J. Biol. Macromol., **3**(3) 83-88 (2003)

Review

Chemical communication via high molecular weight pheromones in mammals

Makoto Kashiwayanagi
Graduate School of Pharmaceutical Sciences Hokkaido University
Sapporo 060-0812, Japan

Introduction

Pheromonal signals provide specific information concerning the identity, gender, endocrine, and social status of different members of the population in a variety of mammals [1;2]. Pheromones have been found in saliva, skin gland secretions, and urine. For example, pheromones in urine excreted from male and female rats induce various changes in gonadal functions such as reflex ovulation in the absence of coitus and mounting, a reduction in the oestrous cycle of female rats from 5 to 4 days, and oestrous synchrony among female rats living together.

The vomeronasal system is a chemosensory system for receiving pheromones organized in parallel with the main olfactory system in most terrestrial

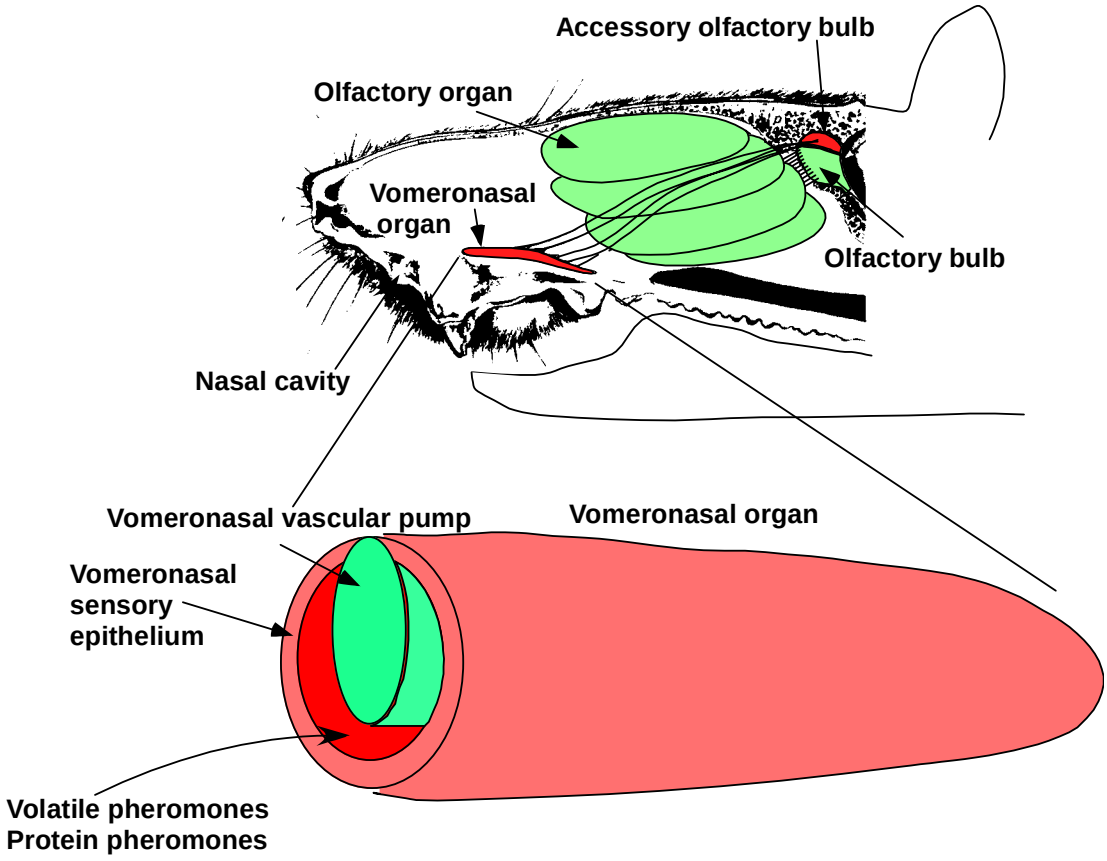

Fig. 1 The vomeronasal organ in the rat

vertebrates (Fig. 1). The vomeronasal organ, which is the peripheral chemoreceptor organ of the vomeronasal system, forms a tubular structure lying bilaterally in the ventral part of the nasal cavity. An autonomically controlled vascular pump can transport nonvolatile and volatile pheromones into the inner lumen of the vomeronasal organ [3]. Pump activation is a prerequisite for normal vomeronasal stimulation in behaving animals. Sensory neurons in the vomeronasal sensory epithelium project information to the accessory olfactory bulb located on the dorso-caudal surface of the main olfactory bulb.

Pheromonal transduction mediated via IP_3 in the mammalian vomeronasal system

The interaction of a pheromone with the receptive membrane of vomeronasal sensory neurons initiates a sequential molecular event leading to action potential initiation, which transmits pheromonal information to the accessory olfactory bulb. Dialysis of inositol-1,4,5-trisphosphate (IP_3) into rat vomeronasal sensory neurons induced inward currents [4]. Increases in the impulse frequency of sensory neurons in response to urinary pheromones were blocked by ruthenium red, an IP_3-dependent channel inhibitor, and U-73122 or neomycin, phospholipase C inhibitors [5]. Male Wistar urinary pheromones as well as female Wistar and male Donryu urinary pheromones induced IP_3 accumulation in the vomeronasal epithelium of the female Wistar rat [6].

IP_3 accumulation in response to pheromones in mammals has also been observed in the hamster and the pig. That is, aphrodisin, a pheromone of 19 kDa protein, excreted from the female hamster, and semen fluid from the pig induce IP_3 accumulation in preparations of male hamster vomeronasal sensory epithelium [7] and porcine vomeronasal epithelium, respectively. The average reversal potential of urinary pheromones- induced current was similar to that of the IP_3-induced current in rat vomeronasal sensory neurons [4;8]. These results indicate that the response of vomeronasal sensory neurons to pheromones is generated via the IP_3-dependent pathway.

Selective pheromone reception in vomeronasal sensory neurons

Single vomeronasal sensory neurons of female Wistar rats responded selectively to urinary pheromones from male and female Wistar rats [9]. Each sensory neuron responded to only one class of urinary pheromones. The neurons also responded selectively to urinary pheromones from male Donryu and Sprague-Dawley rats. Thus, vomeronasal sensory neurons discriminate differences in sex and strains in urinary pheromones.

Cell bodies of vomeronasal sensory neurons are located at various depths in the

cellular layer of sensory epithelium. As shown in Fig. 2, the sensory neurons at the apical and basal layers of the sensory epithelium of marsupial and rodents are immunoreactive to anti-$G_{i2\alpha}$ and anti-$G_{o\alpha}$ proteins, respectively [10]. Localization of the cell bodies of sensory neurons, which responded to male Wistar and male Donryu urinary pheromones, was examined using female Wistar rats [9]. The preponderance of neurons responding to conspecific male urinary pheromones from rats of the same species were found in the $G_{i\alpha}$-positive laminae of the vomeronasal sensory epithelium, and the preponderance of neurons responding to conspecific female rat urinary pheromones and male urinary pheromones from a different strain (Donryu or Sprague-Dawley) were found in the G_o-positive laminae of the vomeronasal sensory epithelium (Fig. 2). Therefore, it is likely that responses of sensory neurons in the apical portion of the female rat vomeronasal epithelium to the male Wistar urinary pheromones were mediated via G_i, while responses of neurons in the basal portion to the male Donryu urinary pheromones were mediated via G_o.

Putative receptor for pheromones

Two families of vomeronasal G-protein coupled receptors (GPCRs) unrelated to olfactory GPCRs were cloned from the rat and mouse vomeronasal epithelium [11]. The human genome contains a vomeronasal GPCR gene. Each family is composed of about one hundred species of vomeronasal GPCR. *In situ* hybridization studies suggested that a single sensory neuron expressed one type of vomeronasal GPCR. One family consists of the sensory neurons expressing $G_{i2\alpha}$ in the upper layer of the epithelium, and another family consists of the neurons expressing $G_{o\alpha}$. It is possible that responses to the male Wistar urinary pheromones are induced via the former type of vomeronasal GPCR, and those to the female Wistar urinary pheromones and the male Donryu urinary pheromones are induced via the latter type of vomeronasal GPCR.

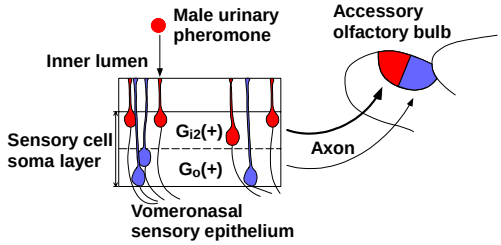

Fig. 2 Selective transmission of pheromonal information

Projection of pheromonal information to the accessory olfactory bulb

Two populations of sensory neurons in the vomeronasal organ project information to different regions of the glomerular layer of the accessory olfactory bulb [10]. These subregions receive different pheromonal information from vomeronasal sensory

neurons [12]. Thus, the response to pheromones in the male Wistar rat urinary pheromones is selectively transmitted to the rostral part of the accessory olfactory bulb of female Wistar rats. It is likely that information from different pheromones is transmitted to the higher brain through the different regions of the accessory olfactory bulb.

Chemical characterization of pheromones

Pheromones have been found to be proteins and low molecular weight molecules [13-17]. At present, pheromones that induce behavioral and endocrinological changes in rats have not been identified. We have characterized active components stimulating the rat vomeronasal organ in urine by examining excitation of neurons in the accessory olfactory bulb. The activity of the component in male urinary pheromones to induce expression of Fos-immunoreactivity, which is correlated with neural activity, in the caudal region of the accessory olfactory bulb of female rats was abolished by papain treatment, while that in the rostral region was not [18]. The pronase treatment of male urine abolished the expression of immunoreactivity in the rostral region as well as in the caudal region, suggesting that at least two urinary peptides (papain-sensitive and -insensitive ones) with the ability to stimulate the vomeronasal organ of female rats are contained in male Wistar rat urine. Exposure of the female rat vomeronasal organ to either the dialyzed urine preparation (< 500 Da) or the remaining substances (> 500 Da) of male rats did not induce expression of Fos-immunoreactive cells in the accessory olfactory bulb, whereas exposure to a mixture of these preparations did induce expression [19]. This suggests that a combination of low and high molecular weight substances is necessary for the increases in Fos-immunoreactivity in the accessory olfactory bulb.

Similar results have been obtained in the mouse and hamster. Thus, the application of urine-derived compounds of low molecular weight such as 2,3-dehydro-*exo*-brevicomin induces only hyperpolarizing responses, that is, inhibitory responses, in the mouse vomeronasal sensory neuron and does not induce c-fos mRNA expression in the mouse accessory olfactory bulb. However, 2,3-dehydro-*exo*-brevicomin in combination with major urinary proteins (~19 kDa) induced significantly greater c-fos mRNA expression. The female golden hamster produces a 17-kDa protein (aphrodisin) that is emitted in vaginal discharge and stimulates sexual behavior in the male hamster. Gel filtration experiments indicated that a mixture of ligands may be present in the purified aphrodisin. Analysis of the crystal structure of aphrodisin revealed the electron density for a small linear ligand in the cavity of the

β-barrel [20]. At present, it is still not clear whether aphrodisin itself or its combination with a low molecular weight ligand is necessary for pheromonal activity.

Decreases in urinary pheromonal activities in male mice after exposure to environmental pollutant

Many classes of environmental pollutants, which are found at significant levels in the environment, affect the reproductive functions and endocrine systems. Exposure of female rats to 3-methylchoranthrene (3-MC) or 4-tert-octylphenol, typical environmental pollutants, has been found to be associated with a significant increase in estrus cycle length. The gonadal functions of various animals are regulated by pheromones excreted from mating partners as described above. Some volatile and nonvolatile pheromones exhibit a strong dependence on the endocrine status of the animals, suggesting that the production of pheromones is controlled by hormones. We have shown that 3-MC interferes with the activity of male urine to stimulate female vomeronasal sensory neurons 1 day after intraperitoneal administration of 3-MC (unpublished data).

Intraperitoneal administration of 3-MC also decreased the levels of mRNA encoding major urinary proteins (MUPs), which are thought to be candidate pheromones themselves or possible carrier proteins of pheromones in urine (Nukazuka et al., submitted for publication). SDS analyses showed that the protein levels in the urine from males 1 day after administration of 3-MC were similar to those from non administered mice, while the levels of a protein with an apparent molecular mass of 19 kDa in urine from 3 and 10 days after the administration were lower than those in the control mice (unpublished data). These results suggest that 3-MC blocks chemical communication between male and female mice by reducing pheromonal activities without changing the total amount of MUPs in male mice 1 day after administration.

Reference List

1) Halpern M. The organization and function of the vomeronasal system. Annu. Rev. Neurosci. (1987) **10**: 325-362.

2) Kashiwayanagi M. Molecular recognition and intracellular transduction mechanisms in olfactory and vomeronasal systems, *In* Hormones, Brain and Behavior, Vol. 2, Academic Press, New York (D. Pfaff, ed), (2002) pp. 1-16.

3) Meredith M, Marques DM, O'Connell RJ, Stern EL. Vomeronasal pump: Significance for male hamster sexual behavior. Science (1980) **207**: 1224-1226.

4) Inamura K, Kashiwayanagi M, Kurihara K. Inositol-1,4,5-trisphosphate induces responses in receptor neurons in rat vomeronasal sensory slices. Chem. Senses (1997) **22**: 93-103.

5) Inamura K, Kashiwayanagi M, Kurihara K. Blockage of urinary responses by inhibitors for IP_3-mediated pathway in rat vomeronasal

sensory neurons. Neurosci. Lett. (1997) **233**: 129-132.

6) Sasaki K, Okamoto K, Inamura K, Tokumitsu Y, Kashiwayanagi M. Inositol-1,4,5-trisphosphate accumulation induced by urinary pheromones in female rat vomeronasal epithelium. Brain Res. (1999) **823**: 161-168.

7) Kroner C, Breer H, Singer AG, O'Connell RJ. Pheromone-induced second messenger signaling in the hamster vomeronasal organ. Neuroreport (1996) **7**: 2989-2992.

8) Inamura K, Kashiwayanagi M. Inward current responses to urinary substances in rat vomeronasal sensory neurons. Eur. J. Neurosci. (2000) **12**: 3529-3536.

9) Inamura K, Matsumoto Y, Kashiwayanagi M, Kurihara K. Laminar distribution of pheromone-receptive neurons in rat vomeronasal epithelium. J. Physiol. Lond. (1999) **517**: 731-739.

10) Halpern M, Shapiro LS, Jia C. Differential localization of G proteins in the opossum vomeronasal system. Brain Res. (1995) **677**: 157-161.

11) Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell (1995) **83**: 195-206.

12) Inamura K, Kashiwayanagi M, Kurihara K. Regionalization of Fos immunostaining in rat accessory olfactory bulb when the vomeronasal organ was exposed to urine. Eur. J. Neurosci. (1999) **11**: 2254-2260.

13) Brownlee RG, Silverstein RM, Muller-Schwarze D, Singer AG. Isolation, identification and function of the chief component of the male tarsalscent in black-tailed deer. Nature (1969) **221**: 284-285.

14) Melrose DR, Reed HC, Patterson RL. Androgen steroids associated with boar odour as an aid to the detection of oestrus in pig artificial insemination. Br. Vet. J. (1971) **127**: 497-502.

15) Goodwin M, Gooding KM, Regnier F. Sex pheromone in the dog. Science (1979) **203**: 559-561.

16) Novotny M, Schwende FJ, Weisler D, Jorgenson JW, Carmack M. Identification of a testosterone-dependent uniue volatile constituent of male mouse urine: 7-*exo*-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-3-octene. Experientia (1984) **40**: 217-219.

17) Singer AG, Macrides F, Clancy AN, Agosta WC. Purification and analysis of a proteinaceous aphrodisiac pheromone from hamster vaginal discharge. J. Biol. Chem. (1986) **261**: 13323-13326.

18) Tsujikawa K, Kashiwayanagi M. Protease-Sensitive Urinary Pheromones Induce Region-Specific Fos- Expression in Rat Accessory Olfactory Bulb. Biochem. Biophys. Res. Commun. (1999) **260**: 222-224.

19) Yamaguchi T, Inamura K, Kashiwayanagi M. Increases in Fos-immunoreactivity after exposure to a combination of two male urinary components in the accessory olfactory bulb of the female rat. Brain Res. (2000) **876**: 211-214.

20) Vincent F, Lobel D, Brown K, Spinelli S, Grote P, Breer H, Cambillau C, Tegoni M. Crystal Structure of Aphrodisin, a Sex Pheromone from Female Hamster. J. Mol. Biol. (2001) **305**: 459-469.

Communicated by Hiroshi Ueno