤギにおける雄効果フェロモンの受容機構に関する研究)

Most mammals have been considered to have two anatomically and physiologically distinct olfactory systems –the main olfactory system (MOS) and the vomeronasal system (VNS). The main olfactory epithelium (OE) of the MOS contains olfactory receptors (ORs) that detect volatile odorants from the environment and send the signals to the olfactory bulb (OB). In rodents, the MOE has a complex structure, which is organized into four topographically distinct zones defined by the expression of ORs. Unlike the OE, the vomeronasal organ (VNO) of the VNS possesses vomeronasal receptors for detecting pheromones, and then relays the information to the accessory olfactory bulb (AOB).

In goats and sheep, resumption of the pulsatile secretion of luteinizing hormone (LH) and ovarian cyclic activity were observed in seasonally anestrous females when exposed to the male. Previous studies implicated the male effect pheromone to be the ultimate factor governing this male effect phenomenon as similar response was observed in the females when only exposed to the male hair/fleece. The male effect pheromone stimulates the neural activity of the gonadotropin-releasing hormone (GnRH) pulse generator accompanied by increased GnRH/LH pulses in females. In goats, the male effect pheromone can be detected as multiple unit activity (MUA) volley, which refers to the neural activity of the GnRH pulse generator. Although the VNS has been conventionally considered to detect pheromones, several reports have shown that the MOS can detect some pheromones in some mammals. Hence, there is a possibility that MOS can detect the male effect pheromone in goats. The present study aimed to investigate the possible involvement of the MOS in the detection of the male effect pheromone in female goats.

In the first study, the morphological structure and zonal organization of the goat OE were examined. The morphological observation revealed that the goat OE has a complex structure comprised of well-developed endoturbinates and ectoturbinates. Specifically, the goat OE has four well-defined endoturbinates (I, II, III and IV) adjacent to the nasal septum and several ectoturbinates lying within the lateral wall of the nasal cavity. The morphological structure of the goat OE is quite similar to that in rodents and sheep as previously reported. To investigate the zonal organization of the goat OE, expression patterns of representative ORs, NADPH: quinone oxidoreductase-1 and olfactory cell adhesion molecule (OCAM) as zonal markers were analyzed by *in situ* hybridization. The spatial distribution pattern of ORs had a topographical preference along the dorsal-ventral and medial-lateral axis and the goat OE appears to be organized into four distinct zones (designated as zone 1-zone 4, from the most dorsomedial extent to the extreme ventrolateral extent). *NQO1* and *OCAM* were both expressed by the OSNs in the OE in a non-overlapping manner. The *NQO1*-positive region appears to correspond to zone 1 while the *OCAM*-positive area covers the expression zones 2-4. The findings in this study suggest that the goat OE has a complex morphological structure that is organized into multiple zones based on the expression of ORs. The well-developed morphological structure and the four-zonal organization in the goat OE were quite similar with those in rodents, implying that these features of OE are highly conserved between mammals, which might provide a fundamental function to discriminate numerous odor molecules in the environment.

The second study aimed to investigate the existence of VNS-related genes in the goat MOS. The expression and spatial distribution patterns of the vomeronasal type-1 receptors (*V1Rs*) and *Gαi2*, a V1R-associated G protein were examined in the goat OE by *in situ* hybridization and immunohistochemistry. Gαi2 immunohistochemistry revealed that a small subset of OSNs express Gαi2 protein and immunoreactivity was localized on the apical surface of the epithelial lining. The Gαi2 protein may be localized on the cilia which is the binding site of odorant molecules and where the ORs are expressed. This result implies that Gαi2 plays a role in signal transduction in the goat OE. Specifically, Gαi2 proteins were observed in all 4 endoturbinates as well as most ectoturbinates of the goat OE but relatively few Gαi2 proteins were observed in endoturbinate I compared to endoturbinates II-IV. These Gαi2 proteins are preferentially distributed in the ventrolateral region of the goat OE. To confirm this distribution pattern of Gαi2 protein, the goat

OE was subjected to *in situ* hybridization for *Gαi2*. The *Gαi2* was expressed in a small subset of OSNs. The *Gαi2*-expressing OSNs appear to be restricted in the ventrolateral region of the OE similar to the spatial distribution pattern of Gαi2 protein. The *Gαi2*-expressing region in the goat OE appears to be similar to the *OCAM*-positive area described in the first study. This finding indicates that Gαi2 may be used for signal transduction by a small subset of OSNs located in the ventrolateral area of the goat OE. *In situ* hybridization for *V1Rs* revealed that *V1Rs* are expressed in a small subset of OSNs in the OE. *V1R*-expressing OSNs were randomly distributed in the OE. Double-label *in situ* hybridization revealed that some of the *V1Rs* co-expressed *Gαi2*. These findings suggest that a *V1R-Gαi2* signal transduction mechanism is employed by a small subset of OSNs in the OE specifically located in the ventrolateral area. Gαi2 immunohistochemistry in the goat OB revealed Gαi2-positive axon terminals situated in the rostral, middle and caudal areas. Moreover, whole mount staining of the OB for dolichos biflorus agglutinin, a lectin that is colocalized with G*α*i2 in the goat OB, showed positively-stained fibers adjacent to the AOB extending to the middle and caudal areas of the OB. These results indicate that the *Gαi2*-expressing OSNs may functionally project their axons to the OB. Taken together, these findings suggest that the goat MOS is equipped with a V1R-Gαi2 signal transduction mechanism and can possibly detect pheromonal cues.

In the third study, the possible involvement of the MOS in activating the neural activity of the GnRH pulse generator in response to the male effect pheromone was examined in female goats. Ovariectomized goats implanted with electrodes in the hypothalamic arcuate nucleus to record the activity of the GnRH pulse generator as MUA were used. Characteristic increases in the MUA are associated with LH pulses and is referred to as the MUA volley. The goats were exposed to hair samples obtained from intact male goat (pheromone source) at ½T (half of the average basal inter-volley interval). One-second exposure to the male hair immediately evoked an MUA volley in all experimental animals at ½T. This result indicates that the male goat hair effectively stimulates the GnRH pulse generator in female goats. To investigate the involvement of the MOS in the male-hair induced activation of the MUA; the MUA was monitored in female goats upon exposure to male hair after VNO occlusion. The VNO occlusion did not prevent the male-hair induced activation of the MUA in female goats. The findings in this study suggest that the goat MOS can detect the male effect pheromone.

Rats, mice, dogs and sheep have a well-developed sense of smell. These species possess a nasal cavity with a complex structure. The present dissertation shows that the goat also possesses an OE with a complex structure and multiple zonal organization suggesting that the goat has a keen sense of smell and that olfaction serves a crucial function in this species. Moreover, the MOS of goats possesses a signal transduction mechanism similar to that of the VNS suggesting that the goat MOS can also process pheromonal cues. Finally, this dissertation demonstrated that the functional blockade of the vomeronasal pathway did not prevent the male effect pheromone-stimulated activation of the GnRH pulse generator suggesting that the MOS might participate in the detection of the male effect pheromone in female goats.

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