

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

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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

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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*

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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

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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 inside 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*

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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*.

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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

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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.