Chem. Senses 25: 207–237, 2000

ABSTRACTS

The 33rd Annual Meeting of the Japanese Association for the Study of Taste and Smell (JASTS XXXIII)

Held in Tokyo, Japan, October 6–8, 1999

T1. Structures and functions of a sweet protein and taste-modifying proteins

Satoru Nirasawa

Applied Entomology Laboratory, National Food Research Institute, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

In general, proteins cannot stimulate taste receptors because of steric hindrance, but exceptionally a few proteins, such as monellin, thaumatin, mabinlin, brazzein, curculin and miraculin, elicit a sweet taste. In this study, four homologues of mabinlin were isolated. In spite of a high similarity among amino acid sequences of the homologues, their heat stabilities were different; one type of the homologues was stable after a 1 h incubation at 80°C and other type was unstable. Comparison of the amino acid sequences of heat-stable homologues with that of an unstable one led to the conclusion that the difference in heat stability is due to replacement of a single amino acid residue. Moreover, cDNAs for mabinlin and miraculin were cloned and sequenced. Expressions of mabinlin, miraculin and curculin genes were performed in various hosts such as *Escherichia coli*, yeast and tobacco.

Enzymatic hydrolysates of various proteins frequently exhibit a bitter taste caused by bitter peptides. This limits their utilization in the food industry. The bitterness of protease hydrolysate solution was significantly reduced by treatment with an aminopeptidase produced by *Aeromonas caviae* T-64. In this study, aminopeptidase-precursor gene was expressed in *E. coli* and expressed protein was activated by a processing enzyme which was isolated from *A. caviae*.

T2. Mechanisms of pheromone perception and discrimination in the mammalian vomeronasal system

Kouhei Inamura

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

We examined the signal transduction mechanism in vomeronasal sensory neurons and the transmission pathway for information of pheromonal substances to accessory olfactory bulb. Using the patch-clamp technique, voltage-activated cationic currents and IP_3-activated currents in sensory neurons in vomeronasal epithelium of female adult rats were identified. Urine preparations involving pheromonal substances induced an excitatory response in vomeronasal sensory neurons. Single vomeronasal sensory neurons specifically and selectively responded to urinary substances from a conspecific and a different strain of rat. Pharmacological experiments on urinary response suggested that the response to urinary pheromones is mediated via the IP_3-dependent pathway. The sensory neurons located in the apical layer (G_{12}-positive) of female rat epithelium preferentially responded to male urine. In addition, following exposure to male rat urine, an increment of Fos expression in the rostral portion (G_{12}-positive) of the accessory olfactory bulb was observed in the mitral/tufted cell layer of the female rat. It is likely that information from male pheromones perceived by the vomeronasal organ is transmitted to higher brain centers through the specific regions of the accessory olfactory bulb.

K1. Gustatory reception of sweet stimuli on the rat soft palate

Shuitsu Harada, Keiko Yamaguchi and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

Gurmarin (10 μg/ml), a protein extracted from *Gymnema sylvestre*, depressed significantly (40–50%) the phasic taste responses to 0.5 M sugars (sucrose, fructose, lactose and maltose) and 0.01 M saccharin-Na recorded from the greater superficial petrosal nerve innervating palatal taste buds in the rat. However, no significant effect of gurmarin was observed for taste responses to 0.1 M NaCl, 0.01 M HCl and 0.01 M quinine–HCl. Phasic responses to 0.1 M D-amino acids that taste sweet to humans (His, Asn, Phe and Gln) were also significantly depressed, but gurmarin treatment was without significant effect on taste responses to D-Trp and D-Ala, six L-amino acids (His, Asn, Phe, Gin, Trp and Ala) and two basic L-amino acid–HCl salts (Arg and Lys) at 0.1 M. With the exception of D-Trp, the selective inhibitory effects of gurmarin on taste responses to D-amino acids and carbohydrates that taste sweet to humans suggest that both rats and humans perceive these substances similarly.

S1.1. Voltage-gated channels and taste responses of mouse taste bud cells in peeled tongue epithelia

Kiyonori Yoshii

Kyushu Institute of Technology, Iizuka 820-8502, Japan

Mouse taste bud cells (TBCs) in fungiform papillae have functional voltage-gated channels. I investigated their distributions in single cells and in single taste buds, and investigated their roles in taste response under in-situ patch clamp conditions. Also, taste responses were investigated with a voltage-sensitive dye, tetra-

methylrhodamine methyl ester (TMRM). The addition of TTX on the basolateral membranes of TBCs blocked Na currents but that on the receptor membranes had no effect, indicating that almost all functional Na channels are expressed on the basolateral membranes. Similar experiments revealed that TEA-sensitive K channels and HVA- and LVA-Ca channels also expressed on basolateral membranes functionally. These results indicate that taste substances never reach these voltage-gated channels freely. TBCs located in the middle part of each taste bud expressed a larger number of these voltage-gated channels than those in the central and peripheral parts. In response to taste substances, some TBCs elicited action potentials, but others stopped eliciting spontaneous discharges. Optical recordings with TMRM showed that taste substances depolarized 25% of TBCs and hyperpolarized 25% of them in the same taste buds. These excitable and inhibitory TBCs may form a network to modulate the synaptic outputs of taste receptor cells to taste nerves.

S1.2. Gustatory neural coding channels: selective synapse formation between corresponding types of taste cells and axons

Yuzo Ninomiya

Department of Oral Physiology, Asahi University School of Dentistry, Hozumi, Motosu, Uifu 501-0296, Japan. e-mail: bye01407@nifty.ne.jp

Taste cells are replaced with an averagc life span of ~10 days in mammals, accompanied by continuing sysnaptic reconnection between newly formed taste cells and gustatory fibers. Little is known about how a stable sensory code for taste quality is maintained under such continual synaptic reconnection. We addressed this issue, first, by using mice operated by cross union anastomoses between chorda tympani (CT) and glossopharyngeal nerves, and studied groupings of regenerated fibers into different tongue regions by examining amiloride inhibition of NaCl responses of the fibers. The results showed that relative abundance of amiloride-sensitive and -insensitive types of fibers was not altered by cross-regeneration of the two gustatory nerves originally possessing different amiloride sensitivities, suggesting that regenerated taste axons selectively recouple with the appropriate type of receptor cells. Next, we studied the issue by using congenic mice whose genetic background was identical to that of BALB strain, lacking gurmarin(G) sensitivity, except for a gene segment containing the locus that controls gurmarin sensitivity that is derived from C57BL mice. The results demonstrated that in congenic mice sucrose-responsive CT fibers were classified into two types: about one-half is G-sensitive and the remaining half is G-insensitive, whereas those in BALB mice were almost exclusively the latter type. This suggests that G-sensitivity was genetically induced in about half of susrose-responsive taste cells and these cells were selectively innervated by a particular type of CT fiber. Both approaches using mice whose innervation or receptor expression was experimentally manipulated demonstrated the existence of selective synapse formation between corresponding classes of taste cells and axons. Such selective synapse formation may help explain the stability of response profiles of taste neurons during continual receptor cell turnover.

S1.3. The cortical representation of taste and flavour in macaques and in humans

Edmund T. Rolls

Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK

In primates the secondary and tertiary olfactory cortices are in the orbitofrontal cortex. Neuronal recordings show that the five prototypical tastes sweet, salt, bitter, sour, and umami are represented here; that the pleasantness or reward value of taste and odour is represented as shown by satiety experiments; and that a representation of the flavour of food is formed, and that this is built for at least 35% of neurons by learned association or odour with taste. Oral somatosensory inputs also provide for a representation of fat in the mouth.

In investigations of whether there are similar areas in humans, we present fMRI results showing a gustatory representation in the medial orbitofrontal cortex distinct from the olfactory representation in the right orbitofrontal cortex, and a separate representation of affectively positive somatosensory stimuli in a different region of the human orbitofrontal cortex (S. Francis *et al.*, 1999, NeuroReport, 10: 453–459). Both pleasant (glucose) and unpleasant (NaCl) tastes are represented in both the orbitofrontal cortex and the amygdala. We also show that olfactory sensory-specific satiety is represented in the human orbitofrontal cortex. The primate orbitofrontal cortex is thus involved in taste and olfactory processing, in the control of food intake, and also in emotion and emotion-related learning.

S1.4. Mapping genes regulating consumption of taste substances

Gary K. Beauchamp, Alexander Bachmanov and Michael Tordoff

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA

Using inbred strains of mice, we have been investigating genetic loci that influence consumption of sweeteners, ethanol and monosodium glutamate (MSG) in two inbred mouse strains, C57BL/6ByJ (B6) and 129/J (129), that differ substantially in intake of these nutrients in 48 h, two-bottle intake tests. B6 mice consume more ethanol and sweetener solution than do 129 mice. There is a positive correlation between ethanol and sweetener intake among F_2 hybrids of these strains, implying that the same or closely linked genes are responsible for strain differences in these traits. Using the F_2 hybrids, we have mapped both traits to the telomeric region of chromosome 4. We created a saturated map of this chromosomal region and verified that both traits mapped to a small region previously suggested to be the Sac (for saccharin preference) locus. The position of the Sac locus has been confirmed by marker-assisted selection of a 129.b6-Sac congenic strain. Recently, a putative sweet taste receptor (TR1) has been mapped to the same chromosomal region (Hoon *et al.*, 1999, Cell, 96: 541). We are currently testing whether TR1 and Sac coincide in the genome. B6 mice also exhibit a preference for lower concentrations of, and consume greater amounts of, MSG than do 129 mice. Unlike the case for sweet and ethanol, there is no correlation between MSG responses and sweetener responses in F_2 hybrids, suggesting that these are independent traits. A genome

screen, designed to investigate possible loci for this trait, is underway.

Supported in part by NIH DC00882 and DK36399.

S2.2. Synaptic mechanisms of an olfactory memory

Hideto Kaba

Department of Physiology, Kochi Medical Schooh Nankoku, Kochi 783-8505, Japan

Female mice form an olfactory memory to the pheromones of the male with which they mate. The pheromonal memory is of critical biological importance, because it prevents any subsequent exposure to the mating male's pheromones from initiating neuroendocrine mechanisms that would terminate pregnancy. Pheromones from strange males, for which no memory has been formed, activate the vomeronasal system, thereby causing pregnancy block. The synaptic changes underlying this memory formation occur in the accessory olfactory bulb (AOB). Memory formation requires glutamatergic neurotransmission from mitral to granule cells via both ionotropic and metabotropic receptors. It is facilitated by the nitric oxide signalling pathway and depends on the balance between kinase and phosphatase enzyme activity. Additionally, memory formation is associated with an increase in length of the mitral-to-granule glutamatergic synapses. Recently, we have investigated a possible involvement of glial cells in the formation of the pheromonal memory. An immunohistochemical analysis revealed that there was an increased expression of anti-glial fibrillary acidic protein in the external plexiform and glomerular layers of the AOB during the critical period of memory formation. Functional analysis in the context of pregnancy block was examined using L-methionine sulfoximine (MSO) and fluorocitric acid (FCA). MSO inhibits conversion of glutamate to glutamine; FCA inhibits *de novo* formation of glutamine from glucose in astrocytes. Both drugs, when infused into the AOB 0 and 1.5 h after mating, produced a memory deficit as revealed by the mating male being able to block his own pregnancy. These results demonstrate a role for glial cells in the formation of a pheromonal memory.

S2.3. Development and modification of behavioral responses to MHC odortypes

Kunio Yamazaki

Monell Chemical Senses Center, 3500 Market Street Philadelphia, PA 19104, USA

The major histocompatibility complex (MHC) imparts to each mouse, rat and perhaps human an individual odor called an 'odortype' which reflects its MHC genotype. Perception of odortypes in mice affects mate selection, maternal–infant interaction and embryonic implantation. Recent studies indicating that volatile carboxylic acids are at least in part responsible for individual odors and what this finding implies about the pathway from gene to odorant are also reviewed. We suggest that odorants or their precursors are bound directly by MHC products and are released into serum and concentrated in urine. When we observed that odortypes were evident in mice as early as 1 day postpartum, we wondered whether they might exist prenatally. We now know that the odortype of a pregnant female is a combination of fetal and maternal odortypes. This discovery could provide a basis for the mother, or other individuals, to determine paternity of the litter prior to its birth. Behavioral responses of other mice to pregnant females may thus be influenced by this information. Such information may also prime females to be responsive to their offspring shortly after birth.

S2.4. Molecular mechanism of olfaction

Katsuhiko Mikoshiba and Mitsuhiro Hashimoto

Brain Science Institute, RIKEN & Institute of Medical Science, University of Tokyo, 2-1 Hirosawa, Wako, Saitama 351-0198 & 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Putative mammalian odorants were cloned, but the physiological function of these receptors in initiating transduction in olfactory receptor neurons remains to be established. Here, a recombinant adenovirus was used to drive expression of a particular receptor gene in an increased number of sensory neurons in the rat olfactory epithelium. A functional expression system for odorant receptors requires both that the receptors are properly targeted to the plasma membrance and that they couple efficiently with a second messenger system that produces a measurable response to ligand stimulation.

We used the internal ribosomal entry site insert to produce a bicistronic transcript that would result in the expression of odorant receptor (I7) and green fluorescence protein as separate proteins in the same cells. Electrophysiological recording showed that increased expression of a single gene led to greater sensitivity to a small subset of odorants: among the 74 odorants, octyl aldehyde (octanal), an eight-carbon, straight-chain, saturated aliphatic aldehyde, showed the highest response. Among the saturated aldehydes from C3 to C12, in addition to octyl aldehyde (C8) there were significantly increased responses to heptyl (C7), nonyl (C9) and decyl (C10) aldehydes, but no increase was detected for aldehydes with <7 or >10 carbons. Other C8 aliphatic compounds with different groups also failed to elicit responses greater than that of the control, suggesting that I7 odorant receptor recognizes odor substance in a three-dimensional manner.

This work was done in collaboration with Drs Haiqing Zhao, Lidija Ivic, Joji M. Otaki, Mitsuhiro Hasimoto and Stuart Firestein.

S3.1. Brain sites responsible for conditioned taste preference in rats

Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita, Osaka 565-0871, Japan

Conditioned taste preference (CTP) is acquired when ingestion of a particular taste solution is followed by ingestion of palatable nutrients. Water-deprived Wistar rats were allowed to drink 0.1 M $MgCl_2$ for 20 min followed by exposure to 0.5 M sucrose or 0.005 M sodium saccharin for 40 min. After pairing of these stimuli four times, the two-bottle preference test was performed for 0.1 M $MgCl_2$ versus water. The mean preference ratio (~5%) increased to ~10% and 20% after pairing with saccharin and sucrose, respectively. When 0.1% isoamyl acetate was added to 0.1 M $MgCl_2$ as an odor, the preference was enhanced to 30% after pairing with sucrose. Commercially available instant coffee (1%, dissolved in water) was also preferred by rats when its intake was followed by ingestion of 0.5 M maltose. Increased intake of 1% coffee was accompanied by an increase in the β-endorphin level in the arcuate nucleus of the hypothalamus and strong expression of

c-*fos* in the dorsal lateral subnuclcus of the parabrachial nucleus (PBN), indicating that the coffee became palatable as a result of the CTP procedure. Ibotenic acid lesions of the lateral part of the PBN impaired CTP, but the rats with lesions of the medial part acquired CTP. Lesions of the right side of the amygdala impaired CTP, but the left side lesions induced no significant effects on the CTP acquisition. These lesion effects are not reported for conditioned taste aversion (CTA), suggesting that different brain mechanisms are involved between CTP and CTA.

S3.2. Chemosensory cues for dietary fat and their relationship with dietary fat preference

Timothy A. Gilbertson

Pennington Biomedical Research Center, LSU, 6400 Perkins Road, Baton Rouge, LA 70808-4124, USA

Recently we demonstrated what we believe to be the first evidence of the cellular mechanism by which the gustatory system recognizes dietary fat. Essential (*cis*-polyunsaturated) fatty acids directly inhibit delayed rectifying K^+ (DRK) channels of the Shaker Kvl.5 subtype in taste cells, leading to their activation (Gilbertson *et al.*, 1997, Am. J. Physiol., 272: C1203). More recently, using a combination of electrophysiological, biochemical and molecular biological techniques, we have investigated the role of this signaling pathway in the ingestion of dietary fat and how generally applicable this mechanism is for other post-ingestive fat responsive organs. Patch clamp recording from taste cells isolated from fat-preferring and fat-avoiding rat strains demonstrated that DRK channels in taste cells from fat-preferrers are significantly less responsive to fatty acids than are those from fat-avoiders. Thus, there is an inverse correlation between dietary fat preference and gustatory responsiveness to fatty acids. Western blotting and RT-PCR have shown that the fat-responsive Shaker Kv1.5 channels are present in several fat-sensitive organs, including the pancreas, duodenum, liver and adipose tissue. To determine if these tissues respond to fatty acids in a manner analogous to the taste cells, we performed patch clamp recording on a pancreatic cell line (HIT-T15) and a cholecystokinin-secreting intestinal cell line (STC-1), both of which contain Shaker Kv1.5 channels. Free essential fatty acids inhibit DRK channels in these cells with an EG_{50} near 2 μM. However, in addition to the *cis*-polyunsaturates, the mono-unsaturated fatty acids also inhibit DRK channels. It appears that cell activation via DRK channel inhibition by fatty acids may represent a universal mechanism by which the body recognizes dietary fat and that this signaling system may contribute to regulation of dietary fat intake.

Supported by NIH NIDCD DCO25O7 and a research grant from Novartis Pharmaceutical Corp.

S3.3. Apolipoprotein AIV—a potent satiety factor

Patrick Tso

Department of Pathology, University of Cincinnati Medical Center, 231 Bethesda Avenue (ML 0529), Cincinnati, OH 45267, USA

Apolipoprotein AIV (apo AIV) was discovered ~22 years ago. It is a glycoprotein secreted only by the small intestine in humans. However, in rodents both the small intestine and the liver secrete apo AIV, but the small intestine is by far the major organ responsible for the circulating apo AIV. A number of research groups have demonstrated that apo AIV production by the small intestine is stimulated by active lipid absorption and appears not to be mediated by the uptake or re-esterification of fatty acids and monoglycerides to form triglycerides. Rather, it is the formation of chylomicrons that acts as a signal for the induction of intestinal apo AIV synthesis. In addition, we have found that a factor, peptide YY (PYY), is released by the ileum and stimulates synthesis of apo AIV by the jejunum.

Work by our research team has demonstrated that apo AIV is involved in the inhibition of food intake after the ingestion of fat. Apo AIV infused into the third ventricle significantly inhibited food intake in a dose-dependent manner. Infusion of antibodies against apo AIV into the third ventricle elicited feeding in all rats tested. Subsequent experiments by Okumura and his colleagues demonstrated that apo AIV inhibits both gastric acid secretion and gastric motility. Thus, apo AIV may inhibit food intake via its effects on the stomach. Recent work from our laboratory has demonstrated that chronic ingestion of a high-fat diet blunts the intestinal apo AIV response to lipid feeding, possibly explaining why the chronic ingestion of a high-fat diet predisposes both animals and humans to obesity.

S3.4. Neural mechanisms of food recognition and feeding behavior

Taketoshi Ono

Department of Physiology, Faculty of Medicine, Toyama Medical & Pharmaceutical University, Toyama 930-0194, Japan

It has been suggested that the lateral hypothalamic area (LHA) be designated as a feeding center and the ventromedial nucleus of the hypothalamic (VMH) as a satiety center based on lesion and stimulation studies. Neurophysiological studies using the iontophoretic application of glucose, insulin and free fatty acids identified glucoreceptor neurons in the VMH, the activity of which is inhibited by glucose, and glucosensitive neurons in the LHA, the activity of which is facilitated by glucose. These dual centers regulate feeding behavior by integrating various intrinsic signals such as inputs from peripheral nerves from the stomach and liver as well as blood glucose levels. Extrinsic signals such as vision, audition and taste also contribute to the regulation of feeding behaviors. Our neurophysiological studies in monkeys demonstrated that these extrinsic signals, such as vision and taste, which are important for discrimination of food from nonfood, were processed and transmitted to the LHA through the inferotemporal cortex–amygdala–LHA axis. The prefrontal cortex (PFC) also interacts with the hypothalamus; the PFC sends highly integrated information, such as decision making and recognition of a situation in which food is available, to the hypothalamus. The basal ganglia (especially the caudate nucleus), based on inputs from the PFC, is involved in complex feeding behavior by contributing to the drive to sustain feeding behavior. These results suggest that the hypothalamus receives and integrates a large amount of information pertinent to the regulation of feeding behavior from the internal and external environments.

S4.1. Taste and palatability perceived during eating behavior

Shizuko Yamaguchi

Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan

They say that most people like taste but few of them know taste. For both manufactures and consumers, it is requisite to *know taste*. In this paper, some important features of taste were discussed. Firstly, it is noted that the word 'taste' has the sense of 'a small amount' or 'hint' both in Japanese and English. It also means wonderfulness that can be realized only through a deep experience. The taste of food is composed of a few components having strong tastes and a number of components having weak ones. The latter contributes to *koku* in Japanese, which is a control factor of palatability. *Koku* includes two factors, deepness or fineness, and concentration. The balance of the two is important. As the tastes of foods are usually not strong, we can distinguish a variety of tastes. Umami as a signal of protein appears with various amino acids, peptides, etc. Thus, it does not become very strong even at high concentrations. The taste of some substances can be sensed in very small amounts. As little as 5×10^{-8} g of quinine sulfate can cause bitterness. Taste perception has a time dimension. Taste changes with time during a single act of tasting. For a short period, time–intensity tracking can reveal a particular pattern for every tastant. On the other hand, it may take a long time for a particular taste to the recognized. For example, umami has been tasted in foods for many centuries, yet its recognition as a basic taste has taken place only in the twentieth century.

S4.2. Contribution of aroma substances to scented teas and the consumers' preference

Dongmei Wang, Akio Kobayashi and Kikue Kubota

Laboratory of Food Chemistry, Department of Nutrition & Food Sciences, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan

Jasmine tea, Earl Gray black tea and apple tea are three typical scented teas, manufactured by different processes, which are scented by living flower, natural essential oil and synthetic flavoring compounds respectively. The contribution of these aroma substances to the original green or black tea were analyzed qualitatively and quantitatively by GC and GC-MS methods. The strength of each aroma character was determined by aroma extract dilution analysis and the selected highly contributing materials in the scented tea were discussed in comparison with those in the original tea.

The results obtained from these different scented teas are consistent with the view that the main aroma compounds do not work to develop the particular aroma in the original tea but to give a different characteristic aroma to it.

S4.3. Sensory and consumer research on beer

Yoshiko Yokomukai

Kirin Brewery Co. Ltd, Marketing Department, Research Laboratory for New Products, 10-1, Shinkawa 2chome, Chuo-ku, Tokyo 104-8288, Japan. e-mail: yyoshiko@ kirin.co.jp

When we enjoy drinking beer, we usually perceive a tasty, pleasant bitterness, we perceive the flavor attributes and we satisfy our thirst. Even when drinking the same amount and same brand of beer, we often experience a different flavor due to our physiological state or degree of satiety. Moreover, there are individual differences in people's ability to metabolize alcohol. Those who cannot easily break down alcohol may become ill as a result of consuming it and this may cause the person to subsequently dislike the beverage.

We can classify sensory evaluation techniques into two types. Analytical sensory evaluation involves highly trained panelists who are required to evaluate the sensory attributes of products as if they were machines. Acceptance sensory evaluation, on the other hand, involves panelists or consumers who are not trained. These individuals evaluate how much they like or dislike a product. Such evaluations may involve either unbranded or branded samples. In the latter case, the samples may include the package, brand name, advertisements etc.

In this symposium, I introduce the principles of successful sensory evaluation. Included are specific examples on how to objectively use consumers' sensory perceptions of beer. Questions addressed include: How can QDA (Quantitative Descriptive Analysis) panelists describe flavor attributes? How can we understand consumers' preference for beer taste and flavor? How can we relate QDA panel data to consumer preferences?

S4.4. Understanding food choice: recent developments in consumer science

Peter Leathwood

Nestlé Research Center, 1000 Lausanne 26, Switzerland. e-mail: peter.leathwood@rdls.nestle.com

Discoveries from basic research in taste, smell and food preference play a key role in product development in the food industry. However, basic researchers are often unaware of elements, such as branding, which have an important influence on how people perceive and select foods.

An examination of food buying motives suggests that, for many people, strong, reliable brands provide points of reference which they use when deciding which foods to buy and consume. This is a major reason why the food industry invests so much in brands. Brands were originally symbols to identify the goods of a seller and differentiate them from those of competitors. For foods, they now aim to provide a guarantee of quality, value and satisfaction. While sensory attributes of a food product may vary with culture and over time as tastes vary, the brand must provide consistent benefits and values. The influence of the brand on preference is rarely considered by researchers outside industry. Brands generate expectations which influence both perception and preference. Thus, if the *actual* intensity of a sensory attribute (i.e. assessed by tasting blind) is not far from the *expected* intensity (generated by seeing the label with the brand), then *perceived* intensity (when tasting branded) tends to assimilate towards expectation. However, if the mismatch is large, there may be a contrast effect with intensity perceived as further away from expected than it really is. Thus what is delivered must correspond closely and consistently with consumers needs and expectations. This is the aim of the product optimization methodologies developed and used in consumer science.

P3. Calcium-activated chloride conductance of olfactory receptor neurons of the rainbow trout: a non-stationary noise analysis

Koji Sato and Noriyo Suzuki

Animal Behavior and Intelligence, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

To characterize properties of Ca^{2+}-activated Cl^--channels of ciliated olfactory receptor neurons (ORNs) of the rainbow trout, we made a non-stationary noise analysis of amino acid-induced whole-cell currents and estimated the unitary conductance (γ), number of channels/ORN (n) and channel density of the ciliary surface (d). Choline-Cl external and CsCl internal solutions were used to remove Na^+- and K^+-current components from the amino acid-induced currents. A mixture of four amino acids (1 mM in ejection pipette; L-glu, L-arg, L-ala and L-norval) were applied repetitively to cilia of ORNs by the pressure ejection system (20 s intervals; 1 kgf/cm^2, 25 ms). The ensemble mean and variance of the series of current responses of ORNs were calculated on Macintosh computers using an acquisition, text conversion and spreadsheet calculation software. At a holding potential of –60 mV (V_{eq} = –10.3 mV), γ, n and d were estimated to be 8.93 ± 2.60 pS, 170.2 ± 131.2 channels/ORN and 5.18 + 3.32 channels/μm^2 (mean ± SD; n = 8), respectively. At +20 mV, the values were 13.0 ± 5.96 pS, 161.2 ± 58.6 channels/ORN and 5.81 ± 2.40 channels/μm^2 (mean ± S.D.; n = 6). The plot of the estimated unitary currents on whole-cell peak current–voltage relations in reduced scale showed that the estimated γ values at different holding potentials were in the appropriate range.

P4. Adenophostin analogues induced inward currents in turtle olfactory sensory neurons

Makoto Kashiwayanagi, Kazuya Tatani, Satoshi Shuto and Akira Matsuda

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

Using the whole-cell mode of the patch-clamp technique, we recorded inward currents in response to adenophostin analogues in turtle olfactory sensory neurons. Dialysis of adenophostin analogues, novel IP_3 receptor ligands, into the neurons induced inward currents with an increase in membrane conductance in a dose-dependent manner under the voltage-clamp conditions (holding potential, –70 mV). The application of Ca^{2+}-free Ringer solution to neurons previously dialyzed with adenophostin analogues induced inward currents that were reversibly inhibited by application of Na^+,Ca^{2+}-free Ringer solution or normal Ringer solution. The magnitude of the responses to the adenophostin analogues varied among these derivatives. The reversal potential of inward currents induced by an adenophostin analogue was similar to that induced by IP_3, suggesting that inward currents induced by the adenophostin analogue were generated by a similar ionic mechanism to that induced by IP_3. The present study demonstrated that IP_3-mediated transduction pathways exist in turtle olfactory receptor neurons and that adenophostin analogues act as agonists of IP_3.

P5. cADP-ribose induces inward currents and increases in intracellular Ca^{2+} concentration in turtle olfactory neurons

Kousuke Sekimoto and Makoto Kashiwayanagi

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

In olfactory neurons, it is well established that cAMP and/or IP_3 act as second messengers during odor responses. We previously showed that cAMP-accumulating odorants induce odor responses even after complete desensitization of the cAMP-mediated pathway. These results suggested that one or both cAMP- and IP_3-independent pathways contribute to the generation of odor responses. The present study explores the role of cADPR in the olfactory transduction pathway. Turtle olfactory receptor cells were isolated with an enzyme-free procedure and loaded with fura-2/AM from the bath solution. The cells responded to dialysis with cADP-ribose with an inward current, an increase in membrane conductance and an increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]$. Dialysis of cADP-ribose induced inward currents in a dose-dependent manner. Flooding of cells with 100 μM cADP-ribose from the pipette also induced an inward current without changes in $[Ca^{2+}]$ in Ca^{2+}-free Ringer solution. In Na^+-free Ringer solution, cADPR induced only small inward currents with increases in $[Ca^{2+}]_i$. Inward currents and increase in $[Ca^{2+}]_i$ induced by cADP-ribose were completely inhibited by removal of both Na^+ and Ca^{2+} from the outer solution. The experiments suggest that cADP-ribose activates a cation channel at the plasma membrane, allowing inflow of Na^+ and Ca^{2+} ions.

P7. TGF-α-positive olfactory neurons exist at the border of the epithelium consisting of supporting cells and horizontal basal cells

Yuko Suzuki

Department of Oral Anatomy, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 061-0293, Japan

In the dorsal fossa (the roof) of the posterior nasal cavity of DDY mice and Wistar rats, we found seven epithelial patches consisting of only non-neuronal cell types, i.e. supporting cells and horizontal basal cells, among the normal olfactory epithelium by a 3-D reconstruction study. In the electron micrographs of these epithelial patches, the supporting cells occupied three or four layers in the apical to middle regions, and in the basal region horizontal basal cells were localized in a single row adjacent to the basement membrane. Bowman's gland ducts were also present in the epithelium. Neuronal cells (olfactory cells and globose basal cells) were totally absent. Light and electron microscopic immunohistochemistry using monoclonal anti-TGF-α (transforming growth factor-α, MAb213) antibody revealed that a group of TGF-α-positive olfactory cells occupied the boundary between the epithelial patches and the normal olfactory epithelium. These TGF-α-positive olfactory neurons were labeled with anti-vimentin antibody but not with anti-OMP, anti-PGP9.5 or anti-neurotubulin antibodies. Since both the epithelium and neurons are unusual, they may subserve some specialized function in the olfactory system.

P8. GABA receptors in the olfactory bulb involved in olfactory learning

Fumino Okutani and Hideto Kaba

Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

After training with an odor paired with foot shock, rat pups show aversion to the odor. The mechanism underlying this aversive olfactory learning is considered to involve disinhibition of mitral cells in the olfactory bulb by the somatosensory stimulation-induced activation of centrifugal noradrenergic fibers originating in the locus coeruleus. Mitral cells are inhibited through $GABA_A$ receptors in the external plexiform layer and secondarily through $GABA_B$ receptors in the glomerular layer. We examined whether GABA receptor agonists could block olfactory aversive learning in young rats, and whether GABA receptor antagonists could in turn induce olfactory learning without somatosensory stimulation. Both muscimol, a $GABA_A$ receptor agonist, and baclofen, a $GABA_B$ receptor agonist, when infused into the olfactory bulb prevented olfactory aversive learning in a dose-dependent manner. Infusion of bicuculline, a $GABA_A$ receptor antagonist, during training with odor exposure alone produced preference or aversion to some odors. Even if bicuculline was infused without odor exposure, pups showed aversion to the strange odors to which they had never been exposed. Saclofen, a $GABA_B$ receptor antagonist, has a similar effect to bicuculline in establishing olfactory learning. These results suggest that olfactory learning in young rats is modulated through both $GABA_A$ and $GABA_B$ receptors in the olfactory bulb.

P9. Responses of the mushroom body Kenyon cell in the intact cockroach to olfactory stimulations onto the antenna

Weiping Chen and Jun Inouchi

Laboratory of Neurophysiology, National Institute of Sericultural and Entomological Science, 1-2 Ohwashi, Tsukuba, Ibaraki 305-8634, Japan

The possible roles of the insect mushroom body in the control of complex motor repertories and in associative olfactory learning are suggested. Physiological and morphological evidence indicates that the intrinsic interneurons (Kenyon cells) of the mushroom body can receive inputs from the projection neurons of the antennal lobe. The mechanisms of olfactory processing of the Kenyon cells, however, are still little understood. Using an *in vivo* patch-clamp recording technique in the soma of the Kenyon cell in the cockroach (*Periplaneta americana*) brain, we found that monomolecular odorants (alcohols: 1-hexanol, 1-heptanol, 1-octanol, 1-octen-3-ol) and a blended odor (an insect chow) delivered onto the antenna elicited odor responses to reveal subthreshold, depolarizing synaptic events (EPSPs) in the neurons sampled (n = 56). Some of these neurons produced EPSPs as well as action potentials during odor responses. A group of sampled Kenyon cells responded to stimulations with the alcohols but displayed different synaptic and firing patterns in response to each odor. Another group of the sampled neurons responded to the alcohols and the food odor. The response patterns of this group are odor-specific as well as neuron-specific. The results suggest that the Kenyon cells probably receive different combinations of converging inputs from the projection neurons in the antennal lobe in response to different odors.

P10. Analysis of the high-frequency electroencephalogram activity in association with odor stimulation in the rat

Yoshiyuki Hirano, Yasutake Shimizu and Keiichi Tonosaki

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

Olfaction is an indispensable sense for many animals for the acquisition of food and for reproductive behavior. Animal that have an excellent sense of smell are reported abundantly. There are various methods being used for the research of the olfactory function. However, it is difficult to study the olfactory sense under natural conditions. In the behavioral study of olfaction, it is necessary to train the animal for a long time. We looked for a simple and noninvasive method. Here we report that the analysis of electroencephalogram (EEG) activity in association with odor stimulation in the rat is useful in this type of experiment. Limonene and various fatty acids were used as the odorants in this study. The EEG was recorded with a rat under slight anesthetization with sodium pentobarbital. The high frequency of EEG elements (40–80 Hz) increased markedly with odor stimulation. As a result, the EEG recording method is more useful and convenient than the usual behavioral method for judging the olfactory sense of animal.

P11. Trial measurement of olfactory event-related potentials: assessment of young and elderly adults

Byung Chan Min, Soon Cheol Chung, Chul Jung Kim, Se Jin Park, Kang In Lee[1], Kyu Sik Kwon[1],Sang Gyun Kim, Byung Woon Min, Ji Young Oh, Jung Sang Shin[2], Jin Kyu Kang[3] and Kazuyoshi Sakamoto[4]

Ergonomics Laboratory, Korea Research Institute of Standards & Science, Taejon, [1]Department of Mechanical and Industrial Engineering, Jeonju University, Cheonju, [2]Department of Physics, Chungnam National University, Taejon, [3]Department of Industrial Engineering, Taejon National University of Technology, Taejon, Korea and [4]Ergonomics Laboratory, The University of Electro-Communications, Tokyo, Japan

We developed an apparatus for odorous stimuli control to record olfactory event-related potentials from the human scalp. Olfactory event-related potentials were recorded from 10 younger subjects (males aged 22–28 years) and 10 older subjects (males aged 60–78 years). All subjects reported normal olfactory senses. Skatole (Olfactometer T&T) was used as the odorant element. We obtained event-related potentials to odorous stimuli using this apparatus from the site of Cz, whose positive peak latencies were ~180 ± 23 ms. Such responses were not recorded if oxygen stimuli were used instead of odorous stimuli or with click sounds produced by the switching electromagnetic valve. Since age increase is associated with prolongation in latency of the later components (N1 and P2) and not the earlier components (P1 and N1), age is possibly related to alterations in central olfactory structures which contribute disproportionately to the decline in olfaction typically seen in the elderly. The results of the present study emphasize the importance of subject parameters (i.e. young and old) as a source of variability in olfactory event-related potential measures. These data suggest that olfactory event-related potentials provide a specific measure of olfactory function.

P12. Brain regions involved in conditioned odor aversion in rats

Tadashi Inui and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Previous reports have suggested the involvement of possible factors in the acquisition and retention of conditioned odor aversion (COA). To reveal critical factors, behavioral experiments were performed in normal and brain-lesioned Wistar rats. In our COA paradigm, 0.001% isoamyl acetate was used as the conditioned stimulus (CS) and an i.p. injection of 0.15 M LiCl was used as the unconditioned stimulus (US). We examined the effects of the length of CS–US interval (ISI) and the simultaneous presentation of taste (0.05 or 0.1% saccharin). The results showed that rats could acquire strong odor aversions when the ISI was 0 min, but no COA was acquired when the ISI was 30 min. When the odor stimulus was dissolved in 0.05% saccharin solution and presented as the CS, rats acquired strong aversions to the CS even with the 30 min ISI. When the stronger (0.1%) saccharin solution was used, stronger odor aversions were acquired. In the behavior–lesion experiments, we examined the effects of excitotoxic lesions of the mediodorsal nucleus (MD) and the parvicelluar part of ventroposteromedial nucleus (VPMpc) of the thalamus, amygdala, orbitofrontal cortex (OFC) and insular cortex (IC) with ibotenic acid. Lesions of the MD, VPMpc, OFC or IC elicited only minor effects, but lesions of the amygdala impaired the formation of COA. These findings suggest that the amygdala is important in the formation of COA.

P13. Brain magnetic active fields of pleasantness/unpleasantness using an olfactory odd-ball paradigm in MEG

Masahiko Yamaguchi[1], Mitsuo Tonoike[1], Naoya Hirata[2], Isao Kaetsu[2], Ritsu Seo[1,3] and Izumi Koizuka[1,4]

[1]Osaka LERC., Eleetrotechnical Laboratory, Amagasaki 661-0974, [2]Faculty of Science & Technology, Kinki University, Osaka 577-0818, [3]Seo Hospital, Amagasaki 660-0839 and [4]St Marianna University Medical School, Kawasaki 216-8511, Japan

We examined how pleasantness/unpleasantness of odors was concerned with from a result of dipole which was estimated using an effective whole-head-type MEG device to detect the activity in a brain. We used two odors: amylacetate (with a banana-like odor) as the pleasant odor, and isovaleric acid (with a stink odor) as the unpleasant odor. The two kinds of odor were stimulated using the blast method by which 300 ms odor pulses were administered into the nasal cavity directly, and the experiment was done in accordance with an olfactory oddball paradigm using the rare stimulation in the rate of 25%.

We found that the pleasantness/unpleasantness of the above cognitive responses depended on the kind of odors and on the stimulated side of the nasal cavity. In the case of the left nasal cavity stimulation, the difference of pleasantness and unpleasantness appeared as a different latency, the pleasant response being as much as 100 ms quicker than the unpleasant one; however, no difference in latency was found between the right and the left hemispheres. On the other hand, in the right nasal cavity stimulation a difference of pleasantness/unpleasantness appeared only at the right side, and the latency of unpleasantness was more shorter than that of pleasantness, being only some dozens of milliseconds. In this case, there was a difference of latency between the right and the left hemispheres, the right side being shorter and dominant.

P15. Change in odor sensitivity of mice vomeronasal receptors

T. Hatanaka and A. Sgyou

Department of Biology, Faculty of Education, Chiba University, Chiba 263-8522, Japan

The mouse shows diurnal periodicity in various routine activities. The urinary marking behavior of mice also changes daily. Particularly, the number of counter urine marks of males to female urinary marks was increased in the evening and maintained at a high level throughout the night, but females did not show any remarkable change. It is thus expected that the sensitivities of the vomeronasal receptor to urinary sex pheromones also change with the day and night shift. To demonstrate this possibility, the response of the mouse vomeronasal receptors to urine odors during daytime and that during the night were compared by labeling the activity of succinic acid dehydrogenase using nitro blue tetrazolium. As a result, it was shown that among the various combinations of preparation sex and stimulant, only the sensitivity of male vomeronasal receptors to female urine odor was increased in night, and this result coincided with the daily change of urinary marking behavior.

P16. The analysis of olfactory-related behaviors in mice lacking gastrin releasing peptide receptor

Kazuyuki Yamada, Etsuko Wada and Keiji Wada

Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira-City, Tokyo 187-8502, Japan

Gastrin releasing peptide (GRP), which widely distributes in the central nervous system, is one of the mammalian bombesin-like peptides. We previously demonstrated that deficiency of GRP-receptor (GRP-R) led to abnormal social behavior in male mice. As it has been reported that social behaviors could be regulated by olfaction, GRP-R-deficient mice may have some dysfunction in their olfactory behavior and/or olfactory systems. Therefore, we performed a series of experiments in order to examine olfactory-related behaviors in female GRP-R-deficient mice. In the food exploration test (finding the hidden cookie method) there was no significant difference between the latency of GRP-R-deficient mice and wild type mice in finding a piece of cookie buried in sawdust. On the other hand, GRP-R-deficient mice showed a higher rate of and longer duration in contact with anesthetized male mice than did wild type mice. Furthermore, GRP-R-deficient mice did not show a salient preference to socially dominant male mice, although wild type female mice prefer socially dominant male mice to socially subordinate male mice in the T-maze choice test setting. These results indicate that deficiency of GRP-R does not have any influence on the olfaction itself, but affects the processing of olfactory information which is related to regulating systems of social behaviors.

P17. Immunoreactivity for G proteins in the vomeronasal organ of human fetuses

Maiko Yukimatsu, Shigeiu Takami, Joji Matsumura[1] and Fumiaki Nishiyama

Department of Anatomy, School of Health Sciences and [1]Department of Anatomy, School of Medicine, Kyorin University, 476 Miyashita-cho, Hachioji, Tokyo 192-8508, Japan

Immunolocalization of Go_α and $Gi_{\alpha 2}$ was examined in the vomeronasal organ (VNO) of human fetuses. Tissue blocks including the VNO were dissected out from formalin-fixed 5-month-old fetuses (n = 10), re-fixed in Zamboni's fixative overnight and cut with a cryostat frontally and sagittally at 14 μm thickness. Using polyclonal antibodies to Go_α and $Gi_{\alpha 2}$, a peroxidase-labeled streptavidin–biotin technique was carried out. Immunoreactivity for Go_α was present in a subpopulation of vomeronasal epithelial cells. The immunoreactivity was frequently observed in their perikarya. In some cases, intense immunoreactivity was observed in apical processes and apical endings of bipolar-shaped cells that were faced to the vomeronasal lumen. Nerve fibers associated with the VNO exhibited intense immunoreactivity for Go_α as well. $Gi_{\alpha 2}$-immunoreacitive cells were observed scattered in the vomeronasal epithelium. Since Go_α and $Gi_{\alpha 2}$ were characteristically expressed and coupled with putative pheromone receptors in rodent vomeronasal receptor neurons, our present results suggest that some vomeronasal epithelial cells in human fetuses express Go_α and $Gi_{\alpha 2}$ and are involved in vomeronasal chemoreception.

P18. Urinary substances induce inward currents in rat vomeronasal sensory neurons

Kouhei Inamura and Makoto Kashiwayanagi

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

Using female rat vomeronasal sensory neurons, we here successfully recorded inward currents in response to urine from various sources. Of the neurons that responded to urine, 77% responded to only one type of urine. Male Wistar urine induced responses preferentially in the apical layer of the sensory epithelium, while male Donryu and female Wistar urine induced responses mainly in the basal layer of the epithelium. The amplitude of inward currents induced by application of male Wistar urine was voltage dependent with an average amplitude of –47.1 ± 6.2 pA (n = 29) at –74 mV. The average reversal potential for male Wistar urine was –9.3 ± 6.1 mV (n = 4), which was not apparently different from the reversal potentials for urine excreted from different species. It is likely that the urine-induced inward currents in response to different types of urine are mediated via a similar channel. The simultaneous removal of Na^+ and Ca^{2+} from extracellular solution eliminated the response. The magnitude of the urine-induced inward current in Cl^--free external solution was similar to that in normal solution, suggesting that the urine-induced current is cation selective. Application of the constant-field equation indicated a very high permeability coefficient for Ca^{2+}. This study first demonstrated that substances contained in urine elicited an excitatory response in vomeronasal sensory neurons through cation-selective channels.

P19. Region-specific expression of Fos-immunoreactive cells induced by protease-sensitive urinary pheromones

Kenji Tsujikawa and Makoto Kashiwayanagi

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

Vomeronasal organs of female Wistar rats were exposed with sprayed urine preparations of male Wistar rats prior to sacrifice. We showed previously that exposure of the vomeronasal organs of female rats to male rat urine induced expression of Fos-immunoreactive cells, which is correlated with cellular activity, in the accessory olfactory bulb (AOB) (Inamura *et al.*, 1999). In the present study, in order to characterize active components stimulating the rat vomeronasal organ, male urine preparation was subjected to ultrafiltration and protease-treatment, and its effects on induction of Fos-immunoreactive cells in the AOB were examined. Exposure to crude urine and ultrafiltrated urine preparation (<5 kDa) induced significant Fos expression, which is correlated with cellular activity, in the mitral/tufted cell layer of the AOB, while exposure to the remaining substances after the ultrafiltration (>5 kDa) and control salt solution did not. Exposure to urine preparation treated with papain induced expression of Fos-immunoreactive cells in the rostral region of the AOB, but did not induce such expression in the caudal region. Exposure to urine preparation treated with pronase did not induce urine-specific Fos-immunoreactivity either in the rostral or in the caudal region. These results suggest that at least two different peptides carrying pheromonal activities are contained in male Wistar rat urine.

P20. Region-specific expression of Fos-immunoreactive cells in sexually experienced male rats after exposure to oestrous urine

Harumi Sakamoto, Kouhei Inamura, Makoto Kashiwayanagi and Kenzo Kurihara

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

We studied Fos-immunoreactive (Fos-ir) structures in the accessory olfactory bulb (AOB) of rats after the vomeronasal organ was exposed to urine. Exposure of the vomeronasal organ of male Wistar rat to oestrous and dioestrous female Wistar urine induced the appearance of many more Fos-ir cells in the rostral region of the periglomerular cell layer, but did not induce Fos-ir cells in the caudal region. These results suggest that the regionalization of Fos-ir cells after exposure to female urine is remarkable in the periglomerular cell layer. Sexually experienced male rats have been shown to prefer oestrous to dioestrous female urine while sexually inexperienced males do not exhibit these preferences. We compared the expression of Fos-ir cells in the AOB of sexually experienced and sexually inexperienced male rats following exposure to oestrous urine. In the localized region (lateral and rostral regions) of the periglomerular cell layer, many more Fos-ir cells were expressed in the sexually experienced rats than in the inexperienced rats. These results suggest that sexual experience promotes the formation of a memory of a pheromone found in oestrous urine at the periglomerular cell layer of the accessory olfactory bulb.

P21. Contribution of NMDA glutamate receptors to oscillatory signal propagation in guinea-pig accessory olfactory bulb slices

Tokio Sugai, Michio Sugitani and Norihiko Onoda

Department of Physiology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan

The role of NMDA receptors in the oscillatory signal propagation evoked by electrical stimulation of the vomeronasal nerve layer (VNL) was studied by an optical imaging method, using a voltage-sensitive dye. Application of APV or MK-801, selective NMDA receptor antagonists, enhanced the oscillatory response in the external plexiform and mitral cell layers (EPL/MCL), where mitral cells make reciprocal dendrodendritic synapses with granule cells. The removal of Mg^{2+}, which blocks the activity of NMDA receptors in a voltage-dependent manner, from the perfusate abolished the oscillatory responses; the subsequent application of APV restored the oscillatory responses, indicating that NMDA receptors mediate inhibition of the oscillation. Furthermore, paired-pulse (conditioning and test) VNL shocks delivered at an interval of 300 ms markedly depressed the oscillations in the test response. After application of APV the oscillatory EPL/MCL responses recovered moderately with a small increase in amplitude. These results suggest that, in the dendrodendritic synapse in the EPL/MCL, NMDA receptor activation on the granule cell spines may enhance GABA release from their spines, which in turn strongly inhibits the activities of mitral cells, resulting in a cessation or a long-lasting depression of the oscillations.

P22. Human olfactory contrast accompanies the menstrual cycle

Mio Moriyama and Takashi Kurahashi

Department of Biophysical Engineering, Osaka University, Toyonaka, Osaka 560-8531, Japan

It has been generally known that there is a remarkable relation between olfactory perception and the menstrual cycle. In the present study, we investigated this possibility by defining the concept of 'olfactory contrast'. Five subjects were selected after filling in detailed questionnaires and attending consultations about the stability of their menstrual cycle. Cyclopentadecanolide was used as the odor (solvent: ethyl alcohol, 99.5%). All subjects were tested every 2 or 3 days. The stimuli were delivered by 0.5×10.0 cm filter papers absorbed odor. At first we estimated the perception threshold by way of a three-option forced choice in a rising dilution series. At higher concentrations, olfactory strengths were asked in six stages of category ratio scale to get strength–odor intensity curves. The slopes of these dose–response curves represent the contrast, as has been shown widely in physiology and engineering fields. Therefore we took derivatives of the resultant curves, and the maximum value was defined as olfactory contrast. As a result, this contrast rose around the menstrual phase, while olfactory thresholds became slightly higher. This result suggests that women can distinguish odors more clearly and their ability to recognize an odor may increase at the menstrual phase.

P23. Positive relationship between menstrual synchrony and ability to smell 3α-androstenol

M. Morofushi and K. Shinohara

Department of Physiology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Although many studies have confirmed women's menstrual synchrony, the underlying mechanism has not been clarified. Recently, a number of studies have reported that human axillary extracts influence women's menstrual cycles. There are two possible pheromones secreted from the axilla, 3α-androstenol and 5α-androstenone. In the present study we examined the relationship between menstrual synchrony and the ability to smell the putative pheromones, 5α-androst-16-en-3α-ol (3α-androstenol) and 5α-androst-16-en-3-one (5α-androstenone).

We checked menstrual synchrony among 67 women living together in a college dormitory in order to classify synchronized and non-synchronized groups. Afterwards, dilution series of androstenol and androstenone and the control odorant (pyridine) were presented to the 64 women and the sensitivity to odors in synchronized and non-synchronized women was compared.

Menstrual synchrony was found in 24 out of 64 (40%) women. No difference was found between synchronized and non-synchronized women in the detection threshold to pyridine, indicating that general olfactory ability did not differ between two groups. The detection threshold to 3α-androstenol was significantly lower in synchronized than in non-synchronized women, but the threshold to 5α-androstenone was not different between them. Some subjects failed to detect either 3α-androstenol or 5α-androstenone, even when presented with the strongest concentration. All the women who were synchronized could detect 3α-androstenol but not 5α-androstenone.

These results indicated that the women who showed menstrual synchrony had a high sensitivity to 3α-androstenol but not to 5α-androstenone.

P24. Effects of human pheromones on pulsatile LH secretion

Kazuyuki Shinohara, Masayo Morofushi and Kimihiko Saito

Department of Physiology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Axillary compounds from donor women in the follicular phase (FP) shorten both the time to ovulation and the length of the menstrual cycle in recipients, and those in the ovulatory phase (OP) delay ovulation and lengthened the total cycle. We examined the effects of axillary compounds on pulsatile luteinizing hormone (LH) secretion. Twenty college female students aged 19–22 years were involved in this study. We collected axillary compounds on cotton pads from five donor women during the FP and the OP. Each pad was treated with 70% isopropyl alcohol. On day 5–7 after the menstrual onset, blood samples were collected from 15 recipients at 10 min intervals. No recipients were exposed to axillary compounds or isopropyl alcohol for the first 4 h. For the next 4 h, FP and OP compounds and 70% isopropyl alcohol were applied hourly to each of five recipients. The frequency of the LH pulse in humans is increased by exposing recipients to axillary compounds in the FP and decreased by axillary compounds in the OP. However, isopropyl alcohol and male compounds had no effect

on the LH pulse frequency. Therefore, in humans, pheromones may regulate the timing of ovulation by changing the frequency of pulsatile LH secretion, which controls the rate of follicular growth and maturation.

P26. Toward selection of standard odor molecules. 1. Calculation of molecular frequency and examination of the odor evaluation method

Fumiko Yoshii, Toshio Ogino[1], Yoshiaki Yamada[2], Takashi Hoshi and Hisahiro Hagiwara

Graduate School of Science and Technology, [1]Faculty of Education and [2]School of Dentistry, Niigata University, Niigata 950-2181, Japan. e-mail: fuyoshii@mb.infoweb.ne.jp

Our future goal is to select standard odor molecules for olfactory experiments or for odor evaluation, based on molecular properties. We calculated infrared (IR) wave-numbers and intensities of iso-valeric acid, beta-phenylethyl alcohol, indole, methyl anthranilate and naphthalene using Gaussian98 with B3LYP, 6-31G(d), since the importance of frequency of odoriferous molecules was suggested (L. Turin, 1996, Chem. Senses, 21: 773–791). The calculated IR wave-numbers and intensities showed rather good agreement with the IR data measured during the gaseous phase. We found that the size and the shapes of low-frequency areas of vibrations varied according to conformers. The relationship between wave-numbers and odor qualities has not yet been recognized. The latter parts of this work focused on the odor-similarity evaluation during the gaseous phase using bags made of polyethylene terephthalate. It was not difficult to decide the dilution factor of odorants that evaluated as 'weak detectable odor' by university students without training. However, odor-similarity scores varied very much depending on subjects. Screening of subjects by odor sensitivity might reduce this variance.

P29. Evaluation of odor quality for five odorants of a T & T olfactometer using 54 concrete odor terms

Shunshiro Ohnishi, Toyohiro Yata[1], Masahito Katto and Yutaka Kurioka

Department of Electrical Engineering, Faculty of Science and Technology, Kinki University, 3-4-1, Kowakae, Higashi-Osaka, Osaka 577-8502 and [1]Nippon OTIS Co., 2-4-1, Nishi-shinjuku, Shinjuku, Tokyo 163-0023, Japan

In a previous paper, the odor quality of five odorants of a T & T olfactometer was evaluated by the semantic differential method using 28 concrete adjectives. Applying principal component analysis to the ratings for 32 subjects, the first component was extracted as 'bad smell' (including rotten smell)–'good odor' (including floral, fruity and sweet odors), and the meanings of higher order components were not clear. This seemed to be caused by the inadequacy of the evaluation items.

In the present paper, 54 concrete odor terms are used for odor-description as proposed by Shimoda and Osajima *et al.*, and two subjects evaluated odor quality for two concentration levels of the five odorants.

As a result of the principal component analysis, the four principal components were obtained. The first component is extracted as 'bad smell'–'good odor', which is similar to that of our previous paper. The second component is 'good odor', and the third and fourth components are 'good odor' or 'bad smell'. The meanings of higher order components show the 'bad smell' or 'good odor' of the first component in detail.

P30. Magnetic resonance imaging for diagnosis of congenital anosmia

Kohji Matsumoto, Tsunemasa Aiba[1], Kazuya Hashimoto and Kenta Tomiyama[2]

Department of Otolaryngology, Osaka City University Medical School, [1]Department of Otolaryngology, Osaka City General Hospital and [2]Department of Otolaryngology, Yodogawa Christian Hospital, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. e-mail: kmatsu@msic.med.osaka-cu.ac.jp

There are few patients who have had no sense of smell since birth. However, it is impossible to accurately determine whether they have genuine congenital anosmia or acquired sensorineural anosmia due to head trauma or viral infection in infancy. Magnetic resonance imaging (MRI) was performed on nine patients who had lacked a sense of smell since birth. Seven of them, including two patients with Kallmann syndrome, exhibited abnormality of the olfactory bulb, olfactory tract, olfactory sulcus or rectus gyrus, with some variation among patients in type and degree of abnormality. The other two patients exhibited normal olfactory pathway morphology, and for them the possibility of acquired sensorineural anosmia could not be ruled out. MRI is useful for determining whether patients with congenital anosmia have olfactory pathway anomalies. Many patients with congenital anosmia and hypoplasty or aplasty of the olfactory pathway nevertheless had no gonadal or endocrinological disorders.

P31. Alterations in olfactory function in workers exposed to heavy metals and organic solvents

Mariko Nishikitani, Shunichi Araki, Kazuhito Yokoyama and Hajime Sato

Department of Public Health and Occupational Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

To evaluate subclinical effects of heavy metals [manganese (Mn), chromium (Cr) and lead (Pb)] and organic solvents on olfactory functions, olfactometry tests (T & T olfactometer, olfactory perception threshold test and toluene threshold test) were conducted in welders (Mn), plating workers (Cr), CRT manufacturing workers (Pb) and rotogravure printers (toluene) in Korea and Japan. Blood concentrations of Mn, Cr and Pb in Mn, Cr and Pb workers were 0.6–2.3 (mean 1.4), 0.2–3.7 (mean 2.3), 11.0–41.6 (mean 24.6) μg/dl, respectively, which were significantly higher than those in control workers ($P < 0.05$). Blood toluene concentrations in solvent workers were 1.3–37.2 (mean 9.1) μg/dl. Results of the analysis of covariance showed that in Mn workers detection and recognition thresholds of the T & T olfactometer were increased; in Cr workers, only the recognition threshold was increased. In Pb and solvents workers, no significant changes in the olfactory thresholds were found. It is suggested that recognition of smell is most sensitive to the neurochemicals examined in this study (Mn and Cr). The T & T olfactometer seems a useful technique for the assessment of olfactory effects of occupational exposures to chemicals.

P32. Suppression of the frog electro-olfactogram by dithizone

Tadshi Ishimaru, Shigekazu Teranishi, Toshiaki Tsukatani, Toshiro Nishimura, Takaki Miwa and Mitsuru Furukawa

Department of Otorhinolaryngology, School of Medicine, Kanazawa University, 13-1, Takara-machi, Kanazawa 920-8641, Japan

Zinc is thought to be the most important element for olfaction but its role is still unclear. We have already reported that the frog EOG was suppressed under zinc-chelated Ringer's solution by dithizone and its suppression was not produced by the transduction channel blocking as reported in the last JASTS meeting. Then, we have investigated the effect of dithizone on the EOG produced by cholera toxin (100 μg/ml). The EOG caused by cholera toxin was also suppressed to 55.5 ± 19.9% (mean ± SD, n = 6) by dithizone. As the Ringer's solution was dithizonated before and after stimulation but non-dithizonated during the stimulating period, the EOG produced by the odor (20 μM n-amyl acetate) was suppressed. Therefore, suppression of the EOG by dithizone was not caused by chemical changing of the odorant. Under this suppression, EOG was recovered to 85.4 ± 14% (mean ± SD, n = 5) with a very small quantity of zinc (1 fM). These results indicate that the effect of the zinc chelator dithizone is the suppression of G-protein activity because cholera toxin is a stimulator of G-protein. Recovery of the EOG with zinc indicates that zinc probably has an important role in the transduction of the olfactory cell.

P33. A clinical study of olfactory disturbance

Seiichi Tomofuji, Megumi Fujii, Keijiro Fukazawa and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan

A consecutive series of 321 patients with olfactory disturbance in the Department of Otolaryngology, Hyogo College of Medicine during the 4 year period from 1995 to 1999 was studied. Of the 321 patients, 165 were male and 156 female. Their olfactory acuity was evaluated with a T & T olfactometer.

The olfactory disturbance of 197 patients was due to chronic sinusitis, in 44 patients it was due to the common cold, 24 patients had allergic rhinitis, 17 patients had head trauma and the reason for the disturbance in the remaining 38 patients was unknown.

Treatment was mainly based on a local injection into the submucosal tissue of the septal mucosa with suspended steroidal aqueous solution. Of 176 patients with chronic sinusitis, 127 underwent the endoscopic sinus surgery. The olfaction of 44 of the 127 patients could be evaluated; of these, 26 patients (59.1%) were improved.

It is important to clarify the cause of disease and the site of disturbance for the treatment of olfactory disturbance.

P34. Olfactory dysfunction following head trauma

Keijiro Fukazawa, Megumi Fujii, Sejichi Tomofuji and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan

A consecutive series of 15 patients with olfactory dysfunction following head trauma in the Department of Otolaryngology Hyogo College of Medicine during the 4 years from 1995 to 1999 was studied. Nine (60%) were male and six (40%) were female. The trauma was frequently observed in the occipital and frontal regions, with a few exceptions. The olfactory acuity was evaluated with a T & T olfactometer and an i.v. olfaction test (Alinamin® test). Improvement of the detection and recognition thresholds occurred in two patients (15.3%), and in four patients (30.8%) only the detection threshold was improved. Two of the four patients (50%) that had responded to the i.v. test were improved, and four of the nine patients that had not responded were improved. There was no definite correlation between the rate of response for some treatments and the degree of consciousness at the time of their injury. It can be said that treatments for the olfactory dysfunction should be performed even though the patients did not respond to the i.v test.

P35. Evaluation of olfaction after total laryngectomy

Megumi Fujii, Keijiro Fukazawa, Hiroki Yasuno, Chihiro Hatta and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan

Olfactory disturbance is common after laryngectomy. This phenomenon is explained by the fact that the patient breathes directly through the tracheostoma and the airway of the nasal cavity is separated from the lower respiratory system. However, there have been few reports regarding the olfactory function following laryngectomy.

In this study, we investigated the olfactory function of 23 patients who had undergone total laryngectomy. We evaluated the olfactory function by using the Jet Stream Olfactometer (JSO) and Alinamin® test (the venous intra-olfaction test) before laryngectomy and at 3, 6 and 12 months after the operation.

JSO is a new method for measuring the olfactory function of patients with a tracheostoma by blowing the odor into the nasal cavity.

All the patients in this study complained of olfactory disturbance after laryngectomy, and the olfactory function of almost all the patients was worse at 3 months after the operation. However, the olfactory function was improved in some patients at 6 months post-operation.

P36. Prediction of sensory odor attributes by an electronic nose

Shoichi Hayakawa, Mitsuya Shimada and Kiyoshi Matsumoto

Graduate School of Bioresource and Bioenvironmental Science, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

The purpose of this work was to predict sensory odor attributes by applying neural network algorithms to the sensory data and the responses obtained from an array of semiconductor tin dioxide gas sensors. Ninety-nine of the odorants whose sensory data were listed in the American Society for Testing and Materials Data Series DS 61 were used. A 79 data set was used as training data, a 10 set was used for validation data and another 10 set was used for test data. This gave a poor sensory prediction when sensory scores on a single odor descriptor (e.g. 'sweet') were used as training data. On the other hand, a good prediction of sensory odor attributes was obtained when similarity indices calculated from the sensory responses on 146 odor descriptors were used as training data. The

correlation coefficients between the sensory similarity indices and the predicted values were $R = 0.717$ for the similarity index to allyl caproate and $R = 0.746$ for the index to eucalyptol.

P37. Odor discrimination: correlation between the human olfactory sense and a fragrance and flavor analyser

Yosihiro Aoyama, Motoo Kinoshita, Miroshi Nakano, Hisamitsu Akamaru, Kurt Kolb and Jun-ichi Kita

Analytical Instruments Division, Shimadzu Corporation, 1 Nishinokyo-Kuwaharacho, Nakagyo-ku, Kyoto, 604-8511, Japan

This paper discusses the results of a new fragrance and flavor analyzer capable of predicting aromas in terms of human sensory values. Some previous fragrance analyzers cannot produce results corresponding to the human olfactory sense. The reasons for this are (i) lower sensitivity than the human nose; (ii) low confidence in the data due to humidity fluctuations; and (iii) difficulty in corresponding data to human terms with conventional analytical methods. This new analyzer combines metal oxide semiconductors, a thermal desorption tube and neural networks. This allows the system to have high sensitivity without being disturbed by humidity. Multi-layer perceptrons or radial basis functions can then be used to produce results that correspond to human sensory values. This is illustrated here with results from canned coffee samples. The coffee was tested by the new fragrance and flavor analyzer and a panel of human judges. The results indicate that the system is able to identify a relationship between 'smell' data and the human conception of the aroma.

P38. Comparison between measurements of coffee aroma by gas sensors and sensory evaluations

Iwao Tago, Masakazu Sakata, Yutaka Ueki, Toshiyuki Tamura and Kiyoshi Fukushima

SANYO Electric Co., Ltd, Commercial Equipment Systems Company, Product Development Center, 1-1-1, Sakata Oizumi-Machi, Ora-Gun, Gunma, Japan

In this study, we measured the aroma of brewed coffee by gas sensors and compared it with sensory evaluations; we also considered the advantages of applying gas sensors.

We stored coffee powder for 3–14 days at temperatures of 20, 35 and 50°C. After storage, we brewed coffee from the powder and measured the aroma by gas sensors. We used coffee powder before storage as a control and its corresponding measured value as a reference (100%). For the samples stored at 35 and 50°C for 14 days, the values decreased to 78 and 66%, respectively. On the other hand, for the samples stored at 20°C, it only decreased to 95% after 14 days.

We next compared these values with sensory evaluations, and found a correlation between them. Most people would not be aware of a change in samples of more than 90%. People who have a keen sense of smell would be aware of a slight difference in samples of between 80 and 90%. Most people would be aware of a serious deterioration in the samples of less than 80%.

As a result, it is thought that the evaluation of coffee flavor would be simpler and more effective by applying gas sensors along with sensory evaluations.

P39. Investigation about 'Sôkô', 2nd report (from the viewpoint of psychophysiology)

Kyoko Ohsawa

Office Harmonia, 3-31-6-404, Nishi-waseda, Shinjuku, Tokyo 169-0051, Japan

'Sôkô' is a system for the study of creating fragrances by self-expression. The system of Sôkô was established 20 years ago in Japan, in order to discriminate it from vocational fragrance design. I suggested in a previous report that the Sôkô system effected a decrease in mental stress similar to sedation, but there was no thorough examination of the process involved. Sôkô seemed to be divided into three stages: the first stage was the creation of an image of fragrance; the second stage was the measurement of the aromatic; and the third stage was the final churning and smelling. The electrodermal activity (EDA, OG-Giken BF-102R) was measured throughout Sôkô, and the psychological test 'Profile of Mood Status' (POMS, Japanese version) was performed before and after Sôkô.

Subjects were 35 females (31.2 ± 5.8 years old) who agreed to the aim of the experiments.

There were significant differences ($P < 0.05$) between stages 1 and 3; between stages 2 and 3, in the amplitude and trembling of the basal line in EDA, subjects seemed to be relaxed throughout Sôkô. There were significant differences ($P < 0.05$) between before and after Sôkô in all of the six scales of the POMS, especially the scale of confusion (C)—subject seemed to exhibit decreased anxiety, depression, hostility and confusion. There were no significant differences among age, period of experience of Sôkô and EDA or POMS.

I conclude that Sôkô has an effect not only of fragrance but also self-creation through the process of Sôkô.

I acknowledge the Miya Fragrance School (Shibuya, Tokyo, Japan) for their co-operation, and Prof. Katsuya Inoue (Tsukuba University, Tokyo, Japan) for academic advice.

P40. Relationships between the structural/electronic properties and odor activities of pyrazine derivatives

Kazuko Shimazaki, Hiroki Shikata, Masaomi Fukamachi, Nobuaki Higashi, Hiroaki Komatu, Fumihiro Omori, Kazuhiro Hayakawa, Tomoko Monobe and Naoki Miura

Tobacco Science Research Laboratory, Japan Tobacco Inc., Yokohama 227-8512, Japan

Pyrazine derivatives are the dominant compounds with a potato-like, earthy or nutty smell. In order to investigate the relation between the structural/electronic properties of pyrazine derivatives and their odor activities, a computational calculation was applied to ~20 pyrazine derivatives. The initial geometries were obtained by the systematic conformational search program CONFLEX combined with MM3. Each stable conformer was geometry optimized by the MP2/6-31G* basis set of *ab initio* calculation (Gaussian 94). The newly proposed parameter (effective lone-pair parameter) of the nitrogen atoms on the pyrazine ring, which includes the stereo and electronic features of the compound, could describe their activities well. To examine the kinetic steric effect of the substituent groups on the pyrazine ring to their odor type (potato-like or earthy), the steric constants (Ωs) were calculated. The activity of potato-like odor could be evaluated by this method. On the other hand, the earthy odor

could be evaluated by the electronic features of the substituent groups. These studies indicate that the stereo-electronic properties of the pyrazine ring and the substituent groups play an important role in the odor activity.

P41. Chemical structure–odor relationships of 'green odor' in green leaves

Kota Sano, Jun Suzuki and Akikazu Hatanaka[1]

Fragrance Laboratory, Soda Aromatic Co., Ltd., Tokyo and [1]Department of Life Science, University of East Asia, Shimonoseki, Japan

C_6-Alcohols and C_6-aldehydes, including leaf alcohol (3*Z*-hexenol) and leaf aldehyde (2*E*-hexenal), have been reported as important volatile components of 'green odor' in green leaves.

Sensory odor characters of 24 kinds of C_6-alcohols and C_6-aldehydes (*n*-hexanol, *n*-hexanal, 7-hexenols, 7-hexenals and 8-hexadienols) were disseminated by using eight sensory descriptive tems: grassy–leafy green, vegetable green, fruity, sweet, fresh, spicy, oily–fatty and herbal. Saturated compounds and each monoen-compound had strong characters in comparison with dien-compounds: *n*-hexanol and *n*-hexanal, oily–fatty; 2*E*-hexenol and 2*E*-hexenal: fruity; 3*Z*-hexenol and 3*Z*-hexenal, grassy–leafy green etc. The data from sensory evaluation were analyzed statistically using principal component analysis and from these results some relationships between chemical structure and odor were found. The double bond at the C-3 position was the important factor for 'green odor' in green leaves. The position of the double bond was more important than geometrical isomerism and the functional group for sensory characteristics. These tendencies may suggest that there are some relationships between chemical structure and human perception via receptor.

P42. The physiological effects of volatile components in a forest

Kazuhiko Sawada, Roichi Komaki, Yoshikuni Yamashita, Yasushi Suzuki and Seiichi Ishiyama[1]

Kanebo Co., Ltd, Cosmetic Laboratory and [1]Reserch Institute of Human Engineering for Quality of Life

The physiological effects of volatile components in a forest were investigated by contingent negative variation (CNV) and blood flow measurement.

After analyzing the atomosphere in the forest and the headspace gas of *Hiba* leaves by gas chromatography, α-pinene, (–)-limonene, bornyl acetate, Δ3-carene, thujopsene, farnesene and cedrol were selected to be tested. α-Pinene, bornyl acetate and Δ3-carene caused a decrease in CNV amplitude while thujopsene, farnesene and cedrol caused an increase. α-Pinene and Δ3-carene caused a decrease in blood flow whereas thujopsene, farnesene and cedrol caused an increase. It is found that many volatile components have physiological effects.

P43. The odor contribution of volatile compounds of marine algae on the seacoast

Yoshihiko Akakabe, Kenji Matsui and Tadahiko Kajiwara

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan. e-mail: akakabe@agr.yamaguchi-u.ac.jp

The characteristic odor components of the fresh marine green alga, *Ulva pertusa*, which are remeniscent of the sea or seacoast, are long-chain aldehydes such as (8*Z*,11*Z*,14*Z*)-heptadecatrienal, (8*Z*,11*Z*)-heptadecadienal, (8*Z*)-heptadecenal and pentadecanal. The biogeneration of these aldehydes that comprises the α-oxidation of fatty acids to (*R*)-2-hydroperoxy acids as an intemediate, followed by decarboxylation with a loss of one carbon atom, has been proposed. The enantioselective 2-hydroperoxylation of fatty acids was observed not only in green algae but also in brown and red ones. Therefore, these volatile aldehydes of marine algae must contribute to the odor of the seacoast.

P44. The examination of a fragrance's sleep-induction effect by using the frontal EEG as an index, 1

Tomoyuki Yoshida and Eri Goto[1]

National Institute of Bioscience and Human-Technology, Higashi 1-4, Tsukuba 305-8566, Japan and [1]Japan Airlines. e-mail: yoshida@nibh.go.jp

This study investigated a fragrance's sleep-induction effect by using the frontal EEG as an index. We used two fragrances, one of which had a waking effect (peppermint oil) and the other a calming effect (sandalwood oil). Twenty healthy students were divided into three groups. The first group smelled peppermint oil (concentration 1%, 0.06 g), the second smelled sandalwood oil (concentration 100%, 0.07 g) and the last group smelled no odor. The odors were presented to subjects with a mask on which an odor-contained filter paper was stuck. EEGs on the left and right frontal areas (Fpl and Fp2), and a heart rate on the left arm were recorded during the experiment (30 min). The subjects were told that they could sleep if they became sleepy in the experiment time. The EEGs were analyzed from a viewpoint of peak-frequency change and frequency fluctuation of a waveband. From the results of the EEG indices, we found that peppermint oil is very effective for the sleep-induction, because the peak frequency was decreased clearly in the peppermint mask in comparison with the other mask conditions. It is concluded that peppermint oil could lower the level of arousal and thus be suitable for sleep induction.

The examination of a fragrance's sleep-induction effect by using the frontal EEG as an index, 2

Eri Goto and Tomoyuki Yoshida[1]

Japan Airlines and [1]National Institute of Bioscience and Human-Technology, Higashi 1-4, Tsukuba 305-85660, Japan

This study was undertaken to elucidate the effect of sleep induction by smelling essential oil (peppermint, sandalwood). The results of study 1 suggested that peppermint oil should have the effect of reducing the arousal level and is suitable for sleep induction. In this study, however, peppermint was prepared for maintaining an awake-state, and essential oil conditions were group factors. We thus tried to study the sleep induction effect of peppermint oil again, with the same person experiencing all essential oil conditions (18 min). Odors were presented to 15 healthy students with the mask in the same way as in study 1. After the experiment, the sleep induction effect was evaluated from the self-evaluation and physiological indices. As a result, we found that the peak frequency of a waveband and heart rate was decreased at the end of the experiment in comparison with the start. The frequency fluctuation of a wave also became 1/*f*-like, which indicated comfortableness. This means that the subjects who smell

peppermint oil become more comfortable and sleepy than the subjects who smell sandalwood oil. As for the experimental procedure of this study, it was reconfirmed that the peppermint condition was more suitable for sleep induction than the sandalwood condition.

P47. Cloning of taste receptor tissue-specific genes of the fleshfly

Masayuki Koganezawa and Ichiro Shimada

Biological Institute, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578, Japan

The taste tissue (labellum) cDNA library was screened with the subtracted probe which enriched taste receptor tissue-specific genes to identify molecules involved in taste reception of the fly. To generate the probe, cDNAs of femur, antenna or eye, which do not contain taste receptor cells, were subtracted from cDNAs of tarsus or labellum containing the taste receptor cell.

Seven cDNAs were identified that showed sequence similarities to pheromone-binding protein (PBPs). The predicted amino acid sequences of these clones contain a putative signal peptide sequence at the N-terminal and the conserved six cysteines commonly shared with known insect PBPs. All seven clones were expressed mainly in taste tissue, labellum or tarsus. Some of them were also present in antenna or gut at a low level. The expression pattern of these clones was different from that of known insect PBPs or general odorant-binding proteins, which were present mainly in antenna. In addition, there was no difference between the expression of these clones in males and females. The unique expression pattern may suggest the role of these genes in taste reception and feeding behavior of the fly.

P48. A sweet saponin for the blowfly, *Phormia regina*

Arifa Ahamed[1], Seiji Tsurumi[2], Mamiko Ozaki[3] and Taisaku Amakawa[1,4]

[1]Graduate School of Science & Technology, [2]Radioisotope Research Center, Kobe University, Kobe 657-8501, [3]Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto 606-8585 and [4]Faculty of Human Development, Kobe University, Kobe 657-8501, Japan

Saponins are known to increase membrane permeability and thus induce cell lysis. However, some saponins, which have been reported as pharmaceutical components in oriental medicinal plants, show biological activities depending on their chemical structures. We introduce chromosaponin I (CSI) as a sweet substance for the blowfly, *Phormia regina*.

CSI is a γ-pyronyl triterpenoid saponin isolated from the pea and other leguminous plants. It stimulates the growth of roots in a variety of plants by interfering with the action of a plant hormone, ethylene. We found that CSI is a unique saponin; it stimulates the growth of plant roots and is also sweet for the blowfly. From these findings, we can say that CSI may function at a common target in the signal transduction systems in both plants and insects. To prove the action of CSI in the taste sensory system, we chose the blowfly chemosensillum on the labellum. The four functionally differentiated taste receptor cells generate distinguishable impulses in their amplitude from each other. The electrophysiological response from a single taste cell is thus easily observed by stimulating the sensillum with an adequate stimulus for each cell.

We found that CSI induces impulses only in the sugar taste receptor cells as well as full proboscis extension of the blowfly. The optimum concentration of CSI in these responses was 0.1 mM, which is much lower than that of sucrose. Based on the comparison of dose–response relationships, CSI is 100 times sweeter than sucrose. As far as we know, this is the first report describing that a natural saponin is sweet for insects, although this saponin is tasteless for humans. However, the mechanism of CSI action is different from that of sugars. Since the impulses induced by CSI appeared after a significant latency, CSI is likely to function inside the taste cells.

P50. Enhancement of salt taste response by organic acids; electrophysiological experiments with taste cells of the blowfly, *Phormia regina*

Yoshihiro Murata[1], Naoko Shirasugi-Kataoka[2] and Taisaku Amakawa[1,3]

[1]Division of Bioscience, Graduate School of Science and Technology, [2]Division of Life Environment, Faculty of Human Development and [3]Division of Science for Natural Environment, Faculty of Human Development, Kobe University, Kobe 657-8501, Japan

Human sensory test results indicate that small quantities of organic acids intensify saltiness. This was examined electrophysiologically in the present study using taste cells of the blowfly, *Phormia regina*. The impulse frequency during 200 ms was defined as salt taste cell response. The organic acids used for the experiment were acetic, succinic and citric acids. We used NaCl and KCl as saltiness materials. Salt taste response to 10 and 50 mM NaCl was increased by small amounts of the above organic acids and decreased with large amounts, these findings being consistent with human sensory test results. The organic acid concentration for maximum response was different, with that of citric acid being least, followed by succinic acid and acetic acid. For example, the most effective concentrations to accompany 10 mM NaCl are: 0.5 mM for citric acid, 1.5 mM for succinic acid and 50 mM for acetic acid. The pH for maximum salt taste response to 10 mM NaCl also differed in each case: 3.36 for citric acid, 3.49 for succinic acid and 3.08 for acetic acid. Salt taste response to 10 mM NaCl was not the same even when the sample pH was the same. Enhancement of salt taste response due to organic acid would thus appear not to depend on pH. All organic acids in this study enhanced salt taste response to 10 and 50 mM KCl.

P51. Control of taste sensitivity by the *Drosophila* taste receptor gene *Tre*

Hiromi Morita, Kunjo Isono, Kouhei Ueno, Masayuki Ohta, Yuka Mikuni[1], Satoshi Nakajima[1], Kazuo Yamamoto[1] and Yasuo Tsukahara

Graduate School of Information Science and [1]Faculty of Science, Tohoku University, Sendai 980-8577, Japan. e-mail: morita@biology.is.tohoku.ac.jp

A gene homologous to a G-protein-coupled receptor was identified in *Drosophila* by analyzing deletions produced by imprecise excisions of P-element which had been inserted near the taste gene *Tre*. *Tre* has been known to be involved in trehalose-specific taste sensitivity in the gustatory sensory neurons. In order to determine

whether the gene codes for the taste gene *Tre*, deletion mutants were genetically analyzed. Flies that carried large deletions uncovering the first exon of the cloned gene with severe reduction of the gene expression level as well showed noticeably reduced gustatory sensitivity to trehalose, which has been known to be present as a spontaneous dimorphism in wild populations. A complementation test between the spontaneous mutation of *Tre* and P-induced mutations further proved that those mutations are mutually allelic. Many internal deletions within the P-construct leading to the loss of the mini-white marker were obtained, but found to be normal with respect to gustatory trehalose sensitivity. Another P-excised mutant that showed a most severe gustatory phenotype was found to have genomic deletion uncovering upstream of the 5′ genomic region of the gene. It was concluded that the G-protein-coupled receptor gene codes for the previously described taste gene *Tre*.

P52. Structure–activity relationships of benzenesulfonic acid related substances in the salt receptor of the fleshfly *Boettcherisca peregrina*

H. Hatano, A. Furuyama and I. Shimada

Biological Institute, Graduate School of Science, Toboku University, Sendai 980-8578, Japan

Monovalent cation (Na^+), nucleotides, some anhydro-sugars and proline are known to stimulate the salt receptor of the fleshfly. We first found that benzenesulfonic acid (BSA) can stimulate the salt receptor in a dose-dependent manner. BSA is much more stimulatory than NaCl. BSA also stimulates the sugar receptor slightly, but does not show any clear dose dependency. The salt response to BSA is almost independent over the wide pH range 5.0–10.0. The experiments of chemical treatment of taste receptor cells of the fly suggest that the receptor site for BSA is different from those for NaCl and nucleotides. The stimulating effect of various derivatives and related compounds of BSA were examined to reveal the structure–activity relationship of BSA. Hydrophobic interaction at the benzene ring and hydrogen bonding at the sulfonic acid group may play a principal role when BSA reacts with the receptor site. A cyclohexane ring can be substituted for the benzene ring of BSA, but a hydrocarbon chain cannot replace it. A sulfinic or phosphonic acid group can be substituted for the sulfonic acid group of BSA, but a carboxy group cannot take its place. Based on the above results, we propose a model of the receptor site for BSA. Stereospecificity of the receptor site is firstly clarified for BSA in the salt receptor of the fleshfly.

P53. Analysis of *Hydra* glutathione-induced response by image moments

Kazumitsu Hanai, Akihiro Shibahara, Yu Iwasaki and Kazuo Sasaki

Department of Physics, Kyoto Prefecture University of Medicine, Kyoto 603-8334 and Division of Bioinformation, Faculty of Engineering, Toyama University, Toyama 930-8555, Japan

The *S*-methylglutathione-induced response of *Hydra japonica* is subject to suppression by a number of biologically active peptides. The suppression appears to occur at very low concentrations of peptides. It was first examined by careful observation by humans, and thus might suffer from human errors. Then we tried to examine the response by computer-assisted image analyses. *Hydra* images were taken as digital images by a black and white CCD camera via a frame grabber. Several image parameters were calculated from the binary images. Among these parameters, the autocorrelation functions calculated from the time series of area and secondary moment showed a difference in the presence and absence of 100 ag of acidic fibroblast growth factor (aFGF). We found a significant difference in the area image parameter obtained in the presence and absence of aFGF. The difference, though small, was dependent on the aFGF concentration, which was similar to that seen by human observations. These observa- tions suggest that the glutathione-induced *Hydra* response is more sensitive to aFGF by six orders of magnitude than conventional cultured cell systems.

P54. Morphology of presumable taste organs in several species of cephalopods

Katsuto Suzuki, Kazuhiko Anraku[1], Noriko Akikusa, Yoshimichi Watanabe and Takayuki Marui

Department of Oral Physiology, Ohu University School of Dentistry, Koriyama 963-8611 and [1]Laboratory of Fisheries Engineering Faculty of Fisheries, Kagoshima University, Kagoshima 890-0056, Japan

Since few morphological studies of chemoreceptor cells of cephalopods have been reported (e.g. Graziadei, 1965, 1976), a light and scanning electron microscopical investigation (LM and SEM) of presumable chemosensory receptor cells in the cuttlefish (*Sepia esculenta*), squid (*Todarodes pacificus*) and octopus (*Octopus vulgaris* and *Paraoctopus dofleini*) was performed. Several types of receptor cells were identified, which were compared with the histological results of the chemoreceptors on Cephalopoda investigated so far. Although chemosensory cells (chemoreceptors) of several kinds of cephalopods have not been clearly identified by this anatomical study, the silver staining method reveals presumed epithelial chemoreceptors, possibly nerves themselves. These presumed chemoreceptors are located on the suckers of their tentacles, lip, oral cavity and the arms, which are involved in a series of feeding behaviors. SEM studies in four species of cephalopods confirmed that the lip, oral cavity and suckers of the tentacles and arms possess presumed chemoreceptors. However, further precise histological investigations have to be done with semi-ultra-thin sections for LM and TEM. The functional characteristics for these putative chemoreceptors should be checked to confirm their chemo- or mechano-receptive role.

P55. Gustatory responses to amino acids from the facial nerve of zebrafish, *Brachiodanio rerio*

Noriko Akikusa, Yoshimichi Watanabe, Katsuto Suzuki and Takayuki Marui

Department of Oral Physiology, Ohu University Dental School, Koriyama 963-8611 Japan

The zebrafish (*Brachiodanio rerio*) is a popular vertebrate for genetic analysis and its genetic control. Important implications regarding the encoding of gustatory signals may be obtained by using this animal in terms of physiology and molecular biology. Thus, we have tried to reveal the gustatory response characteristics in this animal, since little is known regarding the physiology of the taste system of the zebrafish.

The stimulatory effectiveness of amino acids on the external gustatory system of the zebrafish was investigated with extracellular electrophysiological techniques. Integrated responses to the application of ~0.5 ml (flow rate, 0.2 ml/s) taste solution were

analyzed. Twelve amino acids solution at 1 mM among 14 chemicals were highly effective for the system. The relative response magnitudes to the standard (1 mM L-Ala) for the effective compounds are in the order L-Ala (100) > L-Pro (99.0) > L-CysH (79.8) > Gly (79.0) > L-Lys–HCl (57.5) > L-Arg (32.6) > L-Glu–Na (18.5) > β-Ala (18.3). This response spectrum was compared with those of other fish species.

P56. Innervation of taste buds in the barbels of goatfish

Yoko Sakata, Sadao Kiyohara and Junzoh Tsukahara

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

The innervation of taste buds in the barbels of goatfish was examined. The barbel nerve is located in the caudal portion at the base of barbel and sends large nerve branches continuously running to the tip of the barbel. These branches run mediorostrally and divide into small nerve branches running toward the tip of the barbel. Each branch runs a little way and is subdivided into two components which course circularly at a branching site in the opposite direction. Each circular branch ends within one half of the barbel and has two terminal branches. Each terminal branch runs toward the surface of the epithelium to give two sub-branches. Each sub-branch ramifies repeatedly to form seven or eight strands, each of which enters the ventral portion of a taste bud to makes a nerve plexus. Therefore, the longitudinal running nerve branch is a functional unit of taste fibers and innervates four groups of seven or eight taste buds located at almost the same level of the barbel. Small nerve bundles comprising coarser fibers than nerve branches of taste fibers were found to run along an elastic layer of the perichondrium encapsulating the longitudinal cartilage and terminate on or in the perichondrium to function as proprioceptors monitoring the positional state of the barbel.

P57. The primary facial taste center, the facial lobe, in goatfish

Sadao Kiyohara

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

We previously showed that the primary taste center of goatfish is characterized by an extraordinarily developed facial lobe (FL) which occupies the dorsal regions of the medulla. The FL has a surface so highly convoluted it forms lobules. Each lobule consists of three different layers. In this study, peripheral projections to the FL were examined. After application of dextran amine to the central cut end of the ramus hyomandibularis, labeled fibers were found in the descending trigeminal root, facial sensory root and facial motor root. The labeled facial fibers project to the entire regions of the FL and terminate exclusively in layers of neuropile. Most of the labeled trigeminal fibers terminate in their ordinal targets, principal and spinal trigeminal nucleus in the medulla. No direct projections were found to the FL. However, when the entire root of the trigeminal nerve was labeled, minor projections were found to the proximal part of the FL. We also noticed differences in the structures of the primary taste center between *Parupeneus spilurus* and *Upeneus arge*. In *Parupeneus* ventral facial lobe, a columnar nucleus is located between the dorsal FL and vagal lobe, while this lobe is lacking in *Upeneus*. This difference suggests that *Parupeneus* has more developed taste epithelia in the lips and oropharyngeal cavity than *Upeneus*.

P58. Gustatory pathways from the facial primary gustatory nucleus to the spinal cord in the sea catfish *Plotosus lineatus*

Takuji Yoshitomi and Sadao Kiyohara

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

The primary facial taste center, the facial lobe (FL), of the sea catfish *Plotosus lineatus* has a distinct somatotopical map. The entire body surface of the catfish is represented in the FL, and the barbels and trunk-tail are sharply defined in the lobules extending rostrocaudally in the FL. Previous behavioral studies showed that the catfish is able to search and intake food using the gustatory sense only. In particular, the facial gustatory system participates in a food search behavior. This fact suggests that the gustatory information must reach the spinal motor neurons. The aim of this study is to reveal the gustatory pathways from the FL to the spinal cord in *Plotosus*, using horseradish peroxidase, carbocyanine dye and rhodamine-conjugated dextran amine as neurotracers in fixed or live brains. The present results show two faciospinal pathways as shown previously in channel catfish (Kanwal and Finger, 1997). One is a direct projection from the trunk-tail lobule of the FL to the dorsal region of the spinal cord. The other is an indirect projection from the facial lobe except the trunk-tail lobule to both the dorsal and ventral regions of the spinal cord by way of neurons located in the reticular formation of the medulla. Neurons projecting to the reticular formation always located in the ventral margin of barbel lobules and had >200 μm dendric field in each lobule. The projecting neurons to both the rcticular formation and the spinal cord belong to large types of neurons (Kiyohara *et al.*, 1996).

P59. Postnatal development of taste buds in the oral cavity of the postnatal marmoset

Keiko Yamaguchi, Shuitsu Harada, Norikazu Kanemaru and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, 8-35-1Sakuragaoka, Kagoshima 890, Japan

The development of taste buds on the soft palate (SP), fungiform (FF), foliate (FL) and circumvallate papillae (CV) at different postnatal ages was examined histologically in the marmoset. After paraffin embedding, complete serial sections at 10 μm thickness were made and stained by H&E. Digitized images for each section were examined carefully. The number of FF taste buds at 1 day was 334. While only 20% of all the taste buds at birth possessed a taste pore, 39% of 182 taste buds on the SP at 1 day did. The number of taste buds with pores at 1 day was small for the center CV (19 of 59), one side CV (7 of 25) and one side FF (2 of 16). The total number of taste buds increased with increasing age and reached a maximum at 2 months of age: FF, 1069; SP, 609; CV-center, 530; CV-side, 390; FF-side, 201. Virtually all taste buds possessed a taste pore after 2 months of age. The number of taste buds tended to decrease with increasing age after 2 months of age. These results suggest that the functional maturation of SP taste buds might precede maturation in other areas on the tongue, and that the

decrease in the number of taste buds in the oral cavity with increase of age might change taste sensitivity.

P60. Electrophysiological properties of cells in the taste papilla of the bullfrog morphologically identified by intracellular staining

Hiroko Takeuchi, Takashi Tsunenari and Akimichi Kaneko

Department of Physiology, Keio University School of Medicine, Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan

To correlate the function and morphology of cells in the taste papilla, we made a patch clamp recording in the whole cell configuration from a slice preparation of the bullfrog taste papilla. The pipette contained 0.2% Lucifer yellow, which diffused into the cell during the whole cell recording and revealed the entirety of the recorded cell. The mucous cell, the wing-type cell and the rod-type cell were morphologically identified. In mucous cells no voltage-gated current was recorded, but they were dye-coupled. Both wing- and rod-type cells showed voltage-gated Na (I_{Na}) and K (I_K) currents, but the I_{Na}/I_K ratio was different (1.35 in the wing-type and 0.33 in the rod-type cell, $P < 0.001$). The wing-type cell responded to puffer-applied quinine (10 mM), but neither the mucous cell nor the rod-type cell did. Existing morphological reports suggest that the wing-type cell is a supporting cell because it does not have synaptic structures. This suggestion is hardly acceptable because the wing cell responds to bitter taste stimuli and generates action potentials. Rod-type cells may respond to other taste substances, or may be in an immature stage of differentiation.

P61. The change of fungiform papillae of tongue after chorda tympani damage—long-term observation

Masanori Umemoto, Mieko Sone, Megumi Fujii and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8132, Japan

Information was obtained by microscopic observation of the fungiform papillae of the tongue after chorda tympani damage. In previous studies, the threshold of electrogustometry and the filter paper disc test were found to be higher in those areas innervated by the chorda tympani after middle ear surgery. However, changes of fungiform papillae of the tongue were not reported. In this study, we observed fungiform papillae and capillary vessels in the papillae after middle ear surgery. After 10 years of chorda tympani damage, fungiform papillae were of the normal shape, but the capillary vessels in the papillae had disappeared. These results show that fungiform papillae of the tongue were controlled only by the aorta tympanica posterior.

P62. Some fine structural observations of the vallate papillae taste buds of rats with a taste disorder induced by tetracycline

Takuma Yoshikawa, Masato Imada[1], Minoru Ikeda, Tomio Arikuni[1] and Akinori Kida

Departments of Otorhinolaryngology and [1]Anatomy, Nihon University School of Medicine, Oyaguchi-kami-machi, Itabashi-ku, Tokyo, Japan

In the present study we have investigated taste buds of rats with a taste disorder by electron microscopy, with special emphasis on afferent nervous elements in the vallate papillae taste buds. The taste disorder was induced in rats by i.p. injection of tetracycline for 2 weeks and then the degree of the disorder was assessed by a two-bottle preference test. In the normal rat, afferent nerve fibers in the connective tissue core were unmyelinated and surrounded by Schwann cells. After entering the basement membrane of the taste buds, intragemmal nerve fibers were surrounded by dark cells or their processes and approached the receptor clear cells. The terminal portions of the intragemmal nerve fibers contained many synaptic vesicles (round clear and cored vesicles) and mitochondria. Receptor cells also contained many round clear and cored vesicles. Since the apposed cell membranes were postsynaptically dense, both the terminal portion and the clear cell made a reciprocal synapse. Such a reciprocal synapse was also observed in rats with a taste disorder. In the taste disorder, however, afferent terminal portions were frequently observed at the basal part of the taste buds, appeared swollen and were surrounded by processes of dark cells. Some clear cells contained enlarged rough endoplasmic reticulum. Receptor clear cells were decreased in number and made few synaptic contacts on terminal portions. Highly electron dense bodies appeared occasionally in the nerve fibers and in their enlarged terminal potions. There were many vesicles, mitochondoria and high density granules in some enlarged afferent nerve endings, as if they had lost contact with receptor cells. Afferent nerves at the basal portion of the taste buds did not make synapses with the basal cells. Taking these findings together, taste disorder induced by tetracycline is due to damage to the sensory receptor cells in the taste buds.

P63. Optical recording of cytosolic Ca^{2+} concentrations in mouse taste bud cells *in situ*

Satoshi Shiobara, Yoshitaka Ohtubo[1], Kiyonori Yoshii[1] and Takashi Kumazawa[2]

Departments of Materials Science and Engineering and [3]Applied Chemistry, Saitama Institute of Technology, Okabe 369-0293 and [2]Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka 820-8502, Japan

Taste stimuli increase the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{in}$) in isolated taste bud cells (TBCs), where taste stimuli might reach not only their receptor membranes but also their basolateral membranes because they were exposed by the isolation. Many taste substances will damage basolateral membranes and increase $[Ca^{2+}]_{in}$. In order to record the taste response-induced $[Ca^{2+}]_{in}$ change, we developed a method to stimulate receptor membranes only while recording the $[Ca^{2+}]_{in}$ in mouse taste bud cells embedded in peeled lingual epithelia with a calcium indicator, calcium green-1. In 30% of TBCs, 0.5 M NaCl, 1 mM denatonium and 1 mM quinine applied on receptor membranes increased the fluorescent intensities of calcium green-1. The application of 1 mM $CdCl_2$ on basolateral membranes blocked the NaCl-induced increase the $[Ca^{2+}]_{in}$ while the application of 10 μM ryanodine on basolateral membranes had no effect. These results suggest that the elevation of $[Ca^{2+}]_{in}$ in response to NaCl is caused by the influx of the extracellular Ca^{2+}. Although single mouse taste buds contain 50 cells, only a few cells have synaptic contacts with taste nerves. Our data showed that the number of taste bud cells that increased

[Ca^{2+} l_{in} in response to taste substances was larger than that having synaptic connections with taste nerves.

P64. Expression of *Prox1* in rat taste buds

Yuji Seta, Takashi Toyono, Hidemitsu Harada and Kuniaki Toyoshima

Department of Oral Anatomy II, Kyushu Dental College, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803-8580, Japan

Prox1 encodes a homeobox protein that is structurally homologous to *Drosophila prospero*. In fly neurogenesis, expression of *prospero* depends on basic helix–loop–helix (bHLH) genes in neuroblasts and ganglion mother cells (GMCs), and is involved in subsequent specification of neuronal progeny. Using RT-PCR and *in situ* hybridization, we investigated if *Prox1* gene expression occurs in rat taste buds. Further, we examined dynamics of *Prox1* expression in the process of degeneration and regeneration in rat denervated taste buds. The results indicate that *Prox1* is expressed in cells of the taste bud cell lineage. In denervation experiments, *Prox1*-expressing cells constitute an early stage of progenitor (transit amplifying cells) in rat taste buds, but are not self-renewing stem cells. We conclude that the expression of *Prox1* in rat taste buds is required for neural induction.

P65. Bovine circumvallate taste buds: structure and gustducin localization

Shoji Tabata, Takahiko Kobayashi, Takafumi Gotoh, Shotaro Nishimura, Michio Muguruma[1] and Hisao Iwamoto

Department of Animal Science, Kyushu University, Fukuoka 812-8581 and [1]Department of Biological Resource Sciences, Miyazaki University, Miyazaki 889-2129, Japan

The structure of taste cells in bovine circumvallate papillae was studied by light and electron microscopy, and the localization of gustducin was also investigated by biochemical and immunohistochemical methods. Taste buds existed only in the inner epithelium of the trench. Under electron microscopy, two cell types were identified as candidates for the taste receptor cells. The type I cell had electron lucent cytoplasm and possessed many electron dense cored vesicles in the apical cytoplasm. The type II cell had cytoplasm that was more electron dense than that of the type I cell and possessed many electron lucent vesicles in the cytoplasm. Both types of cell protruded microvilli into taste pores and revealed synaptic contacts with nerve fibers. In Western blotting, the specimens with circumvallate papillae revealed obvious immunoreactivity at ~40 kDa against gustducin antibodies, whereas the lingual specimens without taste buds revealed no specific reactions. The immunocytochemical localization of gustducin was investigated using the avidin–biotin complex method and the 1.4 nm gold and silver enhancement method. A subset of taste cells showed immunoreactivity under light microscopy. The electron microscopic specimens showed that only the type II cells exhibited gustducin immunoreactivity and this was localized in the electron lucent vesicles. The reaction products were especially associated with the membrane of the vesicles. The microvilli in the taste pores also showed specific immunoreactivity.

P66. Investigation of the relationship between response type to membrane-permeable cyclic nucleotides and expression of gustducin in rat taste cells

Kumiko Sugimoto and Kirio Kawai

Section of Molecular Neurobiology, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8549, Japan

It has been reported that gustducin (Ggust) plays an important role in transduction mechanisms for sweet and bitter taste via a decrease in intracellular cyclic nucleotide concentration, which may lead to activation of cyclic nucleotide-suppressible conductances. In the present study, to elucidate what type of response induced by cAMP is related to Ggust, the expression of Ggust was investigated by immunocytochemistry in the taste cell after the membrane conductance changes induced by cAMP were recorded in the same cell using whole-cell patch-clamp techniques. Male Wister rats, 8–15 weeks of age, were used for experiments and taste cells were isolated from vallate and foliate papillae by treatment with collagenase and EDTA. To elevate the intracellular cAMP concentration, a membrane-permeable cAMP analogue, 8-br-cAMP, was applied to the bath solution, and changes in whole cell currents were recorded. The remarkable effect of 8-br-cAMP was the suppression of voltage-dependent outward K^+ currents, which was observed in six cells out of 15 cells. Three of these six cells demonstrated positive iminunoreactivity to Ggust. On the other hand, an increase in voltage-dependent outward K^+ currents induced by 8-br-cAMP was observed in the other three cells, all of which did not express Ggust. When K^+ currents were blocked by a pipette solution of CsCl, three out of 11 cells showed a remarkable increase in non-selective cation conductance in response to 8-br-cAMP. The immunoreactivity to Ggust of these cells was negative. Only one cell that expressed Ggust demonstrated a decrease in non-selective cation conductance with 8-br-cAMP, which supports the mechanism that Ggust activates cAMP-suppressible conductances. These results suggest that elevated cAMP in taste cells regulates not only voltage-dependent K^+ channels but also non-selective cation channels in various manners.

P67. Effect of glossopharyngeal efferent fiber-induced slow potential on taste cell response in the frog

Toshihide Sato, Takenori Miyamoto, Yukio Okada and Rie Fujiyama

Department of Physiology, Nagasaki University School of Dentistry, Nagasaki 852-8588, Japan

Stimulation of the glossopharyngeal (GP) nerve of the frog induced positive or depolarizing slow potentials from the tongue surface and the taste cells in the fungiform papillae. The amplitude of the slow potentials was stimulus intensity- and frequency-dependent. The activity of A type afferent fibers in the GP nerve was not related to the slow potential. Intravenous injection of atropine abolished the positive or depolarizing slow potentials evoked by GP nerve stimulation. Therefore, activation of autonomic postganglionic fibers induced the slow potentials. The amplitude and polarity of the slow potentials were changed dependent on the types of adapting solutions on the tongue surface. These results suggest that the slow potentials recorded

from the tongue surface and the taste cells may be due to the liquid junction potential generated between saliva secreted from the lingual glands by GP nerve stimulation and adapting solutions on the tongue surface. The receptor potentials induced by tastants were modulated by depolarizing or hyperpolarizing slow potentials induced by GP nerve stimulation.

P68. Effect of GTPγS on saccharin-induced responses in bullfrog taste cells

Yukio Okada, Rie Fujiyama, Takenori Miyamoto and Toshihide Sato

Department of Physiology, Nagasaki University School of Dentistry, Nagasaki 852-8588, Japan

Taste stimuli interact with receptor sites in the apical taste membrane, triggering a biochemical cascade via G protein activation. We examined the effect of GTPγS on the saccharin-induced current in the taste cells of bullfrogs. The membrane currents of rod-type taste cells were measured using a conventional whole-cell patch-clamp technique. In eight of 30 cells dialyzed with K^+, low Cl^- (10 mM) internal solution, 30 mM saccharin elicited an inward current of –34 ± 14 pA (n = 8) at a membrane potential of –50 mV. The saccharin responses consisted of five transient ones and three sustained ones. In 25 rod cells dialyzed with 0.5 mM GTPγS, six cells displayed a saccharin-induced inward current of –59 ± 36 pA (n = 6). Thus, GTPγS displayed an enhancing tendency for saccharin to elicit an inward current, but there was no difference in the response rate between the control and GTPγS-dialyzed cells. The dialysis of heparin (1 mg/ml, an inhibitor of IP_3 receptor) greatly inhibited the saccharin-induced responses. The dialysis of *Pasteurella multocida* toxin (5 μg/ml, an activator of PLCβl) could not induce any response in the taste cells. The results suggest that saccharin may raise the intracellular IP_3 level via a cascade other than Gqα-PLCPβ1 in frog taste cells.

P69. Comparison between human and gerbil fungiform papillae in the intracellular Ca^{2+} response elicited by sweeteners

Rie Fujiyama, Toshihiro Miyazaki, Takenori Miyamoto, Yukio Okada and Toshihide Sato

Departments of Oral Physiology and [1]Oral Histology, Nagasaki University School of Dentistry, Nagasaki 852-8588, Japan

We examined changes of intracellular free calcium concentration elicited by taste stimuli of sucrose and sodium saccharine in the taste buds of human and gerbil fungiform papillae. The cytosolic Ca^{2+} changes were monitored with the Ca^{2+}-sensitive dye Fura-2 using lingual epithelial sheets containing some fungiform papillae from humans and gerbils. We confirmed the presence of the taste buds in the papilla by histological observation. In the human papillae, sodium saccharine (60 mM) and sucrose (0.5 M) increased intracellular free calcium concentration in 23% (n = 7) and 10% (n = 10) of the taste buds tested, respectively. In the gerbil papillae, sodium saccharine (60 mM) and sucrose (0.5 M) increased intracellular free calcium concentration in 40% (n = 35) and 50% (n = 10) of the taste buds tested, respectively. These results suggest that the gerbil's sensitivity to sweeteners is much higher than the human's. It has been reported that the gerbil's electrophysiological taste threshold to sweeteners is slightly lower than that of other mammals. Our present findings about response rate to sweeteners may be consistent with the nerve response.

P70. KCl response of mouse taste cells is mediated by an intracellular second messenger system

Takenori Miyamoto, Toshihiro Miyazaki[1], Rie Fujiyama, Yukio Okada and Toshihide Sato

Departments of Oral Physiology and [1]Oral Histology, Nagasaki University School of Dentistry, Nagasaki 852-8588, Japan

We have already reported that in taste cells of mouse fungiform papillae, apically applied 0.5 M KCl induces an inwardly rectifying current (fir), which was suppressed not only by a K^+ channel blocker, Cs^+, but also by a Cl^- channel blocker, niflumic acid. The reversal potential of the KCl-induced response was independent of the apical ionic concentration, but rather was close to the mid-point between the equilibrium potentials of Cl^- and K^+ at the basolateral membrane. KCl-induced I_{ir} displayed a fast rundown under the condition of the conventional whole-cell clamp method, but no rundown was observed using the perforated patch method, suggesting that the transduction mechanism for KCl response requires some intracellular factors. The perfusion with the internal solution containing 300 μM GTPγS induced a similar I_{ir} to that induced by KCl. In addition, an enhancement of the L-type Ca^{2+} current was induced by KCl stimuli in some taste cells using the perforated patch method. The immunohistochemical reactivity of the taste bud cells of fungiform papillae was positive to a cloned inwardly rectifying Cl^- channel, CLC-2. On the other hand, some peripheral cells in the taste bud were positive to a cloned inwardly rectifying K^+ channel gated by the G protein, GIRK1. No immunoreactivity to CLC-3, GIRK2 and ROMK1 was observed. These results suggest that the transduction mechanism of the KCl-induced response is mediated mainly by the inwardly rectifying Cl^- and K^+ channels, which are activated by second messenger coupled to G protein, and additionally by the enhancement of L-type Ca^{2+} channels.

P71. Sucrose preferences and salivary constituents of C57BL mice fed a gymnema-containing diet

Hideo Katsukawa, Yuzo Ninomiya, Toshiaki Imoto[1] and Tadataka Sugimura

Department of Oral Physiology, Asahi University School of Dentistry, Hozumi, Gifu 501-0296 and [1]Department of Physiology, Faculty of Medicine, Tottori University, Yonago 683-0826, Japan

Gymnema sylvestre contains a peptide, called gurmarin, that suppresses sweet responses of the chorda tympani nerve of rodents. Our previous studies demonstrated that the sucrose preference of rats decreased immediately after the start of feeding of gymnema diets and subsequently returned to the control level. This suggests that gurmarin-binding proteins induced in their submandibular saliva contribute to the observed recovery of preferences by suppressing the effects of gurmarin. We examined induction of similar proteins in mice fed a 3%-gymnema diet by analyzing preferences for sucrose solutions and saliva constituents. Preference percentages for 0.03 M sucrose significantly decreased at 1–2 days after the start of the gymnema diet in C57BL mice (gurmarin sensitive), whereas there was no change in preference during a feeding trial in BALB mice (gurmarin insensitive). Recovery of preference was recognized at 3–4 days of a trial in mice with

the submandibular gland intact but not in mice with sialadenectomy. When added to the reaction mixture of gumarin and anti-gurmarin serum (ELISA), submandibular saliva of C57BL mice fed the gymnema diet inhibited the reaction more intensely than that of mice fed the plain control diet. This suggests that gurmarin-binding proteins are induced in the saliva of mice fed gymnema diets, as in rats fed the gymnema diet. Probably, saliva compositions modified by dietary ingredients increase the preference for food containing these ingredients.

P72. Effect of zinc deficiency on the lingual trigeminal nerve fibers in rats

Tomoko Goto, Michio Komai and Yuji Furukawa

Laboratory of Nutrition, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

It is well known that zinc deficiency causes abnormalities in general taste sensation. We have reported that the responses of the lingual trigeminal nerves to carbonated water are lower in zinc-deficient rats than in zinc-sufficient rats. We therefore investigated the effect of zinc deficiency on the lingual trigeminal nerve fibers in rats. Male Sprague–Dawley rats, 4 weeks old, were divided into four groups (Zn-Def, Low-Zn, Zn-Suf, Pair-fed). After feeding on the experimental diet for 42 days, the rats were anesthetized and their lingual trigeminal nerve was cut and fixed by formalin solution. Paraffin sections were prepared and stained with luxol fast blue. The area of the lingual trigeminal nerve was significantly smaller in Zn-Def rats than in Zn-Suf and Pair-fed rats. Myelin sheaths were significantly thinner in the Zn-Def group than in the other three groups, their thickness being maximum in Zn-Suf and Pair-fed groups and intermediate in the Low-Zn group. These results indicate that the myelination of the lingual trigeminal nerve fiber is affected by dietary zinc content.

P73. Undissociated acid-induced chorda tympani nerve response in rats

Kazuma Ogiso, Yasutake Shimizu and Keiichi Tonosaki

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

We investigated whether undissociated acid is capable of exciting the chorda tympani nerves (CTN) in rats, using buffered acid solutions as taste stimuli. These solutions were prepared by adding alkali to weak acids (NaoH, KOH or Tris, pH-adjusted from 3.0 to 7.0 at 0.5 intervals) such as acetic acid, so that the proportion of undissociated and dissociated acids was varied while keeping the total acid concentration constant (0.1 M). When acetic acid solutions, adjusted to wide ranges of pH by NaOH, were applied to the tongue the response magnitude of the CTN did not vary systemically with pH changes. However, if the sodium effect was eliminated by amiloride or replacement of the cation by potassium or Tris, the CTN response was reduced systemically as pH increased. Similar results were obtained with citric acid and ascorbic acid. These pH-dependent changes in the CTN responses to acid cannot be solely attributed to the proton gradient, because the response magnitude induced by hydrogen itself, which was estimated from responses to strong acids (HCl), was much smaller than that by equi-pH acetic acid (~85% less). Thus we cannot explain the pH-dependent responses of the CTN to weak acids unless effects of undissociated acid molecules are postulated. It is therefore concluded that undissociated acids in weak acid solutions can be a stimulant to taste receptor cells.

P74. Chorda tympani nerve responses to mixtures of a metabotropic glutamate receptor agonist and sweet substances

Noritaka Sako and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita 565-0871, Japan

We recent by found that the synergistic responses to binary mixtures of monopotassium L-glutamate (MPG) and 5′-inosine monophosphate (IMP) were suppressed by gurmarin, an anti-sweet peptide, but were not suppressed by *S*-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4), a metabotropic glutamate receptor antagonist (Sako and Yamamoto, 1999). These results suggest that the synergism of umami substances occurs on the sweet-responsive macromolecule. In the present study, to investigate the role of the umami receptor and the sweet-responsive macromolecule in the synergistic effect, integrated chorda tympani nerve responses to the binary mixture of metabotropic glutamate receptor agonist (L-2-amino-4-phosphonobutyric acid; L-AP4) and sweet substances were measured. Results were as follows: (i) the mixture of L-AP4 with one of 0.1 M sweet substances—sucrose, glucose, fructose and maltose—showed strong synergistic effects, but the mixture of L-AP4 and 5% Polycose showed negligible synergistic effects. (ii) The synergistic responses to mixture of sweet substances and L-AP4 were not suppressed by 50 μM gurmarin or 2% pronase E, an anti-sweet enzyme. These results suggest that synergistic mechanisms for L-AP4 and sweet substances are different from those for glutamate and IMP.

P77. Responses of the glossopharyngeal nerve in the aquatic toad, *Xenopus laevis*, to dipeptides

Y. Sakata and S. Yamashita

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kohriyama 1-21-35, Kagoshima 890-0065, Japan

The dipeptide sensitivity and specificity of the glossopharyngeal taste system of the aquatic toad, *Xenopus laevis*, was characterized. The taste system of *Xenopus* responded to most of 68 dipeptides tested at 1 mM, but was highly sensitive to ~20 of these stimuli. In general, dipeptides with hydrophobic or basic amino acids residues in the N- or C-terminal were more effective than those with neutral amino acids residues having short side chains. Dipeptides composed of amino acid residues with an aromatic ring were also highly stimulatory. These amino acid residues were more effective in the C-terminal than in the N-terminal. The estimated electrophysiological threshold for Gly–L-Arg, the most effective dipeptide, was ~1 μM and did not saturate at concentrations up to 3 mM. Higher cross-adaptation coefficients were identified between dipeptides and between dipeptides and their component amino acids. In contrast with this, cross-adaptation coefficients were lower between dipeptides and non-component amino acids and quinine–HCl. These results suggest that receptor sites for

dipeptides have a stimulatory process more similar to amino acid than to quinine–HCl.

P78. Enhancement of NaCl responses by ANS in the frog glossopharyngeal nerve

Kazuhisa Okuda-Akabane and Yasuyuki Kitada

Department of Oral Physiology, School of Dentistry, Iwate Medical University, 1-3-27 Chuodori, Morioka, Iwate 020-8505, Japan

In salt taste reception, it has been proposed that salt stimuli can use the submucosal pathway as well as the mucosal pathway. If chemicals were large molecules unable to pass through the tight junction between taste cells, they could not affect the salt response via the submucosal pathway. It has been reported that treatment of the tongue surface with 1-anilinonaphthalene-8-sulfonate (ANS), a large molecule, enhances the NaCl response in the frog glossopharyngeal nerve (GL) (Kashiwagura *et al*., 1977). In the present study, we investigated the properties of the enhancement of NaCl responses by ANS in the frog GL in order to determine the pathway responsible for the NaCl response. The summated responses from GL were recorded in anesthetized frogs. The enhancing effect of 1 mM ANS on the 100 mM NaCl response was reversible, and disappeared within 2 min. So, it is unlikely that the ANS enhancing effect is due to the action of ANS through the submucosal pathway. Amiloride at 0.1 mM failed to inhibit the enhanced response to 100 mM NaCl by 1 mM ANS. These findings suggest that amiloride-insensitive Na^+ channels (or receptors) are present in apical membranes of frog taste cells.

P79. Effects of antidromic electrical stimulation of the superior laryngeal nerve on the nerve activity of the superior laryngeal nerve in rats

Takamitsu Hanamori

Department of Physiology, Miyazaki Medical College, Miyazaki 889-1692, Japan

It is known that the single fiber of the taste nerve branches and innervates several taste buds. Immunohistochemical studies have shown that the fibers innervating around the taste buds contain various neuropeptides such as substance P. Thus, it is supposed that afferent taste signals may be modified at the level of the peripheral nerve. Some electrophysiological studies have shown the depression of nerve activity in the taste nerve following antidromic electrical stimulation. Although the mechanism of this antidromic depression has not yet been resolved, there is a possibility that some neuropeptides may contribute to this depression. In the present study, the effects of the electrical repetitive stimulation of the superior laryngeal (SL) nerve on the nerve activity in the SL nerve were investigated in anesthetized rats. The left SL nerve was put on a pair of platinum wire electrodes for recording and for stimulation. The data from the integrated and rate (spikes/s) recordings of the whole SL nerve were analyzed. The taste stimuli applied to the larynx were deionized water, 0.9% NaCl and 0.1 M substance P (dissolved in 0.9% NaCl). Repetitive antidromic electrical stimulation of the SL nerve depressed the spontaneous discharge rate and the water response in the SL nerve. The spontaneous discharge rate in the SL nerve was increased by ~17% of the basal activity by the application of 0.1 M substance P to the larynx. These results suggest that some neuropeptides and antidromic impulses modify the afferent nerve activity in the SL nerve in rats.

P80. Chemoreception of the taste nerve in the pharynx of the rat

T. Shingai, J. Kitagawa, Y. Takahashi and Y. Yamada

Department of Oral Physiology, Faculty of Dentistry, Niigata University, Niigata 951-8514, Japan

Pharyngeal chemoreception is important in the perception of the taste of food. However, very few reports are available for the afferent activity of the taste nerve in the pharynx. To my knowledge, no work has been done on the sense of fat in the pharynx. Therefore this study was designed to investigate the responsiveness of the pharyngeal branch of the glossopharyngeal nerve to fat and fatty acids. The pharynx and larynx of urethane-anesthetized rats were surgically opened and fat stimuli were applied to the internal suface of the pharynx. Application of oleic acid produced vigorous discharges in the nerve. The activity was rapidly increased after the application and continued for >10 s. Linoleic acid also produced an excitatory response. Triolein, which was used as a pure fat, had no effect on the nerve activity. Vegetable oil and paraffin oil also did not evoke any response. It was found that only fatty acids had potent excitatory effects on the pharyngeal nerve. The findings provide new evidence supporting the existence of the pharyngeal gustatory mechanisms for the detection of fat.

P81. Chemical stimulation of the larynx inhibits vagal preganglionic neurons

Motoi Kobashi, Ryuji Matsuo and Masatoshi Mizutani[1]

Department of Oral Physiology, Okayama University Dental School, Okayama 700-8525 and [1]Okayama Prefectural University, Soja 719-1197, Japan

Neural responses of the dorsal motor nucleus of the vagus (DMV) to the administration of water into the posterior oral cavity were investigated using animals which had undergone bilateral sectioning of both the chorda tympani and glossopharyngeal nerves. All experiments were performed under urethane–chloralose anesthesia. Seventy-eight DMV neurons which showed antidromic response to electrical stimulation of the anterior subdiaphragmatic vagus were recorded. Among them, 34 neurons showed a decrease in the firing rate in response to the administration of water (water-responsive neurons). These neurons did not respond to the administration of 0.15 M NaCl. The administration of taste solutions elicited an inhibition of the firing rate of water-responsive neurons. The administration of HCl (0.03 M) and NaCl (1.0 M) induced a marked inhibition of the firing rate. The administration of sucrose (1.0 M) and quinine–HCl (0.03 M) had weak effects. A similar inhibition in gastric contractility was observe in response to the administration of taste solutions. Electrical stimulation of the left superior laryngeal nerve (SLN) elicited inhibition in all water-responsive neurons tested (10 neurons). These results suggest that the inhibition of vagal excitatory fibers are responsible for the inhibition of the gastric motility to the administration of water and other taste solutions.

P82. BDNF is expressed in the petrosal ganglion of the glossopharyngeal nerve in adult rat

Miyoko Okada, Sigeru Takami and Fumiaki Nisiyama

Department of Anatomy, School of Health Sciences, Kyorin University, 476 Miyasita-cho, Hachiouji, Tokyo 192-8508, Japan

Our previous study demonstrated that gustatory nerve fibers and taste receptor cells in the vallate papilla exhibit immunoreactivity for brain-derived neurotrophic factor (BDNF) (Takahasi *et al.*, 1977, Chem Senses, 22: 349). To test the hypothesis that BDNF is produced in ganglion cells of the petrosal ganglion (PG) and transported via post-ganglionic gustatory fibers to the vallate papillae, BDNF protein and BDNF mRNA were examined in the PG of adult Sprague–Dawley rats by using immunohistochemical and in *situ* hybridization (ISH) techniques. Rats were fixed by transcardial perfusion with Zamboni's fixative, tissue blocks including PGs were microdissected out and 14-μm-thick cryostat sections were made. Immunoreactivity for BDNF was present in ganglion cells of the PG and post-ganglionic nerve fibers. By use of digoxigenin (DIG)-labeled cRNA probes to BDNF mRNA (antisense and sense probes) and anti-DIG IgG, the ISH technique demonstrated that DIG immunoreactivity was localized in perikarya of ganglion cells of the PG only when the antisense probe was applied. Our present study suggests that ganglion cells of the PG express BDNF and transport it to the taste buds of the vallate papilla.

Supported by a project research grant from Kyorin University (98-06-09).

Expressions of neuropeptides in geniculate ganglion after chorda tympani injury

Kenzo Tsuzuki[1,2], Hiroki Yasuno[1,2], Koichi Noguchi[2] and Masafumi Sakagami[1,2]

Departments of [1]Otolaryngology and [2]Anatomy & Neuroscience, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan

It is well known that substance P decreases in axotomized somatosensory neurons, such as the dorsal root ganglion and trigeminal ganglion. In this study we compared the regulation of this neuropeptide in geniculate ganglion (gustatory) neurons in a chorda tympani transection (CTT) model and an ischema-induced facial nerve paralysis (IFNP) model. In the CTT model, the expression of preprotachykinin (PPT) mRNA significantly decreased 3 days after surgery, whereas in the IFNP model, the reduction of PPT mRNA was transient. The expression of GAP-43 mRNA, a sign of cell damage, was increased and maintained in CTT model, while this change was also transient in the IFNP model. In the CTT model, intragemmal PGP9.5 immunoreactive fibers decreased and fungiform papillae were atrophic. These changes were not observed in the IFNP model. These data suggest that gustatory neurons down-regulate the expression of PPT mRNA in response to axotomy as well as somatosensory neurons. The IFNP model is a mild and transient injury model for geniculate ganglion neurons. Geniculate ganglion neurons contribute to the maintenance of fungiform papillae as well as the lingual nerve.

P84. Connections between the insular cortex and the thalamic taste relay in rats

Tomio Hayama and Hisashi Ogawa

Department of Physiology, Kumamoto University School of Medicine, Kumamoto 860-0811, Japan

We re-examined projections of the thalamic taste relay, the parvicellular part of the posteromedial ventral thalamic nucleus (VPMpc), to the insular cortex in rats with a tract tracing method using WGA-HRP. After tracer injection into the VPMpc, we saw labeled cell bodies and axon terminals in a rostrocaudally elongated portion of the insular cortex in flattened cerebral cortices. Close examination of the label distribution clearly revealed two foci of the projection: one with denser label was in the cortical taste area (CTA) and the other was located approximately at the level of the bregma in the caudalmost portion of the insular cortex. Following injections into the CTA, many labeled cell bodies were observed in the VPMpc. When injected into the caudal portion of the insular cortex, labeled cell bodies were found in the peripheral portion of the VPMpc. Tracer injections into a portion rostral to the CTA did not produce labeled cell bodies in the VPMpc but did in the surrounding nuclei. The findings indicate that the VPMpc does not project to the portions rostral to the CTA and further that the central and peripheral portions of the VPMpc differentially project to the CTA and caudal portions of the insular cortex.

P85. Cortical areas related to taste, measured by fMRI and MEG

Tatsu Kobayakawa[1], Yasushi Yamauchi[1], Akira Takahashi[1], Hirotaka Kaneda[2], Saho Ayabe-Kanamura[1,3], Hisashi Ogawa[4] and Sachiko Saito[1]

[1]National Institute of Bioscience and Human Technology, [2]Sapporo Breweries Ltd, [3]University of Tsukuba and [4]Kumamoto University School of Medicine, Higashi 1-1, Tsukuba, Ibaraki 305-8566, Japan

Magnetoencephalography (MEG) has a good temporal resolution and can give a good estimation of the location of activity because the magnetic field generated from the living brain is free from distortion by the skull. But when the number of activated regions increases to more than two, the authenticity of estimation of the equivalent current dipoles might decrease. Functional magnetic resonance imaging (fMRI), on the other hand, has good authenticity for detecting many activated regions at a time, but very poor temporal resolution. In the present study, we tried to measure changes in the regional cerebral blood flow (rCBF) induced by gustatory stimulation, using the fMRI technique to find activated areas, and compared the results with the findings by MEG.

Changed rCBF was observed at the transition between the parietal operculum and the insular cortex. These are the regions which our MEG study estimated to be activated in a short latency. The activations were also observed in the pre-central sulcus, the post-central sulcus, the frontal operculum, the anterior part of the insula, the angular gyrus and the intraparietal sulcus. The MEG study located activity on some of the latter regions in a long latency after stimulation.

The present study showed that MEG and fMRI compensate for each other's shortcomings to raise the authencity of the method.

P86. Taste nerve responses and starch appetite in ventromedial hypothalamic obese rats

Yasutake Shimizu, Mifumi Yamazaki, Maki Sakurai, Keiji Nakanishi and Keiichi Tonosaki

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan

The chorda tympani nerve responsiveness in ventromedial hypothalamic (VMH) lesioned obese rats was examined. The integrated nerve response to sucrose was greater in VMH-lesioned rats than in control animals. In contrast, there was no remarkable difference in responses to NaCl, HCl, quinine–HCl and monosodium glutamate between these two groups. The enhanced chorda tympani response was not specific for sucrose but common to sugars such as glucose, maltose and fructose. To obtain the basis for the starch appetite, which is one of the typical feeding characteristics of obese rats, we then used a conditioned taste aversion technique. Establishment of taste aversion to starch did not affect intake of sugar solutions, indicating that rats discriminate the starch taste from the taste of sugar. Thus, the increase in sugar responsiveness of the chorda tympani nerve would not directly explain the starch appetite in obese rats. Interestingly, the VMH-lesioned obese rats with taste aversion to starch avoided sugar solutions as well as starch solution, suggesting that obese rats confuse starch taste with sugar taste. The starch appetite in obese rats may be related to confusion between starch and sugar.

P87. Effects of gurmarin on behavioral responses in C57BL, BALB and congenic-*dpa* mice

Kiyohito Nakashima[1], Yuko Murata[2], Toshiaki Imoto[3] and Yuzo Ninomiya[1]

Departments of [1]Chemistry and [4]Oral Physiology, Asahi University School of Dentistry, Gifu 501-0296, [2]Natural Research Institute of Fisheries Science, Yokohama 236-8648 and [4]Department of Physiology, Faculty of Medicine, Tottori University, Yonago 683-0826, Japan

Our behavioral and electrophysiological studies have indicated that taste responses to a sweet-tasting amino acid, D-phenylalanine (D-Phe), are strain-specific and closely link with sensitivity to gurmarin, a specific inhibitor for sweetener responses. Taste receptor cells in C57BL mice possess sensitivities to D-Phe and gurmarin but those in BALB mice lack them. We developed a congenic strain whose genetic background is identical to that of the BALB strain, except for a gene segment containing the locus controlling sensitivities to D-Phe and gurmarin that is derived from C57BL mice. In this study, effects of gurmarin were examined on behavioral responses in C57BL, BALB and congenic mice. In both C57BL and congenic mice, a conditioned taste aversion of D-Phe generalized to sucrose and quinine, but not to NaCl, HCl and monosodium glutamate. The generalization of sucrose was significantly decreased by treatment with 30 μg/ml gurmarin for 10 min and recovered to near basal levels by treatment with 15 mM β-cyclodextrin for 10 min. The suppressive effect of gurmarin on the aversive response to D-Phe lasted for ~3 h. In BALB mice, a conditioned taste aversion of D-Phe generalized only to quinine. Treatment with gurmarin or β-cyclodextrin had no effect on the generalization of quinine in BALB as well as in C57BL and congenic mice. These results indicate that gurmarin inhibition of sweet responses was similar between C57BL and congenic mice, but that the response to bitter substance was similar between BALB and congenic mice. The present results further support the idea that a *dpa* gene may code for gurmarin-sensitive receptor proteins that recognize sweet- tasting molecules.

P88. The effect of oral capsaicin stimulation on sodium chloride preference in Sprague–Dawley rats

Michio Komai[1,2], Hiro-umi Ishikawa[1], Ken-ichiro Yamanaka[1], Tomoko Goto[1], Eiko Hatakeyama[2] and Yuji Furukawa[1]

[1]Laboratory of Nutrition, Division of Life Sciences, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555 and [2]Kansei Fukushi Research Center, Tohoku Fukushi University, Japan. e-mail: mkomai@biochem.tohoku.ac.jp

We have already shown that NaCl preference decreased in rats stimulated with capsaicin both on the tongue and administered intragastrically, compared with the solvent-treated counterparts. The mechanism involved in the salt preference reducing effect of capsaicin especially through oral stimulation for 10 s without ingestion is still unknown, so the present study was undertaken to understand it.

In each experiment ten Sprague–Dawley male rats, 6 weeks old (180 g), were used. They were separated into two groups (five rats each), and one group was stimulated daily on the tongue surface with a swab dipped into 140 ppm capsaicin solution, while the other group received a vehicle, for 10 s for 10 days. The sodium chloride preference rate was measured by a four-bottle (water and 86, 154, 239 mM NaCl) preference test for 2 h after the treatment.

It was clearly shown that capsaicin pungent stimulation for 10 s lowered the sodium chloride preference. Capsaicin-treated rats siginificantly avoided the concentrated NaCl solutions such as the 239 and 154 mM solutions. This is possibly attributable to the increased catecholamine secretion due to oral pungent stimulation with capsaicin as indicated by our previous results.

P89. Properties of taste preference and effect of sugar diet on blood chemistry in the spontaneously hypertensive rat

Kiyoaki Katahira and Kunjo Yamazakii[1]

Experimental Animal Center, Fukushinia Medical University, 1 Hikari-ga-oka, Fukushima 960-1295, Japan and [1]Monell Chemical Senses Center, Philadelphia, PA 19104, USA

The present study was conducted to clarify the relation between the genetic background and food factors which cause geriatric diseases. In the first experiment, preference tests were performed in a free choice situation with two bottles among 5% glucose, 5% sucrose, 0.9% saline and water. Daily intake of the solution was measured both in spontaneously hypertension (SHR, six males) and Wistar rats (six males). In the infant and adult stages both of SHR and Wistar rats there was a greater intake volume of glucose and sucrose solution than water. Also, there was a greater intake volume of water in the SHR group than Wistar. In comparison with Wistar, SHR showed a remarkable taste preference to both sweetness and saltiness. In the second experiment, we examined the effect of administering glucose, sucrose and water on the blood chemistry of the SHRs. Each solution was provided *ad libitum*, from 3 to 21 weeks of age. Nine items in the plasma were analyzed with an autoanalyzer. The glucose group displayed a high level of plasma glucose. Total cholesterol and HDL choresterol in the sucrose group were higher than in the other groups. The above

results suggest that dietary factors are important to prevent geriatric diseases in the genetic background.

P90. Effect of umami taste on diet-induced thermogenesis to rats

Hitoshi Murakami, Miro Smriga, Masato Mori and Kunio Torii

Central Research Laboratories, Ajinomoto. C., Inc., Kawasaki, Japan

We previously showed that monosodium glutamate (MSG)-containing ('umami') solution was selected by rats that were offered a choice of 15 amino acids solutions when dietary protein balance was complete. Thus, we thought that MSG-containing solution was signaling the ingestion of proteins. On the other hand, diet-induced thermogenesis (DIT) was especially elicited by ingestion of protein and so it is thought that brown adipose tissue (BAT) is associated with DIT. Given the above notion, in the present study, we investigated the effect of MSG solution on DIT by using a thermocamera, an experimental cage and a hairless strain of Sprague–Dawley rats (9–22 weeks old). Thermography was recorded for 2 h once a week in the upper back of rats in a position that corresponded to the location of BAT. The thermogenesis increased dramatically during the first 5–15 min of eating and drinking, and MSG solution promoted a greater thermic response than did distilled water. However, the speed of the thermic response and the amplitude of the thermic capacity were impaired in parallel with aging. We suggested that by using thermography, the thermic effect on BAT was associated with DIT and this effect was impaired in parallel with aging. It is possible that the MSG-containing ('umami') solution enhanced the thermogenesis and so accelerated the metabolism of ingested nutrients.

P91. c-*fos* gene expression in forebrain structures to retrieval of conditioned taste aversion in rats

Yasunobu Yasoshima, Thomas R. Scott[1], Yoshimitsu Suzuuki and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita, Osaka 565-0871, Japan and [1]Department of Psychology, University of Delaware, Newark, DE 19716, USA

To elucidate the involvement of forebrain areas in conditioned taste aversion (CTA) learning, we studied the induction of the immediate–early genes c-*fos* and *zif268* (*NGFI-A*, *egr-1*) in forebrain structures as a marker of neuronal activation. In the CTA conditioning procedure, rats ingested the conditioned stimulus (CS, 0.5 M sucrose) and received an i.p. injection of 0.15 M LiCi (2% of body weight) as the unconditioned stimulus (US). The CS and US were paired twice. We compared expressions of c-*fos*-like (c-FLI) and ZIF (ZIR) immunoreactivities induced in naive rats that drank (1) distilled water, (2) 0.5 M sucrose, (3) 0.2 M NaCl (4) in rats administered quinine–HCl intraorally, (5) in rats given LiCl intraperitoneally and (6) in CTA-trained rats that were re-exposed to the CS. The number of c-FLI- and ZIR-positive cells in the supramammillary area (SuM) of those rats re-exposed to the CS was significantly larger than in those of other rats. In the behavioral study, lesions of the SuM before the single CS–US pairing caused no disruption of the acquisition of the aversion but accelerated its extinction. On the other hand, lesions of the SuM after the two CS–US pairings did not impair the expression or retention of the CTA. The present results suggest that the SuM cells participate in the acquisition mechanism of CTA formation to strengthen long-term gustatory memory.

P92. Analysis of the CNS information processing mechanisms in human brain magnetic fields evoked by food-related visual stimuli

Hajime Nagai, Chizuko Yamamoto[1], Seiji Nakagawa[2], Masahiko Yamaguchi[2], Mitsuo Tonoike[2] and Takashi Yamamoto[1]

Suntory Research Center, [1]Faculty of Human Sciences, Osaka University and [2]LERC Electrotechnical Laboratory, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan. e-mail: hajime_nagai@suntory.co.jp

We examined the effects of the visual information process which are related to the recognition of foods. To understand the mechanism of gustatory related brain neural responses, we have already tried to make a new gustatory stimulation system for measuring the brain magnetic field, and found that the positions of signal sources evoked by the taste solution were around the insula cortex or operculum area. From the results of the taste-evoked brain magnetic field, we hypothesized that food images also activate the taste cortex through the visual cortex.

Visual stimuli were both food and non-food pictures which were shown to subjects randomly by LCD projector. The brain magnetic field was measured by Neuromag-122, and averaged up to 80 times by each stimulation trigger.

The positions of the signal sources on the subject's MRI moved from the calcarine sulcus area to the inferior temporal gyrus area in accordance with the visual information processing. After activation of the visual cortex, four out of seven subjects had a signal source on the insula cortex or operculum area. The latencies of these signals were between 340 and 675 ms. This would suggest that food images activated the primary gustatory cortex like gustatory stimuli did.

P93. Neuronal responses during a stimulus–reward association task in the rat posterior cingulate cortex

Eiichi Tabuchi, Etsuro Hori, Katsumi Umeno, Taketoshi Ono and Hisao Nishijo

Department of Physiology, Toyama Medical & Pharmaceutical University, Toyama 930-0194, Japan

Neuronal activity in the posterior cingulate cortex was recorded during a stimulus–reward association task using awake rats. After implantation of bilateral bipolar concentric electrodes for intracranial self-stimulation (ICSS) and a chronic head folder, the rats were trained to learn the association of a 2 s sensory stimulus (tone or light) with ICSS or 0.3 M sucrose solution in a stereotaxic apparatus. Following completion of the training, single neurons were recorded from the posterior cingulate cortex. Of a total of 88 neurons recorded, 75 (85%) responded to one or more conditioned sensory stimuli and/or reward stimuli. Most of these responsive neurons (70/75, 93%) responded to reward stimuli (reward-responsive neurons). These reward-responsive neurons responded differentially to sucrose solution and ICSS; some neurons responded to only the sucrose solution, and the other neurons to the ICSS only. On the other hand, only a few neurons responded during presentation of conditioned sensory stimuli, and few displayed tonic responses during the 2 s conditioned stimulation. The lack of sensory-responsive neurons in the

posterior cingulate cortex is consistent with previous neuroanatomical studies indicating no direct connections between the posterior cingulate cortex and the sensory areas. These results suggest that the posterior cingulate neurons did not respond to simple somatosensory stimuli on a tongue during licking, but might discriminate the quality of the rewards.

P94. Contributions of strains and vagus on MSG preference in rats

Takashi Kondoh, Tian-Lu Zhou[1], Taketoshi Ono[1] and Kunio Torii

Basic Research Laboratories, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681 and [1]Department of Physiology, Toyama Medical & Pharmaceutical University, Toyama 930-0194, Japan

Monosodium L-glutamate (MSG) elicits a unique taste, called umami. Mechanisms of preference for MSG, however, have not yet been fully understood. In the present study, we used 13 strains and vagotomized rats to investigate the contribution of genetic factors and the vagus on MSG preference using a short-term single-bottle preference test. Ingestion of one of 13 concentrations of MSG (6, 12, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540 and 600 mM) was measured daily in each 30 min test session. The preferences for MSG solutions were distinctly different in the 13 strains tested. Brown–Norway rats showed a strong preference for 60 mM MSG and did not show any aversive behavior for up to 600 mM MSG. Sprague-Dawley (SD) rats showed a moderate preference for 60 mM MSG and a weak aversion for MSG concentrations higher than 240 mM, while Long–Evans Agouti (LEA) rats showed a moderate preference for 60 mM MSG and a marked aversion for MSG concentrations higher than 120 mM. After dissection of gastric branches of the vagal nerve as well as total vagotomy, MSG became aversive to SD rats, just like intact LEA rats. In LEA rats, MSG intake after total vagotomy was more reduced. These results suggest that the preference and aversion for MSG are determined by genetic factors, as well as by vagal nerve function.

P95. Monosodium L-glutamate modulates vagal nerve activity in the rat

Hisayuki Uneyama, Kunio Torii and Akira Niijima[1]

Basic Research Laboratories, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681 and [1]Department of Physiology, School of Medicine, Niigata University, Niigata 950-2181, Japan. e-mail: clk_torii@te10.ajinomoto.co.jp

Monosodium L-glutamate (MSG) is popular as a food additive that produces umami taste so as to improve the taste quality. It is assumed that umami taste may serve as a marker for a protein-rich diet like sweetness is the marker for a carbohydrate-rich diet. It has been reported that glutamate sensors exist in the oral cavity as well as the gastrointestinal canal and the hepatoportal region.

The effects of oral, gastric and intestinal infusion, as well as intraportal (i.p.v.) and intravenous (i.v.) administration of MSG on vagal gastric and vagal pancreatic nerve activity were observed. Oral, gastric and intestinal infusion of MSG solution (0.15 M, isotonic solution) facilitated efferent activity of the gastric branch of the vagus nerve. Further, i.p.v. and i.v. injection of 10 mM MSG (0.1 ml) originated a reflex activation of the efferent discharges of the gastric branch of the vagus nerve; however, in hepatic vagotomized rat i.p.v. or i.v. injection of the same or a larger amount of MSG (100 mM, 0.1 ml and 0.5 ml) showed no effect on gastric vagal nerve activity.

The results of experiments demonstrate the importance of the peripheral MSG sensors in the reflex regulation of gastrointestinal and pancreatic functions by MSG administration.

P96. Circadian norepinephrine release in the lateral hypothalamus of umami solution-drinking rats

Miro Smriga, Masato Mori and Kunio Torii

Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681, Japan

A novel combination of behavioral and analytical techniques was developed to continuously measure food/drink intake and brain neurotransmitter release during eating/drinking in non-stressed rats. Computerized mierodialysis apparatus and HPLC system were connected with an operant type box. The circadian drinking/feeding patterns of the operant housed rats revealed a strong preference for 0.06 M monosodium L-glutamate (MSG) solution during the first phase of night. Norepinephrine (NE) release within the lateral hypothalamus (LH) also showed a significant circadian pattern, with the highest values recorded immediately before and during the initial period of the dark phase. There were no significant differences of the NE LH release between distilled water- and 0.06 M MSG-drinking rats. Thus, although NE diurnal oscillations were food-intake-related, the present data do not suggest participation of the hypothalamic NE system in the preferential intake of the low-concentrated (0.06 M) MSG solution.

P97. Effect of L-lysine on afferent activity of the hepatic branch of the vagus nerve in normal and lysine-deficient rats

Tatsuro Tanaka, Kunio Torii and Akira Niijima[1]

Basic Research Laboratories, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681 and [1]Department of Physiology, Niigata University, Niigata 951, Japan

It was reported that an essential amino acid deficiency causes changes of taste preference towards a diet that favors the deficient amino acid. For example, L-lysine (Lys), an essential amino acid with a bitter taste, was most preferred during its deficiency in a choice paradigm. We had already hypothesized that the Lys concentration in the circulating blood is sensed by peripheral Lys sensors. We speculated that the Lys sensors send information on the concentration of Lys in the portal venous blood to the brain through hepatic vagal afferents. This report deals with sensory signals from hypothesized hepatoportal Lys sensors.

Normal and Lys-deficient male Wistar rats (300 g) were used. To develop the Lys-deficient rats, Lys-deficient food (formulated as previously reported) was supplied for 7–10 days. Food, but not water, was withdrawn at least 6 h before the beginning of each experiment. The hepatic vagus nerve filament of anesthetized rat was isolated and the responses were recorded by electrodes before and after ingestion of amino acid solution into the cannulated portal vein. Results show that in the Lys-deficient rat the sensitivity to Lys increased 100-fold from that observed in the normal animal.

D-Lysine, L-alanine and L-leucine sensitivities did not change. This increase in Lys sensitivity may accelerate selective intake behavior for Lys in diet to aid recovery from the Lys-deficient state.

P98. Long-term norepinephrine changes in the hypothalamus of rats fed a lysine-deficient diet for 1 week

Masato Mori, Miro Smriga and Kunio Torii

Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681, Japan

An amino acid (AA) deficiency is induced by feeding an essential AA-deficient diet to rats. The anorectic response to such a diet is brain-mediated. By using an L-lysine (Lys)-deficient diet, we demonstrated that specific hypothalamic centers integrate signals about AA availability. We showed that the levels of blood and plasma Lys in Lys-deficient rats significantly declined. Moreover, by the MRI technique, we found enhanced neuronal activity in hypothalamic neurons, when Lys-deficient rats were exposed to cues associated with the deficiency. Presently, we conducted microdialysis evaluation of norepinephrine (NE) release in the lateral (LH) and ventromedial (VMH) hypothalamus of Lys-deficient rats. We measured dynamic NE changes during a chronic (1 week) deficiency. Within 48 h after exposure to the Lys-deficient diet, rats significantly decreased their dietary intake. This decrease was associated with a profound decline of NE release within the VMH but not the LH, suggesting the specific involvement of VMH NE in the regulation of food intake during an AA deficiency. Depression of VMH NE release lasted during the whole period (1 week) of Lys deficiency and recovered immediately after rats were provided with complete diets. Thus, VMH NE participated in both the initiation and regulation of anorexia that characterized Lys deficiency.

P99. Pedunculopontine tegmental nucleus lesions disrupt normal intake of palatable solutions in rats

Tsuyoshi Shimura and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Science, Osaka University, Suita 565-0871, Japan

The pedunculopontine tegmental nucleus (PPTg) has been implicated in the control of a variety of behavioral functions including reinforcement. In the present study, we examined the effects of electrolytic lesions to the PPTg and some related structures on intake of taste solutions in rats. Two-bottle preference tests revealed that PPTg-lesioned animals drank significantly less sucrose (0.1 M) than controls. The consumption of other solutions (NaCl, HCl and quinine–HCl) was not different between lesioned and control animals. Injection of midazolam, a benzodiazepine agonist, significantly increased the consumption of 0.1 M sucrose solutions in control animals. The same injections, however, failed to increase intake of 0.1 M sucrose in PPTg-lesioned animals. Midazolam did not modify intake of an unpalatable quinine solution in both lesioned and control animals. Lesions to other structures (the prefrontal cortex, orbitofrontal cortex, nucleus of accumbens, bed nucleus of the stria terminalis or ventral pallidum) had no effects on the consumption of palatable and unpalatable solutions. These results obtained in PPTg-lesioned animals are the same as in those with lesions of the ventral tegmental area (VTA) reported previously. Because the VTA is one of the projection sites from the PPTg, the enhanced intake of highly palatable solutions is likely to be mediated by both areas.

P101. A bitter substance in mature ovaries of the sea urchin

Yuko Murata, Noriko U. Sata, Yuzo Ninomiya[1], Masaki Kaneniwa, Ryuji Kuwahara and Masahito Yokoyama

National Research Institute of Fishery Science, Fukuura, Kanazawa, Yokohama 236-8648 and [1]Department of Oral Physiology, School of Dentistry, Asahi University, Hozumi, Gifu 501-0296, Japan

The sea urchin (*Hemicentrotus pulcherrimus*) is widely distributed among the Japanese coastal seas and is, therefore, an important coastal fishery product in various places. However, in some areas, sea urchins of which the gonad tastes bitter are often found in the catch. Such sea urchins are not accepted as food and have no commercial value. Our previous study demonstrated that the bitter-tasting compounds of such sea urchins may specifically be included in their mature ovaries, and is probably not one of the known amino acid components of the sea urchin, such as Val, Leu, Ile and any peptides. In this study, the bitter-tasting fraction was separated from the mature ovaries of sea urchins by extraction with 80% ethanol, Sephadex G-10 gel filtration and TLC using Kiesel gel 60. Behavioral experiments using a conditioned taste aversion (CTA) paradigm in mice demonstrated that aversion conditioning to the fraction strongly generalized to PTC and $MgSO_4$, which taste bitter to humans and contain sulfur. A more purified chemical was subsequently obtained from the fraction using RP-HPLC with a bitter taste and was determined as a novel sulfur-containing amino acid. These results suggest that a bitter-tasting component of the bitter sea urchin may be the sulfur-containing amino acid which may stimulate a receptor mechanism common to other sulfur-containing compunds, such as PTC and $MgSO_4$.

P102. New candidate substances related to the taste of green tea

Hideki Horie and Katsunori Kohata

National Research Institute of Vegetables, Ornamental Plants and Tea, 2769 Kanaya, Shizuoka 428-8501, Japan

The balance of astringency and umami is important for the taste of Japanese green tea. The astringency of tea is thought to be caused by catechins; however, we suppose that oxalic acid is also related to the astringent or harsh taste of teas. We could taste oxalic acid at a concentration of 50 mg/l, and normally brewed teas contained more than that. Oxalate-less tea brewed with hard water tasted less astringent. The other candidate substance is pectin, which inhibited the turbidity formed by the mixing of tea catechins and gelatin. Both the roasting and steaming processes of tea increased the content of pectin, while the turbidity formed with gelatin decreased. Since the taste of such teas is less astringent, we suppose that pectin can modify the astringency of tea catechins.

P103. The investigation of taste for extracted coffee and the correlation between taste and ingredients

Masakazu Sakata, Iwao Tago, Yutaka Ueki, Toshiyuki Tamura and Kiyoshi Fukushima

Sanyo Electric Co., Ltd, Commercial Equipment Systems Company Product Development Center, 1-1-1 Sakata, Oizumi-machi, Oura-gun, Gunma 370-0596, Japan

The effects of storage on roasted coffee were investigated. Using multichannel lipid membrane taste sensors and titratable acidity for coffee infusion after storaging at 20°C, 35°C or 50°C for several days, it was confirmed that the sourness of the infusion coffee increased by storing at 50°C.

The correlation coefficients between outputs of the sensors and titratable acidity showed high values. That means it is efficient to utilize lipid membrane taste sensors for evaluating the sourness of infusion coffee.

The outputs of the sensors or titratable acidity were saturated in ~7 days for medium roast coffee beans, but saturation points were indefinite for Italian roast coffee beans. These results mean that Italian roast coffee beans denatured over a longer time in comparison with medium ones. As Italian roast coffee beans have less moisture and more pores than medium ones, adsorption of water continues longer in Italian roast coffee beans.

In order to detect the cause of the rise in sourness, we determined the amount of some organic acids in infusion coffee by liquid chromatography. As a result, quinic acid increased due to storage but lactic and other acids decreased. The correlations between outputs of the taste sensors, titratable acidity and amount of quinic acid were high.

From these results, it is expected that quinic acid is one of the causes of increased sourness.

P105. The relationships between 6-*n*-propylthiourea sensitivity and sensitivity for four basic taste stimuli

Nobuyuki Sakai and Sumio Imada

Department of Psychology, Hiroshima Shudo University, 1-1-1 Ozuka Higashi, Asaminami, Hiroshima 731-3195, Japan

In this study, we investigate the variation of 6-*n*-propylthiourea (PROP) taste sensitivity and the relationships between PROP sensitivity and sensitivity for the four basic tastes. We found that Japanese subjects (n = 395; 309 females and 86 males) could be classed into three groups based on their PROP sensitivities: nontaster (14.2%), taster (64.1%) and supertaster (21.8%). These results are in accordance with those shown by Bartoshuk and her colleagues (Bartoshuk *et al.*, 1994, Physiol. Behav., 56: 1165–1171). The supertasters who showed high sensitivity for the PROP taste also showed stronger evaluation for the taste of sucrose solution than the nontasters and tasters ($P < 0.05$). Several researchers suggest that there are some relationships between PROP sensitivity and the preferences for foods. We also asked subjects to answer a questionnaire including the Food Neophobia Scale and the food attitude survey for several foods (Bartoshuk *et al.*, 1994). However, we could not clarify the relationships between PROP sensitivity and the food attitudes. We intend to investigate the preference and intake behavior to the foods by presenting real foods (not by questionnaire) and their relationships with PROP sensitivities.

P106. Enhancement of sweetness with soluble starch

Norikazu Kanemaru, Shuitsu Harada and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890, Japan

The effect of soluble starch on sweetness intensity was investigated in 135 human subjects. The starch solution was made up with distilled water (DW) and was gelatinized by heating to 80°C. Sucrose (Suc) and sweeteners were dissolved in either DW (standard) or starch solution (test solution) at different concentrations. The solutions were presented to naive subjects, and each subject was requested to taste and compare the sweetness intensity between the standard and test solutions based on a scale ranging from +3 (strong sweetness) to –3 (no sweetness). A greater sweetness intensity occurred for 1 M Suc dissolved in a soluble starch (0.125–4.0%) than for 1 M Suc in DW. Similarly, five other different products of soluble starch at 0.25 and 4% showed enhancement of sweetness for 0.3 and 1 M Suc. In contrast, the enhancement effect was not observed for 0.3 and 1 M Suc dissolved in any of the four products of refined starch (wheat, potato A and B, corn) at 0.25 and 4%. With the sole exception of the taste of 0.3 M Suc, sweet enhancement did not occur for 0.43 M fructose, 0.82 M glucose, 0.82 M sorbitol, 0.0037 M aspartame, 0.0042 M saccharin-Na and 0.016 M cyclamate. These results suggest that the enhancing effect of soluble starch on sweetness for Suc might depend on the molecular structures of soluble starch and sucrose.

P107. The importance of texture in food selection behavior in rats

Kaori Okamoto, Noritaka Sako[1], Tomohiko Mori and Takashi Yamamoto[1]

Research Institute for Food Science, Kyoto University, Uji 611-0011 and [1]Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita 565-0871, Japan

Sensations elicited by chemical and physical properties of food, such as taste, odor and texture, are important in ingestive behavior. In the present study, to examine the importance of texture, we conducted behavioral experiments in Wistar male rats using three kinds of pellets, A, Bs and Bh. When the rats were presented with two types of pellets, A and Bh, which have different ingredients but have similar hardness, they preferred Bh to A. When both foods were smashed into powder, the rats preferred A to Bh, indicating that the taste of A was better than Bh. On the other hand, when the rats were presented with two types of pellets, Bs and Bh, which have the same ingredients but have different hardness, they preferred the soft food (Bs) to hard one (Bh). When both were powdered, the rats consumed them similarly because of the same taste.

After ingestion of Bs was paired with an i.p. injection of 0.15 M LiCl (2% of body weight), the animals preferred Bh to Bs. This finding suggests that the rats can discrimination foods on the basis of their texture. The present study suggests that the texture of food plays an important role in its selection and ingestion.

P108. The effect of physical exercise on the preference of mixed solutions of sweet and sour taste substances

T. Horio

College of Nutrition, Koshien University, Takarazuka 665, Japan

The effect of physical exercise on the preference of mixed solutions of sweet and sour taste substances was examined. After 30 min of exercise using a bicycle ergometer, at intensity 50% $\dot{V}O_2max$ (maximal oxygen uptake), a taste preference test was performed in 40 healthy university students, aged between 19 and 24 years. Test solutions were sucrose (sweet), citric acid (sour) and L-malic acid (sour).

Preference scale values of mixed solutions of 0.15 M sucrose and 0.01 M citric acid, 0.01 M sucrose and 0.0025 M citric acid, and 0.15 M sucrose and 0.0025 M L-malic acid increased after exercise compared with the scale values before exercise. The preference scale values of controls who sat for 30 min were not changed between pre- and post-exercise in any matching of concentrations.

These results suggest that the effect of preference of the mixed solutions of sweet and sour taste substances by physical exercise was different among the matching of concentrations.

P109. Taste interaction between sweet L-α-amino acids and IMP

Misako Kawai, Atsushi Okiyama and Yoichi Ueda

Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210-8681, Japan

It is known that a strong synergistic interaction occurs between umami compounds, acidic amino acids, such as L-Glu or L-Asp, and 5′-mononucleotide. In our psychophysical study, we examined the taste interaction between L-α-amino acids and a 5′-mononucleotide, inosine 5′-mononucleotide (IMP). It was found that the taste intensity of several L-α-amino acids, such as L-Ala, L-Ser and Gly, which have a dominant taste of sweetness, was enhanced by adding IMP. Enhanced taste was recognized as umami, which was not blocked by the sweetness inhibitor ±2-(4-methoxyphenoxy) propanoic acid.

Furthermore, total taste intensities of amino acid and IMP mixture solutions at various concentrations were measured using magnitude estimation [amino acids (L-Ala/L-Ser/Gly/D-Ala): 0–200 mM; IMP: 0–2.0 mM]. The results showed potentiation ratios (= taste intensity of the mixture solution/arithmetic sum of those individual components in the mixture) were >1 in the cases of L-Ala, L-Ser and Gly. However, the potentiation ratio was ~1 in the case of D-Ala, which had an enhanced taste of sweetness. Thus, umami-taste enhancement of sweet L-α-amino acids by IMP is synergistic rather than additive as that of acidic amino acid by IMP.

P110. The effect of umami seasoning added to a low salt diet for the elderly

Teruko Ito, Kimiyo Katsura, Kyuuei Iino, Reiko Ueda[1] and Hiroko Sakamoto[1]

Seitoku University, 550 Iwase, Matsudo, Chiba 271-8555 and [1]Ajinomot Co., Japan. e-mail: itter@seitoku.ac.jp

It was recognized from a previous sensory test by an adult panel that adding umami seasoning to a low salt diet improved the palatability of dishes. In this paper, we tried to ascertain if these effects would reappear with an elderly panel.

Samples were (A) Japanese 'kakitamajiru' soup with a 0.7% salt concentration—the 'standard sample'; (B) one with 0.48% salt concentration—the 'low salt sample'; and (C) one with 0.48% salt concentration + 'Ajinomoto' (monosodium glutamate 97.5%, disodium ribonucleotides 2.5%) 0.04%—the 'Ajinomoto added low salt sample'. The panel consisted of 61 assessors (27 men and 34 women) with an age of >60 years (74.8 ± 7.0 years old). We adopted sensory evaluation of samples according to the ranked data test. The following results were obtained. It was found statistically significant that the (C) sample was recognized as tasty, and that the (B) sample was recognized as tasteless. We divided the panel into two groups: one group who preferred (A) to (B) and the other group who preferred (B) to (A). Then we tried to see if there was a statistical difference between the two groups. We found in the first group that (B) was not preferred, just as we had found in the adult panel test. In the latter group, we assumed that the salt density which assessors liked was less than in the (A) sample. We therefore tried another sensory evaluation using the standard sample (A′) with the salt density lowered to 0.6%. The low salt sample (B′) and the Ajinomoto added low salt sample (C′) were also prepared with lower salt density. With these samples, we obtained the same result as described above, that is, we found with statistical significance that (C′) was recognized as tasty and that (B′) was recognized as tasteless.

P111. Ibuprofen can modify taste intensity in humans

Kazumi Osada, Michio Komai[1], Kenji Tsunoda, Hitoshi Suzuki[2], Bruce P. Bryant[3] and Yuji Furukawa[1]

Resource Exploration Laboratory, Taisho Pharmaceutical Co., Ltd, Ohmiya, [1]Laboratory of Nutrition, Department of Applied Biological Chemistry, Tohoku University, Sendai, [2]Laboratory of Science and Engineering, Ishinomaki Senshu University, Ishinomaki, Japan and [3]Monell Chemical Senses Center, Philadelphia, PA, USA

In our previous study, ibuprofen mainly suppressed the tonic response to NaCl of the chorda tympani (CT) nerve fibers of Sprague–Dawley rats. This suppressing effect could be attributed to the non-specific action of ibuprofen in the periphery, possibly through the effects of trigeminal nerve stimulation on gustatory end organs. In addition, ibuprofen showed strong suppressing effects on both the phasic and the tonic CT nerve responses to sucrose. Thus, it is considered that ibuprofen has a taste suppressing effect by two different manners, i.e. stimulation of trigeminal nerve endings and direct inhibition of the sweet taste receptor. The present study evaluates the taste decreasing effect of ibuprofen on NaCl and sucrose perception in human subjects using a labeled magnitude scale. Forty-two healthy subjects volunteered to participate in this study. All 42 subjects were tested with four kinds of solution (0.1 and 1 M sucrose, 0.05 and 0.25 M NaCl), and taste intensity was scored immediately after each trial. Then, 0.3 g of ibuprofen was applied to the tongues of each subject for at least 30 s. After spitting out the ibuprofen powder, these subjects tasted and scored the sucrose and NaCl solutions. From the present study it was shown that ibuprofen had a taste suppressing effect in humans in a manner similar to the electrophysiological observations of the rat CT nerve responses. These results indicate that

ibuprofen is a sweet and salt taste inhibitor for both humans and rats.

P112. Differences of taste disorders with human aging

Kazuya Hashimoto, Tsunemasa Aiba[1], Kohji Matsumoto and Humiyuki Okuda[2]

Department of Otolaryngology, Osaka City University Medical School, [1]Department of Otolaryngology, Osaka City General Hospital and [2]Department of Otolaryngology, Minamiosaka Hospital, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan

It is well known that the sense of taste deteriorates physiologically with aging. We examined how the pathology of dysgeusia differed with age. Subjects were 628 patients who were examined for the sense of taste at the outpatient clinic of the Department of Otolaryngology, Osaka City University Medical School. The parameters examined were the number of patients, sexual difference, degree of disorder, diagnosis, cause of disorder and degree of improvement with respect to age. The percentage of the taste disordered patients increased with aging, and the men to women ratio was 1:2.. Degree of disorder decreased with aging in normal cases, increased with aging in the mild-to-moderate cases and did not differ in severe or ageusia cases. With respect to the cause, idiopathic disorder showed a similar distribution to that in the whole patient population. Taste disorder from head injury and common cold tended to increase slightly in younger patients and taste disorder from zinc deficiency and drugs tended to increase slightly in older patients. There was no difference in the degree of improvement after treatment, indicating that therapeutic effects were not bad even in the aged.

P113. Importance of palatability and deglutition for cancer patient care: a case report of advanced esophageal carcinoma metastasized to the oral soft palate

Masa-aki Takita

Saiseikai Nakatsu Hospital, Osaka Department of Oral Surgery, 10-39 Shibata 2-chome, Kitaku, Osaka 530-0012, Japan

Oral metastases from esophageal carcinomas are extremely rare, and are considered to indicate a poor prognosis; in the previously reported cases most patients died within 6 months of the diagnosis of metastasis. Oral surgical treatment, radiation therapy and aggressive chemotherapy for the metastatic focus cause difficulty with the oral functions (including deglutition) related to pleasure responses to food taste, which induces negative emotions. Negative emotions depress the immune status, and the condition of patients often rapidly deteriorates.

Case. An esophageal carcinoma (stage IV) with oral soft palate metastasis in a 52-year-old man was observed. Early diagnosis of the oral small focus was made 1 month after radical surgery of the primary tumor. The patient received palliative chemotherapy (low-dose cisplatin and 5-fluorouracil) with effective maintenance of oral functions. The patient developed recurrence of the esophageal tumor 1 year after the operation and died 2 months later (13 months survival duration after detection of oral metastasis). However, he had continued to receive oral nutrition on his palatability until the terminal final stage in a nursing home setting. His survival period was more prolonged than in previously reported patients with esophageal carcinomas with oral metastases. It is considered that maintenance of oral functions contributes to positive emotions which induce good status on a psycho-neuroimmunological level and supports cancer patients' quality of life in cases with oral metastasis.

P114. Gustatory function measured by electrogustometry after middle ear surgery

Mieko Sone, Masanori Umemoto, Keijirou Fukazawa and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, Nisinomiya, Hyogo 663-8138, Japan

The chorda tympani nerve (CTN) is frequently damaged in various inflammatory middle ear disorders. After middle ear surgery, some patients noticed taste disturbance. To examine the function of CTN in various grades of middle ear inflammation, the threshold of taste function was measured pre- and post-operatively by electrogustometry (EGM).

Subjects were 177 ears of 162 (80 male, 82 female) patients with various middle ear disorders. Their age ranged from 5 to 60 years. The thresholds in EGM were examined 2 days before the operation and 2 weeks and 6 months after the operation. We classified diseases into five groups: non-inflammatory diseases (15 ears; normal), chronic otitis media (70 ears; COM), cholesteatoma (55 ears; chole), operated ears in which the CTN was detected [20 ears; P.O. (N+)] and operated ears in which the CTN was not detected [17 ears; P.O. (N–)]. The mean thresholds of normal and P.O. (N+) in EGM were within the normal range (<8 dB). Those of COM and chole were <8 dB, and that of P.O. (N–) was almost scaled out.

After the operation, we classified ears in which the CTN was cut or preserved. Two weeks after the operation, the mean thresholds in EGM were elevated in all groups. Thresholds of ears in which the CTN was cut were higher than those of ears in which the CTN was preserved in each group. Subjective taste disturbance was more frequently found in the group of ears in which the CTN was preserved than in the group in which the CTN was cut. Six months after the operation, the recovery of the EGM threshold was significantly greater ($P < 0.01$) in younger patients than in older ones.

P115. Bitter evaluation of beer by measuring its adsorption on a lipid membrane

Hirotaka Kaneda[1,2], Ken Shinotsuka[1], Tatsu Kobayakawa[2], Sachiko Saito[2] and Yoshio Okahata[3]

[1]Brewing Research Laboratories, Sapporo Breweries Ltd, 10 Okatohme, Yaizu-shi, Shizuoka 425-0013, [2]Human Informatics Department, National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba-City, Ibaraki 305-8566 and [3]Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501, Japan

The adsorption ability of beer on a lipid membrane was studied using a lipid-coated quartz crystal microbalance. The adsorption ability of beer showed a significant correlation with its bitter intensity and bitter duration in a sensory evaluation. The iso-humulone standard increased the adsorption of beer on the lipid membrane. It appears that the sensory bitterness can be objectively

evaluated by measuring the adsorption ability of beer on the lipid membrane, which simply modifies the gustatory reception system.

P116. Quantification of suppression of bitterness by phospholipids using a taste sensor

Sou Takagi, Kiyoshi Toko, Koichi Wada[1] and Toshimitsu Ohki[1]

Department of Electronic Device Engineering, Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka 812-8581 and [1]Nippon Boehringer Ingelheim, Co., Ltd, Kawanishi 666-01, Japan

It is important for pharmaceutical and food sciences to measure the strength of taste. To date, a main measurement method of taste strength has been human sensory evaluation. However, the evaluation of strong bitterness causes heavy stress in evaluators and its result depends on the mental and physical conditions of individuals. Thus, taste-sensing devices have been desired for a long time. A multichannel taste sensor, whose transducer is composed of several kinds of lipid/polymer membranes with different characteristics, can detect taste in a manner similar to human gustatory sensation. The taste of several foodstuffs was studied using this sensor. Recently, it was found that phospholipids such as phosphatidic acid suppress the bitterness of hydrophobic bitter substances without affecting other taste qualities, and they are expected to be used as a bitter masking substance. Here we obtained the results, which are similar to human sensation, using the taste sensor: the bitterness of quinine and L-tryptophan was suppressed, but other samples such as $MgCl_2$ (salt-type bitter substance) were not affected by phospholipids. The present method using the electronic tongue can be expected to provide a new automated method to measure the strength of the bitterness and take the place of human sensory evaluation.

P117. Study on amino acids and dipeptides with a taste sensor

Tomomi Nagamori, Kiyoshi Toko and Yukiko Kikkawa

Department of Electronic Device Engineering, Graduate School of Information Science and Electrical Engineering, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan

Amino acids and peptides are important in producing taste in various kinds of foods, typically, fermented foods such as beer, wine and cheese. There are detailed data on their taste intensity and taste quality from sensory panel tests. Taste is comprised of the five basic qualities of sourness, saltiness, sweetness, bitterness and umami. To date, however, the main methods of measurement are sensory evaluations by humans and conventional chemical analyses.

A multichannel taste sensor, i.e. an electronic tongue, whose transducer is composed of several kinds of lipid/polymer membranes with different characteristics, can detect taste in a manner similar to human gustatory sensation. Taste information is transformed into the pattern composed of electric signals of membrane potentials of the receptor part. Several kinds of foodstuffs such as beer, sake, milk, miso and soy sauce have been measured using the taste sensor; the differences of not only the brands but also the factories could be detected. In this paper, amino acids, dipeptides and typical taste-producing substances such as NaCl (saltiness), citric acid (sourness) and quinine (bitterness) are clearly classified into five groups of basic taste qualities using the taste-sensing system (SA401, Anritsu Corp.).