

enzymes that may cleave the peptide at the positions of N- and C-terminal end.

POSTNATAL DEVELOPMENT OF SEPTO-MESENCEPHALIC CONNECTION IN RATS: TRACT-TRACING ANALYSIS WITH DI1

Tom Kouki, Korehito Yamanouchi

Laboratory of Neuroendocrinology, School of Human Sciences, Waseda University, Mikajima 2-579-15, Japan

In the rat, neural connection of the lateral septum (LS) and the mesencephalic central gray (MCG) is known to play an important role in regulating lordosis behavior. The number of LS neurons projecting MCG is larger in females than that in males. In the present study, to investigate the development of LS-MCG connection, a lipophilic fluorescent dye, Di1, was pasted on the cut surface of the LS of 1, 5, and 15 day-aged rats. In the birthday, a few labeled axons were found in the rostral part of the MCG. The hippocampus (HPC) also contains small amount of labeled axons. In the rat of 5 and 15 day-age, the number of labeled fibers in the MCG were still small, although increase of labeled fibers in HPC was seen according to the age. Thus, in this experiment, remarkable development of LS-MCG connection was not seen from day 1 to day 15 after birth.

MALE MICE HAVE BETTER MEMORY IN OBJECT RECOGNITION TEST AND ESTROGEN IMPROVES MEMORY SCORES ONLY IN FEMALES.

Akiko Hattori, Shinji Hayashi

Endocrinology Laboratory, Graduate School of Integrated Science, Yokohama City University, Yokohama, Kanagawa 236-0027, Japan

By using an object recognition test (ORT), which was designed for assessment of non-spatial memory, we investigated in mice whether there is any sex difference in recognition and memory. Male and female mice were gonadectomized at 6-10 weeks of age, and received subcutaneous injections of estradiol-17 β (E2, 1 μ g / 30 g b.w.) for 2 days beginning on 15 days after gonadectomy, then subjected to ORT on the 3rd day. Mice received vehicle injections were used as controls. Objects were composed from plastic toy blocks almost in similar height and width. We could not detect any difference in discrimination of two given objects between sexes and with or without E2 administration. However, in memory task test, male mice showed significantly higher scores than females. E2 improved the scores only in females up to those in males but not in males. It is well established that brain sex difference is due to the exposure to androgen in critical period and affects various reproductive parameters. The present observation extended the similar concept to the ability of object recognition memory and the sensitivity to estrogen treatment at adult.

VITELLOGENIN RELATED PROTEINS IN THE LIVER AND TESTIS OF 17 β -ESTRADIOL EXPOSED MALE MEDAKA (*ORYZIAS LATIPES*)

Kayo Kobayashi, Atsushi Sakai, Satoshi Tamotsu, Tadashi Oishi

Graduate School of Humanities and Science, Nara Women's University, Nara 630-8506, Japan

Vitellogenin (VTG) is an egg yolk precursor. It is known that VTG is produced in male medaka when they were exposed to estrogen or estrogen like endocrine disrupters. In the present study, we examined the localization of VTG in the liver and testis of 17 β -estradiol (E2) treated male medaka by immunohistochemistry. In the liver, the cytoplasm of the cells surrounding the capillaries was immunopositive to the antiserum against medaka VTG. The intensity of immunoreactivities was stronger at the cell membrane close to the capillaries than in the cytoplasm. In the testis, the endothelial cells, probably the blood vessels surrounding the seminiferous tubules and the inside of the cells in the seminiferous tubules were immunopositive. The testis of E2 treated medaka was shown to take up VTG as well as the ovary. We analyzed the VTG and VTG related proteins in the liver and testis of E2 treated male medaka by Western blotting. A band corresponding to VTG and several bands of smaller molecular weights were detected in the liver and testis samples. There were some differences in the band pattern between the two organs.

EXPRESSION OF STEROIDOGENIC ENZYMES IN THE QUAIL BURSA OF FABRICIUS

Yuri Oeda, Tetsuya Kobayashi, Takeo Machida

Department of Regulation Biology, Faculty of Science, Saitama University, Saitama 338-8570, Japan

The bursa of Fabricius (BF) provides the microenvironment for B-cell maturation. The BF produces the tripeptide hormone bursin, which induces phenotypic differentiation of avian B-lymphocyte precursor cells. Previously, we further identified the presence of α -MSH and melatonin in the BF. However, the factors in the BF that promote B-lymphocyte maturation and/or mediate the endocrine effects of the organ are largely unknown.

Sex steroid hormones are synthesized from cholesterol by the sequential action of P450_{scc}, 3 β -HSD, P450_{c17}, 17 β -HSD and P450_{arom} in the gonad, adrenal glands and the brain. To clarify the possibility of steroid hormone synthesis in the immune system, especially in the BF, and the mechanism of neuro-immuno-endocrine interactions, the present study investigated the expression of steroidogenic enzymes in the BF.

The expression of P450_{scc}, 3 β -HSD, P450_{c17}, 17 β -HSD and P450_{arom} mRNA was detected by RT-PCR in the BF of Japanese quails (*Coturnix coturnix japonica*) aged 5 to 6 weeks. P450_{scc}-immunoreactive cells were also found in the BF by the PAP method. These results suggest that steroid hormone synthesis may occur in the quail BF.

HYPO-ACTIVITY OF LIVER INSULIN RECEPTOR IN GOTO-KAKISAKI RATS IMPROVED BY NATURAL VANADIUM CONTAINING WATER

Katsuhiko Kato¹, Shizuo Yamada², Eiichi Kawamoto³, Kazuhiko Shirama⁴, Yasuo Watanabe⁵

¹Department of General Education, School of Veterinary Medicine, University of Azabu, Kanagawa 229-8501, Japan, ²Department of Biopharmacy, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan, ³Division of Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan, ⁴Department of Anatomy, Tokyo Medical University, Tokyo 160-8402, Japan and ⁵Department of Pharmacology, Tokyo Medical University, Tokyo 160-0023, Japan

For evaluating the prophylaxis treatment for diabetes mellitus, the effects of Mt. Fuji ground water containing natural vanadium on hypo-activity of insulin receptor of Goto-Kakisaki (GK) rats, a genetic model of NIDDM, were examined. Following the consecutive oral administration of vanadium containing water at the dose of 0.53 μ g/kg/day for 12 weeks, the levels of blood glucose, serum insulin and pancreatic insulin in GK rats were significantly improved. Furthermore, the alterations in the insulin receptor binding parameters and the levels of insulin receptor β subunit and primary insulin-like growth factor-I β measured by a radio receptor assay and western blotting, respectively, all recovered to their normal levels in Wistar rats. This observation may indicate the potentiation of cytosolic tyrosine kinase activity and the subsequent amelioration in the uptake of insulin in the liver by the vanadium-containing water administration, as previously reported for vanadium. These results suggest that the consecutive intake of relatively low concentrations of natural vanadium-containing water exerts anti-hyperglycemic effect by ameliorating the liver insulin receptor activity.

DEVELOPMENT OF THE PRIMARY CULTURE MODEL OF STROMAL CELLS IN MOUSE UTERUS AND VAGINA

Keiko Inada, Tomomi Sato, Shinji Hayashi

Laboratory of Endocrinology, Graduate School of Integrated Science, Yokohama City University, Yokohama 236-0027 Japan

Effect of 17 β -estradiol (E2) on uterine and vaginal epithelial (ep) cell proliferation could be mediated by stromal (st) cell-derived paracrine factors. To study the ep-st interactions in mice, we developed a primary culture model of st cells and examined cultured st cells in a response to E2. Uterine and vaginal st cells of immature mice were isolated using trypsin. To examine the effect of E2 *in vivo*, cells were grafted into the subrenal capsule of the host mice and grown for 2 weeks. Hosts were ovariectomized and treated with 5 μ g/kg E2 once. The BrdU labeling in cultured st cells was significantly increased by E2. To examine the effect of cultured st cells on ep cell proliferation, uterine and vaginal epithelia of adult mice were separated and recombined with cultured st cells. The recombinants were grafted and grown under the renal capsule of hosts for 3 weeks. The ep cells recombined with cultured st cells proliferated under the influence of hormonal environment of hosts, suggesting that cultured st cells still have the ability to stimulate ep cell proliferation. In conclusion, this culture system is useful to investigate uterine and vaginal ep-st interaction.

DEVELOPMENTAL EFFECTS OF ETHYNYL ESTRADIOL(EE2) ON REPRODUCTIVE ORGANS IN FEMALE MICE

Akiko Kirigaya, Tomomi Sato, Shinji Hayashi

Endocrinology Laboratory, Graduate School of Integrated Science, Yokohama City University, Yokohama 236-0027, Japan

Reproductive organs can be developed abnormally in the female mice that were exposed to estrogen or estrogen-like substances perinatally. In the present study, effects of EE₂, a synthetic estrogen, given by daily injections during gestational days 10-18 were examined in C57BL/6J mice. Fetuses from the mothers exposed to high doses of EE₂ or DES showed a low survival rate. Body weights of alive offsprings were less than those of the oil-exposed mice. The number of fetuses per litter was similar among all groups. At 30 days of age, polyovular follicles (PF) were found in the ovaries of all groups. Mice exposed to high doses of EE₂ showed a high incidence of PF. Moreover, the number of oocytes in a follicle showed a slight increase in a dose-dependent manner. At 40 days, vaginal epithelia of some of the EE₂-exposed ovariectomized mice showed ovary-independent stratification and cornification. The number of BrdU-labeled cells in the vaginal epithelium was increased in a dose-dependent manner. These results showed the prenatal exposure to EE₂ or DES induces reproductive abnormalities, including PF, ovary-independent vaginal stratification and cornification.

AN INCREASE OF THYROXINE 5'-DEIODINASE ACTIVITIES IN VARIOUS TISSUES OF CHIPMUNKS DURING A HIBERNATION CONDITION

Miho Sato, Noriaki Kondo

Mitsubishi Kagaku Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194-8511, Japan

Our previous study demonstrated that in rodents, hibernators had high activities of thyroxine 5'-deiodinases. Additionally, in chipmunks during hibernation,

plasma T_4 levels decreased to low values, whereas T_3 levels were maintained high. Since we have already established that even in euthermic chipmunks, physiological hibernation states were defined by a reduction of hibernation specific protein (HP) levels in blood, type 1 and 2 thyroxine 5'-deiodinase (D1 and D2) activities of various tissues in nonhibernation and hibernation states were examined in male euthermic chipmunks. Animals were kept under conditions of 23 degrees C and 12hrs light dark cycle throughout the experiment. D1 activities of liver in chipmunks in hibernation states were higher than those of animals in nonhibernation states. D2 activities of brown adipose tissue, brain, heart and skeletal muscle were increased in chipmunks in hibernation states. These results suggested that T_3 production in most of extrathyroidal tissues were facilitated in hibernation states even under euthermic conditions.

THE GENE EXPRESSION IN THE BRAIN AND CHOROID PLEXUS OF CHIPMUNK DURING HIBERNATION

Yasuhiko Kanno, Noriaki Kondo

Mitsubisikagaku Insutitute of Lifescience, Minamioya 11, Machida, Tokyo 194-8511, Japan

Hibernation-specific protein (HP) produced in the liver of chipmunks (*Tamias asiaticus*) is regulated by thyroxine (T_4) and testosterone, and T_4 promote HP transport into cerebrospinal fluid (CSF) through the blood-CSF barrier, choroid plexus. As we previously obtained 33 genes in the brain and choroid plexus, which were differentially expressed by T_4 , the expression profiles of these genes in hibernation cycle were analyzed by semi-quantitative RT-PCR. Most of these differentially expressed genes, including 11 novel genes, were positively and negatively regulated in association with hibernation. In some of the genes, changes in expression by hormone treatment were similar to those observed during hibernation. In many of the genes, there were differences in expression patterns of euthermic animals between hibernation and nonhibernation seasons. These results suggest that the gene expression in the brain and choroid plexus in hibernating animal is regulated even in suppressed physiological condition during hibernation, and that in hibernation season, the gene expression levels are adjusted to hibernation state without body temperature alteration under the control of hormones.

THE JAPANESE MONKEY AS A NON-HUMAN PRIMATE MODEL FOR THE STUDY OF OBESITY

Tomoko Takahashi, Juri Suzuki, Yoshirou Kamanaka, Masamitsu Abe, Norikatsu Miwa, Kaori Takagi, Yuzuru Hamada, Takashi Kageyama

Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan

Obesity is defined as a state that excess adipose tissue is accumulated, and is one of the important risk factors associated with type2 diabetes hypertension and cardiovascular disease. Obesity is known to occur frequently in non human primates in the laboratory. Some have been reported to develop obesity-induced diseases. In the present report, we describe the physiological analyses of Japanese monkey (*Macaca fuscata*) reared in outdoor open enclosures in the Primate Research Institute of Kyoto University. Body weight, body fat mass, and the levels of blood biochemicals such as leptin, adiponectin, insulin, glucose, triglyceride, cholesterol, and were examined. The results show that a significant high proportion of obese monkeys were found in a specific group, showing high values of body fat mass and serum leptin level. These Japanese monkeys are thought to be an ideal population for the examining obesity under the influences of genetic, environmental and social factors, and suitable animal models of human obesity.

HORMONAL CONTROL OF MELANIZATION IN THE LARVAL BODY MARKING OF A SWALLOWTAIL BUTTERFLY, *PAPILIO XUTHUS*

Ryo Futahashi, Haruhiko Fujiwara

Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Bioscience Bldg. 501, Kashiwa, Chiba 277-8562, Japan

A swallowtail butterfly, *Papilio xuthus* changes a larval body pattern drastically during the 4th ecdysis. We induced the precocious 4th molt by the injection of 20-hydroxyecdysone (20E), and found that the body pattern of the molted larva depended on the timing of the 4th ecdysis. When the 4th ecdysis was induced from 2 to 5 days after the 3rd ecdysis, the newly appeared epidermis showed the intermediate-type body patterns which varied from the 4th-larval type to the 5th-larval type continuously. The results of *in situ* hybridization and immunohistochemistry suggested that the black pigmentation until the 4th instar larva is controlled mainly by tyrosine hydroxylase (TH), and that of 5th instar larva mainly by dopa decarboxylase (DDC). To understand the functional roles of insect hormones, ecdysteroid and JH, on the body marking formation, we cultured the epidermis of 4th instar larva and examined the expressions of TH and DDC gene by quantitative RT-PCR. It is revealed that the induction of both TH and DDC gene is triggered by a declining ecdysteroid hormone titer.

PUPAL WING MORPHOGENESIS CONTROLLED BY REGION-SPECIFIC EXPRESSION OF ECDYSONE RECEPTOR (EcR) ISOFORMS

Eigo Suyama, Tomoko Matsunaga, Hiroyuki Shirai, Haruhiko Fujiwara

Department of Integrated Biosciences, Graduate School of Frontier Sciences, Kashiwa 277-8562, Japan

The complicated shapes of lepidopteran wings are defined by bordering lacuna (BL). In the pupal stage, cells outside of BL trigger programmed cell death whereas inside of BL proliferation. Both programmed cell death and cell proliferation are induced by ecdysone. According to the results of *in situ* hybridization, we found that A isoform of ecdysone receptor of *Bombyx mori* (BmEcR-A) expresses only outside of BL in the early pupal stage while B1 isoform (BmEcR-B1) expresses only outside of BL. To find the promoter regions which regulate the region-specific expression of BmEcR isoforms, we have constructed baculovirus-clones which have promoter regions of BmEcR isoforms and EGFP, and examined the region-specific EGFP expression. In the wing infected AcNPV(EcRA-1750/+ 50EGFP), EGFP expressed only outside of BL, and in the wing infected AcNPV(EcRB1-1950/+ 60EGFP), EGFP expressed only inside of BL. In the promoter region of BmEcR-B1 isoform, we have detected dpp (decapentaplegic) responsive sequence (DRS). When a baculovirus-clone with a mutation the DRS are infected into the *Bombyx* larva, we found that the EGFP signal was expressed in whole region of wing without the region-specificity.

EXPRESSION PROFILES OF ECDYSONE RECEPTOR ISOFORMS AND EARLY GENES ASSOCIATE THE PUPAL COMMITMENT IN THE WING DISCS OF THE SILKWORM, *BOMBYX MORI*

Takashi Koyama¹, Takayuki Sekimoto¹, Masafumi Iwami¹, Sho Sakurai^{1,2}

¹Division of Life Science, Graduate School of Science and Technology, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan and ²Department of Biology, Faculty of Science, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan

Progression of pupal commitment is under the control of 20E and JH in the wing discs of the silkworm, *Bombyx mori*. Before head capsule slippage (HCS) in the fourth instar, which is a sign of molting process, the disc cells are not sensitive to 20E. After HCS, they begin to respond to 20E with progression of pupal commitment. The disc cells thus acquire responsiveness to 20E at around HCS. In order to examine the molecular basis of acquisition of 20E responsiveness of wing disc cells, we analyzed the developmental profiles of EcR isoforms and early genes. Before HCS, EcR A isoform is at a low level, and EcR B1 is predominant, while EcR A increased immediately before HCS and became predominant at HCS, and the predominance of EcR A continued for 12 h. Topical application of JH to larvae 12 h before HCS caused a delay of acquisition of 20E responsiveness and prolonged the EcR A predominance. In addition, expression levels of BR-C Z2 and Z4 isoforms were highly reduced by topical application of JH. These results suggest that expression profiles of EcR and BR-C isoforms may have an important role in the acquisition of 20E responsiveness in the disc cells.

COMPARISON OF DISTRIBUTION AND RELEASE SITE OF PROTHORACICOSTATIC PEPTIDE BETWEEN *BOMBYX MORI* AND *MANDUCA SEXTA*.

Yoshiaki Tanaka¹, Yue-jin Hua², Ladislav Roller³

¹National Institute of Agrobiological Science, Tsukuba Ibaraki 305-8634, Japan, ²Institute of Nuclear-Agricultural Science, Zhejiang University, 310029 Hangzhou, People Republic of China and ³Institute of Zoology, Slovak Academy of Sciences, Dúbravská 9, 84506 Bratislava, Slovakia

Prothoracicostatic peptide (PTSP) was isolated from the larval brain of the silkworm, *Bombyx mori*, and has the same structure with myoinhibitory peptide of the tobacco horn worm, *Manduca sexta*. However, MIP was isolated from the nerve cord of *M. sexta* and is mainly present in the abdominal ganglion and epiproctodeal gland, but not in the brain. To compare the distribution and release site of PTSP/MIP between *B. mori* and *M. sexta*, we investigated the distribution and gene expression of PTSP in *B. mori*. PTSP gene is mainly expressed in the brain as well as in the abdominal ganglion and the depletion of staining of epiproctodeal gland with PTSP antibody was not observed during the molting period in *B. mori* although the depletion of staining was observed in *M. sexta*. These results may suggest the different function of the same structure of neuropeptide between the two lepidopteran insects.

ANALYSIS OF THE HORMONAL CONTROL OF PROTHORACIC GLAND ACTIVITY USING A LONG-TERM IN VITRO CULTURE SYSTEM

Akira Mizoguchi

Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan

The prothoracic glands (PGs) of the silkworm, *Bombyx mori*, is inactive at the beginning of the final larval instar but are gradually activated in a few days. To study the endocrine mechanism that regulates this gradual activation of PGs, a long-term *in vitro* culture system has been developed. The PG was dissected out together with surrounding tissues and cultivated in Grace's medium with 2% BSA under a high oxygen partial pressure. The medium was replaced with fresh one every 24hr and the ecdysteroid titer in every culture supernatant determined by RIA. When the PGs from larvae immediately after ecdysis were cultivated, they did not secrete detectable amounts of ecdysteroid for the first 1-2 days but started secretion thereafter. The ecdysteroid secretion continued for at least 6 days. When the gland was co-cultivated with corpus allatum (CA), the ecdysteroid secretion was completely suppressed. If the CA was subsequently removed, however, the gland started secretion. These results suggest that the PG is spontaneously activated without stimulation by PTTH. It is likely that the main regulator of the PG activity during early fifth instar is juvenile hormone secreted by the CA.