

THE COELOMIC FLUID OF THE EARTHWORM *Eisenia foetida* IS TOXIC TO VERTEBRATES BUT NOT TO INVERTEBRATES.

H.Kobayashi. 3-16-17 Kamisaginomiya, Nakano-ku, Tokyo, 165-0031

The coelomic fluid (CF) of the earthworm *Eisenia foetida* is known to have cytolytic, antibacterial, agglutinating, proteolytic and mitogenic activities. We have demonstrated that CF induces smooth-muscle contraction (Biomed Res, 17:197,1996) and has lethal effects on spermatozoa of vertebrates (J Exp Zool, 286:538,2000). However, all these activities were shown at the cellular or tissue level *in vitro* and the effects of CF on whole animals remain to be investigated. If we are to understand its biological significance in nature, it is important to define the effects of CF on live animals. This study was designed to survey its effects in invertebrates and vertebrates. The CF was not toxic to 42 species, which belonged to seven invertebrate phyla, when predominantly aquatic adults and larvae were exposed to it. Eleven teleostean species tested died in 0.2 to 1 % CF within two hours. The larvae of *Bufo japonicus formosus*, *Hynobius tokyoensis* and *H. lichenatus* died in 1 to 2 % CF within 3 hours. The Okinawa tree lizard, Japanese quail, mouse and rat all died within one hour after i.v. injection of CF with 5-10 μ l/kg. Thus, animals are grouped into two categories, invertebrates and vertebrates, depending on the non-lethal or lethal response of animals to the CF. The results will be discussed in relation to lysenin, a novel protein which we isolated from *Eisenia foetida* (Gene 191:97,1997).

MEASUREMENT OF MOUSE ERYTHROPOIETIN BY A SANDWICH ELISA.

S. Sakata, T. Morimoto and M. Takaki

Department of Physiology II, Nara Medical University, Kashihara.

Erythropoietin is well-known to be a renal hormone which regulates the production of red blood cells in humans and animals. We have examined whether mouse plasma erythropoietin (EPO) can be measured by a sandwich-type enzyme-linked immunosorbent assay (ELISA) using two anti-EPO monoclonal antibodies against recombinant human EPO (rHuEPO) and milk proteins as a blocking reagent and as a diluent for standard rHuEPO or for plasma samples. The milk proteins brought about both high slope- sensitivity of the standard curve and low background. The dose-response curves of anemic mouse plasma and of rHuEPO were linear and parallel to each other. The anemic plasma had an additive effect with rHuEPO in increasing the absorbance at 405 nm. The coefficients of variation in the intra- and inter-assays ranged from 4.2% to 15.3%. The plasma EPO levels in 22 normal mice were 18.3 ± 10.3 (SD) mU/ml. The inverse relationship between the logarithm of plasma EPO concentrations and the blood hemoglobin concentrations, red blood cell counts or packed cell volume was found in normal and iron-deficient anemic mice. These results show the validity for the use of this improved ELISA for measuring circulating murine EPO.

EXPRESSION OF NEUROSTEROIDOGENIC ENZYME IN THE AVIAN BRAIN -P450_{17 α} LYASE-

M. Matsunaga, K. Ukena, K. Tsutsui

Lab. of Brain Sci., Fac. of Integrated Arts and Sci., Hiroshima Univ., Higashi-Hiroshima.

New findings have been obtained that the brain can synthesize steroids *de novo*, and such steroids are called neurosteroids. In the avian brain, we have demonstrated that cytochrome P450 side-chain cleavage enzyme (P450_{sc}) and 3 β -hydroxysteroid dehydrogenase/ Δ^4 - Δ^5 -isomerase (3 β -HSD) are expressed and produce pregnenolone, pregnenolone sulfate and progesterone. To clarify the biosynthetic and metabolic pathway of neurosteroids in the avian brain, we examined the expression of messenger RNA (mRNA) encoding for the enzyme cytochrome P450 17 α -hydroxylase/C17,20-lyase (P450_{17 α} LYASE) using the quail. RT-PCR analysis followed by Southern hybridization indicated the expression of P450_{17 α} LYASE mRNA in the brain of sexually mature birds without a clear-cut sex difference. Although P450_{17 α} LYASE mRNA was expressed in various brain regions, the expressions in diencephalon and mesencephalon were higher than those in other brain regions, unlike 3 β -HSD mRNA. *In situ* hybridization showed that P450_{17 α} LYASE mRNA expression was localized in restricted brain regions, such as the hyperstriatum accessorium (HA), the ventral portions of the archistriatum (A), the preoptic area (POA), the septum (S), the anterior hypothalamus (AHy), and the dorsolateral thalamus (DL). Furthermore, P450_{17 α} LYASE mRNA was expressed in Cerebellar Purkinje cells. These results suggest that the avian brain possesses P450_{17 α} LYASE as well as P450_{sc} and 3 β -HSD, and produce dehydroepiandrosterone and androstenedione from pregnenolone and progesterone, respectively. Biosynthesis of dehydroepiandrosterone and androstenedione in the avian brain may be region-dependent.

IDENTIFICATION AND LOCALIZATION OF A NOVEL RFamide PEPTIDE IN THE GOLDFISH BRAIN

K. Sawada, K. Ukena, and K. Tsutsui.

Lab. of Brain Sci., Fac. of Integrated Arts and Sci., Hiroshima Univ., Higashi-Hiroshima.

We isolated novel RFamide peptides, which include RFamide at their C-termini and control the pituitary function, from the quail and frog brains. In this study, we investigate the presence of the novel RFamide peptide in the goldfish brain using an antibody raised against the frog novel RFamide peptide. Immunoreactive cell bodies were localized immunocytochemically in the nucleus posterioris periventricularis (NPPv) of the hypothalamus and the olfactory bulb. Many immunoreactive fibers of NPPv neurons were detected in the ventral diencephalon, and some immunoreactive fibers projected into the pituitary. Those results suggest that the fish brain also contains the novel hypothalamic peptide having RFamide at the C-terminus which may control the pituitary function.

NEUROENDOCRINE REGULATION OF DIAPAUSE-PUPAL CUTICLE COLORATION OF THE SWALLOWTAIL BUTTERFLY, *PAPILIO XUTHUS* L

A. Yamanaka, H. Imai, and K. Endo.

Dept. Physics, Biology & Informatics, Fac. of Sci, Yamaguchi Univ., Yamaguchi.

Papilio xuthus show color polymorphism in the non-diapause pupal body, such as green and brown types. The non-diapause pupal body is determined by a hormone producing brown pupae (pupal-cuticle-melanizing hormone : PCMH) which is secreted from brain suboesophageal ganglion-prothoracic ganglion (Br-SG-PG) complexes in the pharate pupal stage. On the other hand, the diapause pupal body show brown, orange and green types.

In this study, we investigated the neuroendocrine regulation of diapause pupal cuticle coloration in *P. xuthus*, using the ligation and the injection of Br-SG extracts from *P. xuthus*, and *Bombyx mori*. In addition, we examined the effects on the diapause pupal cuticle coloration of *P. xuthus* of various insect hormonal factors.

SEARCH FOR HOMOLOGUES OF VERTEBRATE PITUITRY HORMONES FROM A COMPLIMENTARY DNA LIBRARY OF HATSCHEK'S PIT IN AMPHIOXUS

K. Kubokawa and T. Umeda.

Ocean Res. Inst., the Univ. Tokyo.

Amphioxus is the only invertebrate group in which a homologue to the pituitary gland has been found. This suggests a possibility that primitive pituitary hormones are synthesized and secreted in Hatschek's pit. Peptidergic granules found in the pit were classified into four types according to the size. A strong positive immunocytochemical reaction was obtained with anti-human LH beta and anti-rat pit-1. However, identity of peptidergic granules and positive reaction of antibody has not been clarified. We have tried to survey cDNA encoding functional proteins in a cDNA library constructed from Hatschek's pit and peripheral tissue. Some cDNA clones isolated were cDNA for the functional proteins related to endocrine function in various vertebrate tissues. They are for GDP dissociation inhibitor, which is suggested to regulate GnRH recently. Inhibin, insulin-like growth factor binding protein like protein, an insulin-like peptide isoform, members of the olfactory receptor family and serotonin receptor family. Unfortunately, no cDNA for homologous or similar proteins to vertebrate pituitary hormones have been detected by now. Sequences of many other clones showed no relation with any of known substances.