

to sucrose. In contrast, the majority of the long type sensilla in *P. polytes* did not respond to the extracts of *Citrus* spp, but responded to an alkaloid, brucine.

INVOLVEMENT OF GLUTATHIONE S-TRANSFERASES IN GUSTATORY RECEPTION OF RAT TASTE BUDS

Tomoko Nishino¹, Hideaki Kudo¹, Takahisa Nagata³, Yoshiaki Doi¹, Sunao Fujimoto²

¹Department of Anatomy, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan, ²Health and Nutritional Sciences, Nakamura Gakuen University Graduate School, Fukuoka 814-0198, Japan and ³Department of Surgery, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan

Glutathione S-transferases (GSTs) are the xenobiotic metabolizing enzymes that catalyze conjugation between various ligands and glutathione in several tissues. Recent studies have reported high expression of GSTs in several sensory organs in the vertebrates. However, the presence of GSTs in mammalian taste bud (TB) cells, generally classified into four types (I-IV), remains to be fully investigated. We investigated the expressions of GST isoforms (GSTalpha, GSTmu and GSTpi) in TBs of normal and organic solvents-exposed rats by both Western blotting and immunocytochemistry. In Western blotting, immunoreactivities (IRs) for GSTmu as well as for GSTpi were detected in soluble extracts from rat TBs. However, IR for GSTalpha was not detected. By immunocytochemistry, GSTmu- and GSTpi-IRs were preferentially seen in type II cells and a part of type III cells of TBs in both rats, and significantly increased in these cells in organic solvents-exposed rats. Since IRs for GSTs were detected in the rat TB cells and increased in organic solvents-exposed condition by the present study, we consider now that these GSTs may be involved in the gustatory reception by the xenobiotic metabolism.

DIETARY EXPERIENCE WITH SMELL ALTERS THE FEEDING SENSITIVITY AND BIOGENIC AMINES IN THE BLOWFLY *PHOMIA REGINA*

Tomoyosi Nisimura¹, Kyoko Nakamura¹, Atsushi Seto¹, Takashi Nagao², Satoshi Tamotsu³, Ryohei Yamaoka¹, Mamiko Ozaki¹

¹Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto 606-8585, Japan, ²Human Information Systems, Kanazawa Institute of Technology, Ishikawa 924-0838, Japan and ³Department of Biological Science, Nara Women's University, Nara 630-8506, Japan

Experience of some odors with taste of food affects the feeding preference in the blowfly *Phomia regina*. When the flies fed on sucrose solution with the smell of the limonene, the feeding sensitivity reduced even without the smell. To investigate the changes of the central nerves system in the flies showing the feeding sensitivity reduction, we quantified the biogenic amines in the brain and suboesophageal ganglion. Of all measured biogenic amines, the amount of octopamine and tyramine significantly decreased in such flies. Then, we tested whether injection of two amines recover the feeding sensitivity or not. After injection of octopamine, the feeding sensitivity was not recovered, although injection of tyramine recovered the feeding sensitivity into the normal level. Using immunohistochemical methods, the tyramine positive cells were found in the brain and suboesophageal ganglion. They were classified into seven groups. It is probable that some of them are concerned with the feeding sensitivity regulation in the fly.

SPATIAL COHERENT OSCILLATIONS IN THE SENSORY EPITHELIUM OF THE TERRESTRIAL SLUG *LIMAX VALENTIANUS*

Iori Ito, Satoshi Watanabe, Yutaka Kirino

Laboratory of Neurobiophysics, Department of Bio-molecular Functions, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

Tentacles are olfactory sense organs of slug. The tentacular ganglion and its digitate-like extensions (digits) to the sensory epithelium in each tentacle are synaptic target regions of the olfactory receptor neurons, and their field potentials spontaneously oscillate at 1-2 Hz. In the present study, to examine the relationship between the spontaneous oscillations and the receptor neurons we extracellularly recorded from the two spatially separated sites on the olfactory epithelium in the terrestrial slug *Limax valentianus*. We found that the sensory epithelium displayed spatially coherent oscillations at the same frequency to the tentacular ganglion. The surgical ablations of the digits and the tentacular ganglion from the sensory epithelium abolished the oscillations in the epithelium, indicating that the receptor neurons could not produce the spontaneous oscillations per se. Nearly 27% of the synapses between the receptor neurons and the secondary neurons are symmetrical synapses in *Achatina fulica*, suggesting that the coherent oscillations in the tentacular ganglion is transmitted to the receptor neurons via symmetrical synapses in *Limax valentianus*.

ANNUAL CHANGES IN THE MORPHOLOGY OF OLFACTORY EPITHELIUM IN THE JAPANESE TOAD (*BUFO JAPONICUS*): A STUDY WITH ANTISERUM TO OLFACTORY MARKER PROTEIN

Hideo Nakazawa¹, Masumi Ichikawa², Takatoshi Nagai¹

¹Department of Biology, School of Medicine, Keio University, Kouhoku-ku, Yokohama 223-8521, Japan and ²Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183-8526, Japan

During the breeding season in toads (*Bufo japonicus*) the electro-olfactogram (EOG) recorded from the olfactory epithelium accompanies marked oscillatory potentials. We collected the toads in the field throughout a year and examined morphology of the olfactory epithelium of toads in relation to oscillatory potentials in the EOG. Olfactory receptor neurons in the epithelium were visualized by using rabbit polyclonal antiserum to olfactory marker protein. Cross sections (10 µm) at the central portion of the ventral epithelium were cut on a cryostat. Immunolabeling was visualized by the secondary antibody-Alexa Fluor 488 conjugate and examined with a confocal laser microscope. The olfactory neurons increased by 8% from January to February and rapidly decreased by 28% from the breeding season (February) to the early foraging period (April). The number of the neurons gradually declined throughout the foraging period (April - October). We previously showed that the oscillatory potentials were not accompanied by the EOG in the foraging period. Cell dynamics of the olfactory receptor neurons may relate to induction of the oscillatory potentials in the breeding season.

AMINO ACID RESPONSES IN AMPHIBIAN OLFACTORY RECEPTOR NEURONS

Ritsuko Inoue¹, Hiroshi Yamada², Kei Nakatani³

¹Division of Functional Biosciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, ²Yamanouchi Pharmaceutical Co., Tsukuba, Ibaraki 305-8585, Japan and ³Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

It is known that vertebrate olfactory receptor neurons respond to volatile odorants and the signal transduction mechanism is mediated by cAMP system. Recent studies have shown that amphibians can smell not only airborne odorants but also amino acids. In this study, we have recorded electrical responses of newt and bullfrog olfactory receptor neurons to amino acids using whole-cell patch clamp technique. Amino acids elicited excitatory responses in dose-dependent manner, and patterns of the responses appeared to be cell- and odorant-specific. Moreover, responses to both amino acids and forskolin, a stimulator of adenylate cyclase, were observed in the same cell. Our data suggest that the cyclic AMP system might underlie the signal transduction pathway of amino acid responses in amphibian olfactory receptor neurons.

THE LOCALIZATION OF G-PROTEIN IMMUNOREACTIVITIES IN THE OLFACTORY EPITHELIUM OF AMPHIBIANS

Keiko Okano¹, Masumi Ichikawa², Tadashi Nakamura¹

¹Department of Applied Physics and Chemistry, The University of Electro-Communications, Chofu 182-8585 and ²Department of Developmental Morphology, Tokyo Metropolitan Institute for Neuroscience, Fuchu 183-8526

To investigate molecules involved in signal transduction pathways in the olfactory neurons of lower vertebrates, we performed immunocytochemical analysis of the olfactory epithelium of amphibians, by using an antibody against α -subunit of rat olfactory-specific G-protein (G_{olf}) and antiserum against rat olfactory marker protein (OMP). Because G_{olf} is expressed at high levels in olfactory cilia of rodent, it is most likely that G_{olf} couples with odorant receptors to increase intracellular cAMP levels by stimulating adenylyl cyclase. In many vertebrates, OMP is expressed almost exclusively in mature olfactory neurons. In the olfactory epithelium of newt, anti- G_{olf} strongly immunostained most cilia layer of sensory epithelium. Similar results were obtained in the olfactory sensory epithelium of bullfrog. The staining patterns in the two animals were similar to that reported for the mammalian olfactory sensory epithelium. On the other hand, no OMP immunoreactivities were observed in the olfactory sensory neurons of newt or bullfrog, possibly due to divergence in amino acid sequences of OMPs between mammals and lower vertebrates.

EXPRESSIONS OF MULTIDRUG RESISTANCE-RELATED PROTEIN 1 IN THE RAT OLFACTORY EPITHELIUM

Hideaki Kudo¹, Tomoko Nishino¹, Hiroshi Furukawa², Kunishige Hamasaki², Yoshiaki Doi¹, Sunao Fujimoto³

¹Department of Anatomy, School of Medicine, University of Occupational and Environmental Health, Japan (UOE), Kitakyushu 807-8555, Japan, ²Department of Clinical Pathology, School of Health Sciences, University of Occupational and Environmental Health, Japan (UOE), Kitakyushu 807-8555, Japan and ³Health and Nutritional Sciences, Nakamura Gakuen University Graduate School, Fukuoka 814-0198, Japan

The xenobiotic metabolizing system is considered to play important roles in olfaction by chemical homeostasis. Several phase I and phase II xenobiotic metabolizing enzymes were expressed in the olfactory epithelium. Multidrug resistance-related proteins (MRPs) are the phase III xenobiotic metabolizing pump that extrude some conjugated ligands from cells. However, MRP expressions in the olfactory epithelium have not been confirmed in the mammals. We first detected MRP type 1 isoform (MRP1) mRNA in the adult rat olfactory epithelium by reverse transcriptase polymerase chain reaction (RT-PCR). The nucleoside sequence of the RT-PCR product was completely identical to that found in other organs. By immunohistochemistry using specific antibody to MRP1, MRP1-immunoreactivities were seen on the patch-like structures around the olfactory epithelial cells. By in situ hybridization using digoxigenin-labeled MRP1 cRNA probe, signals for MRP1 mRNA were observed mainly in the perinuclear regions of the olfactory receptor cells. These findings indicate that MRP1 is related to the olfaction as a part of the perireceptor events (i.e. chemical homeostasis) in the olfactory epithelium.