## 2.7.6 Laboratory : Physiological Function of Food

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| | Assistant Professor | Yuko Yamada, Ph.D |
| | Master's Program | 4 |
| | Undergraduate | 3 |
| | Researcher | 2 |

**A. Research Activities (2009.4-2010.3)**

**A-1. Main Subjects**

a) Rubiscolin-6, a d opioid peptide derived from Rubisco, a major protein of green leaves, stimulates food intake.

Rubiscolin-6 (Tyr-Pro-Leu-Asp-Leu-Phe) is a d opioid agonist peptide derived from the D-ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). We have previously reported that rubiscolin-6 has memory-enhancing and anxiolytic activities. Here we show that rubiscolin-6 stimulates food intake after oral at a dose of 0.3 mg/kg in non-fasted male mice. Orexigenic activity after oral administration of rubiscolin-6 was blocked by intracerebroventrical (i.c.v.) administration of naltrindole, an antagonist for d opioid receptor, suggesting that orally administered rubiscolin-6 stimulates food intake via central d opioid receptor activation. The orexigenic effect of rubiscolin-6 after oral injection was also blocked by i.c.v. administration of BW A868C or BIBO3304, antagonists for DP1 receptor for PGD2 or for Y1 receptor for NPY, respectively. Taken together, orally administered rubiscolin-6 may stimulate food intake through activating DP1 and Y1 receptors, downstream of the central d opioid receptor.

b) Soymorphin, a m opioid agonist peptide derived from b-conglycinin, a major storage protein of soybean, decreases food intake

We previously reported that soymorphins, μ opioid agonist peptides derived from soy b-conglycinin b-subunit, have anxiolytic-like activity. We found that soymorphins decrease food intake after oral administration in fasted mice. Orally administered soymorphins suppressed small intestinal transit at lower dose than that of anorexigenic activity. Suppression of food intake and small intestinal transit after oral administration of soymorphins were inhibited by naloxone or naloxonazine, antagonists of non-selective μ or μ1 opioid receptor,

respectively, after oral but not intraperitoneal (i.p.) administration. The inhibitory activities of small intestinal transit by soymorphins were also inhibited by WAY100135, raclopride, or saclofen, antagonists for serotonin 5-HT1A, dopamine D2, or GABAB receptor, respectively. We conclude that orally administered soymorphins suppress food intake and small intestinal transit via μ1 opioid receptor coupled to 5-HT1A, D2 and GABAB systems.

c) b-Lactotensin, a bioactive peptide derived from bovine milk b-lactoglobulin, has anxiolytic activity

b-Lactotensin (His-Ile-Arg-Leu, b-LT) is a bioactive peptide derived from bovine milk b-lactoglobulin acting as a natural agonist for neurotensin receptors. We found that b- LT has anxiolytic-like activity in elevated plus-maze test after i.p. administration in mice. b-LT (10 mg/kg) was also orally active. The anxiolytic-like activity of b-LT after i.p. administration was blocked by levocabastine, an antagonist for neurotensin NT2 receptor. b-LT had anxiolytic-like activity in wild-type littermates but not in NT2 receptor-knockout mice. The anxiolytic-like activity of b-LT was also blocked by SCH23390, an antagonist for dopamine D1 receptor. Taken together, b-LT may exhibit anxiolytic-like activity via activating D1 receptor, downstream of the NT2 receptor.

d) Anti-opioid mechanism of rapakinin

Rapakinin (Arg-Ile-Tyr) is an anorexigenic and vaso-relaxing peptide derived from rapeseed protein we previously isolated. We have found that rapakinin inhibits analgesic effect induced by morphine after i.c.v. administration in mice. The anti-opioid activity of rapakinin was blocked by a cholecystokinin (CCK)2 receptor antagonist, LY225910 but not by a CCK1 receptor antagonist, lorglumide. RIY does not have affinity for the CCK2 receptor, suggesting that the anti-opioid activity of RIY is mediated by CCK release and the CCK2 receptor. We have also found that vaso-relaxing activity of rapakinin is mediated by CCK and the CCK1 receptor followed by PGI2 and the IP receptor. It is well known that PGI2 is involved in pain sensation, then we examined whether PGI2 is involved also in anti-opioid activity. We found that the anti-opioid activity of rapakinin was inhibited by an antagonist of IP receptor for PGI2, suggesting that system is involved in the anti-opioid activity of rapakinin. We examined which system, CCK-CCK2 receptor system or PGI2-IP receptor system, is located upstream, and found that the anti-opioid activity of iloprost, an PGI2 analogue, was inhibited by the CCK2 receptor antagonist, whereas the anti-opioid activity of CCK-8 was not inhibited by the IP receptor antagonist. It suggests that the anti-opioid activity of rapakinin is mediated by the CCK-CCK2 receptor system preceded by PGI2-IP receptor system.

e) Vaso-constricting mechanism of angiotensin II

Angiotensin (Ang II) is a key regulator of blood pressure. We found that Ang II shows vaso-relaxing activity after a transient contractile activity in mesenteric artery isolated from

spontaneously hypertensive rats (SHRs). There are two major isoforms for Ang II receptors, AT1 and AT2. It is known that the contractile activity of Ang II was mediated by the AT1 receptor and the vasorelaxing activity was mediated by the AT2 receptor. The vaso-constricting activity induced by Ang II is inhibited by ONO-AE3-240, an antagonist of the PGE2 EP3 receptor, suggesting that the vaso-constricting activity of Ang II is mediated by the PGE2 and the EP3 receptor downstream of the AT1 receptor. The vaso-constricting mechanism that the AT1 receptor and the EP3 receptor are coupled is the novel pathway we found for the first time. The vaso-relaxing activity of Ang II is mediated by the PGI2 and the IP receptor downstream of the AT2 receptor. The fact that the different prostaglandins are involved in the vaso-constricting and the vaso-relaxing activities of Ang II means that the vaso-constricting and the vaso–relaxing activities of Ang II are regulated by independent mechanisms each other.

**A-2.Publications and presentations**

a) Publications

Books

- Suzuki C, Yoshikawa M, Ohinata K. [Trp5]-oryzatensin(5-9), an Agonist Peptide for complement C3a Receptor, Exhinits Anxiolytic-Like Effect Mediated by Prostaglandin E2. Peptide Science 2009: The Japanese Peptide Society (2010) p269-272
- Kaneko K, Miyamoto C, Yang S, Yoshikawa M, Ohinata K. Rubiscolin-6, a d Opioid Agonist Peptide Derived from Rubisco, Stimulates fod Intake after oral Administration via Central Prostaglandin D2 and Neuropeptide Y System. Peptide Science 2009: The Japanese Peptide Society (2010) p277-280.
- Yamada Y, Yamauchi D, Usui H, Ohinata K, Yoshikawa M. Vaso-constricting and Relaxing Activities of Angiotensin II Are Mediated by Prostaglandin E2 and I2. Peptide Science 2009: The Japanese Peptide Society (2010) p285-288.
- Ohianta K, Yoshikawa M. Update of applied technologies on bioactive peptides acting on the nervous system and functional peptides -development of foods, cosmetics and pet foods-（2009）p123-133.

Original Papers

- Yamada Y, Ohinata K, Lipkowski AW, Yoshikawa M. Angiotensin AT2 receptor agonists act as anti-opioids via EP3 receptor in mice. Peptides. 2009 Apr;30(4):735-9.

- Ohinata K, Fujiwara Y, Fukumoto S, Iwai M, Horiuchi M, Yoshikawa M. Orally administered novokinin, an angiotensin AT2 receptor agonist, suppresses food intake via prostaglandin E2-dependent mechanism in mice. Peptides. 2009 Jun;30(6):1105-8.

- Hou IC, Yoshikawa M, Ohinata K. b-Lactotensin derived from bovine b-lactoglobulin suppresses food intake via the CRF system followed by the CGRP system in mice. Peptides. 2009 Dec;30(12):2228-32.
- Ohinata K, Takagi K, Biyajima K, Kaneko K, Miyamoto C, Asakawa A, Eguchi N, Urade Y, Inui A, Yoshikawa M. Complement C5a stimulates food intake via a prostaglandin D2- and neuropeptide Y-dependent mechanism in mice. Prostaglandins Other Lipid Mediat. 2009 Dec;90(3-4):81-4.
- Kanegawa N, Suzuki C, Ohinata K. Dipeptide Tyr-Leu (YL) exhibits anxiolytic-like activity after oral administration via activating serotonin 5-HT1A, dopamine D1 and GABAA receptors in mice. FEBS Lett. 2010 Feb 5;584(3):599-604.

Reviews

- Yamada Y、Ohinata K, Yoshikawa M :Anti-opioid effect of AT2 receptor agonists and PGE2-EP3 receptor, Seitai no Kagaku, 2009;60(5):468-469.

- Ohinata K, Yoshikawa M :Novel central actions of prostalgandin D2 -orexigenic and anxyolytic activities-, Seitai no Kagaku, 2009;60(5):494-495.

- Masaaki Y, Ohinata K : Immuno-stimulating and opioid peptides, Daizu no subete (2010）

- Komai M, Goto T, Ohinata K, Shirakawa H：The contribution of zinc enzyme : carbonic anhydrase, to normal taste sensation, and related topics, Biomed Res Trace Elements, 2010;21(1):38-42.

Reports

- Annual Study Reports on Milk Nutrition
sponsored by Japan Dairy Association 2008（Ohinata）

- Annual Report 2008 entitled "Basic studies on role of zinc in food intale regulation sponsored by Nestle Nutrition Council, Japan. (Ohinata)

b) Conference and seminar papers presented

- Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 6 papers
- 46th Japanese Peptide Symposium: 3 papers
- 63th Annual Meeting of Japanese Society of Nutrition and Food Science 2009: 2papers

**A-3.Off-campus activities**

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Young Scientists (B) : Yuko Yamada : Novel function of angiotensin system and the control by food-derived components

2.Other Research Grants

- Grant from Core Research for Evolutional Science and Technology (CREST) : Kousaku Ohinata (member) : Biocommunication between mother and child supporting brain development.
- Grant from Japan Dairy Association : Kousaku Ohinata : Studies on anxiolytic peptides derived from milk proteins.
- Grant from Food Scienece Institute Foundation : Kousaku Ohinata : Studies on milk component acting on the nervous system.
- Grant from Fuji Foundation for Protein Research : Kousaku Ohinata : Search for anxiolytic peptide derived from soy protein and their application.
- Grant from Kiei Research Foundation : Kousaku Ohinata : Basic studies on egg component having anxiolytic activity.
- Grant from the Skylark Food Science Institute : Kousaku Ohinata : Studies on orally active orexygenic peptides aiming for food development for the elderly.

**A-4.International cooperation and overseas activities**

Visiting Research Scholars

- Guest Scholar 1（Bangladesh）

**B.Educational Activities(2009.4-2010.3)**

**B-1.On-campus teaching**

a) Courses given

- Undergraduate level : Principles of Biochemistry in Food Science I (Ohinata), Physiological functions of foods (Ohinata), Seminar in Food Science and Biotechnology (Ohinata), Introduction to experimental food bioscience (Ohinata), Laboratory course in food and nutrition chemistry (Ohinata, Yamada)
- Graduate level : Seminar in physiological function of foods (Ohinata), Experimental course in physiological function of foods (Ohinata), Advanced

course in health science of foods (Ohinata), Advanced course in physiological function of foods (Ohinata)

**B-2.Off-campus teaching etc.**

Open lectures, etc.

- Kousaku Ohinata: Digestion, absprption and function of peptides, TechDesign, Lecturer, (Dec. 22, 2009, Oishisa Kagakukan, Tokyo)
- Kousaku Ohinata: Short Course for Development of Peptide-based Functional Foods, Japanese Society of Food Engineering, Lecturer, (Jan. 22, 2010, Osaka International Convension Center, Osaka)
- Kousaku Ohinata: Lecture on milk component for nutritionists, Japanese Dairy Assiciation, Lecturer, (Feb.. 19, Okayama Eisei Kaikan, Okayama)