novel neurosteroid 7α-hydroxypregnenolone may be synthesized actively in the brainstem involving in instinct behavior that may increase the locomotor activity of newts.

INCIDENCE OF RETRIEVING BEHAVIOR IN JUVENILE FEMALE AND MALE RATS: INFLUENCE OF NEONATAL ANDROGEN

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Juvenile rats are known to show maternal like behavior, retrieving. However, the incidence has not been reported, yet. In this experiment, incidence of retrieving in 20 day-aged female and male Wistar rats were observed. Furthermore, in order to examine the effect of neonatal androgen on incidence of juvenile retrieving behavior, 100 μg or 1 mg testosteron propionate (TP) was injected to female rats 5 days after birth. As a result, 24.0% female and 44.7% male rats showed retrieving behavior. In TP-treated females, 47.8% and 25.0% animals showed the behavior in 100 μg and 1 mg TP groups, respectively. These results suggest that there is no sexual difference of the incidence of retrieving in juvenile rats and is no influence of neonatal androgen on the incidence.

EFFECT OF 5-HT_{1A} RECEPTOR- OR $GABA_B$ RECEPTOR- AGONIST ON SEXUAL BEHAVIOR IN FEMALE RATS WITH LESIONS IN THE DORSOMEDIAL MIDBRAIN TEGMENTUM.

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In the dorsomedial midbrain tegmentum, an inhibitory influence exists in the raphe nucleus in regulating sexual behavior in female rats. In this experiment, to investigate the role of 5-HT_{1A} receptor and $GABA_B$ receptor in the area, this area was lesioned by a radiofrequency lesion generator and lordosis behavior was observed before and after treatments with 1 mg/kg bw 8-OH-DPAT or 10 mg/kg baclofen. As a control, these drugs were treated to female rats without brain surgery. As a result, both 8-OH-DPAT and baclofen decreased lordosis and soliciting behavior in control rats. In contrast, in some rats with lesions, the inhibitory effect of these drugs was not observed.

THE ROLE OF THE HYPOTHALAMUS IN METAMORPHOSING RANA JAPONICA

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In view of the contradictory reports as to the role of the hypothalamus in metamorphosing amphibians, the effect of surgical hypothalectomy was re-examined in *Rana japonica*. Removal of the hypothalamic rudiment was carried out at the open neurula stage according to Hanaoka (1967).

The result of surgery differed among different larvae. Some hypothalectomized larvae remained in prometamorphic stage as reported by Hanaoka. Other larvae, on the other hand, entered into the stage of forelimb eruption (i.e., metamorphic climax) with a marked delay. In the latters, however, the regression of the tail, gill and intestine failed to occur. Thus some but not all hypothalectomized *Rana* tadpoles could advance to metamorphic climax without the hypothalamus but they failed to accomplish the final regressive events of metamorphosis. The relationship between the degree of post-surgical regeneration of the hypothalamus and the metamorphosing ability is discussed.

MOLECULAR MECHANISM OF MUSCLE CELL DEATH IN A REGRESSING TAIL DURING AMPHIBIAN METAMORPHOSIS

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The tadpole tail, which is twice as long as the body, is induced to resorb completely by thyroid hormone within several days during the anuran metamorphosis. To investigate the underlying mechanism, we undertook two approaches. First, we examined the effect of dominant-negative thyroid hormone receptor (DNTR) on muscle cell death in vitro. The overexpression of DNTR suppressed the death of a tail-derived myoblastic cell line induced by thyroid hormone. Secondly, tadpole tails were injected with a reporter gene and the DNTR expression construct. DNTR overexpression inhibited a decrease of the reporter gene expression which began at stage 57 in the control tadpoles, but only delayed massive muscle cell death at stage 63 when tails shrink very rapidly. Some remained even a few weeks after the metamorphosis, although most of DNTR overexpressing cells died by the end of the metamorphosis. These results let us to propose that thyroid hormone induces the suicide of muscle cells (the cell-autonomous death) in the tail between stage 57 and 62, and that both the murder and suicide mechanisms execute muscle cell death in stage 62-64 to remove muscle promptly and completely.

REGULATION OF TYPE II IODOTHYROINE 5'-DEIODINASE GENE EXPRESSION BY STEROIDS IN *XENOPUS* METAMORPHOSIS

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Sequential metamorphic changes of the tissues or organs are thought to be an event regulated by activities of two types of iodothyronine deiodinases. Type III deiodinase (D3) inactivates thyroid hormones whereas type II deiodinase (D2) converts thyroxine into an active form of 3, 5, 3'-triiodo-L-thyronine. Recently, we have reported that D3 gene, but not D2 gene, is tissue-specifically regulated by prolactin and growth hormone. The tail is one of the latest metamorphosing organs. This organ expresses high levels of 5D mRNA throughout metamorphosis. When the tail starts to regress, 5'D mRNA level sharply increases. In the present study, we aimed to elucidate what stimulus regulated the D2 expression in the tail.. It is well known that adrenal steroids promote the tail regression. Therefore, we focused on effects of the steroids and found that D2 gene expression was up-regulated in the organ-cultured tails by mineralocorticoid or glucocorticoid, only in the presence of thyroid hormones. The results suggest that the in vivo increase in D2 mRNA level upon the tail regression may be caused by synergistic effects of endogenous steroids and thyroid hormones.

SUPPRESSION OF T_3-INDUCED TADPOLE TAIL ATROPHY BY PARA-NONYLPHENOL

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This study attempts to clarify the suppression of triiodothyronine (T_3)-induced tail atrophy by ρ-nonylphenol (NP). *Xenopus laevis* stage 54 tadpole tails were severed at the base of the hind limb and transferred into a culture medium (70‰minimum essential medium (MEM) containing 100 units/ml penicillin and 100 μg/ml streptomycin). After 2 days incubation, the healthiest tails were selected and cultured at 22°C in Petri dishes containing various solutions as follows. Group-1 tails were cultured in the above mentioned medium. Group-2 and -3 tails were cultured in the same medium to which was added10^{-6} M of NP, and group-4 and -5 tails were cultured in the medium to which was added 10^{-6} M of 17β-estradiol (E_2) . After 1 day, 5×10^{-8} M of T_3 was added to the culture mediums of groups 1, 3 and 5. Groups 2 and 4 continued treatment with no addition of T_3. Control tails were cultured in the culture medium and not exposed to T_3, NP or E_2. Treatment for all groups was continued for a total of 7 days. The results showed that both NP and E_2 suppressed the enhancing effects of T_3 on tail shortening.

INHIBITORY EFFECTS OF BISPHENOL A ON T3-INDUCED TAIL RESOPTION AND ELEVATION OF THYROID HORMONE RECEPTOR MRNA LEVELS IN *XENOPUS* LARVAE

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Previously we demonstrated the suppression of spontaneous and thyroid hormone-induced metamorphosis in *Xenopus* larvae by bisphenol A (BPA;10^{-5}-10^{-4} M). In the present study, we investigated anti-metamorphic effect of BPA (10^{-7}-10^{-4} M) by monitoring resorption of tail fragments from stages 52-54 *Xenopus* tadpoles *in vitro*. BPA blocked 10^{-7} M triiodothyronine (T_3)-induced tail resorption concentration-dependently, whereas in the absence of T_3, BPA had no effect on the tail. RT-PCR was performed for semi-quantitative analyses of gene expression of thyroid hormone receptor (TR) α and β in the cultured tail segments. BPA down-regulated TR mRNA levels either in the presence or absence of T_3. Thus BPA seems to act as an anti-metamorphic agent at least partly through the suppression of TR gene expression.

EFFECTS OF BISPHENOL-A AND CADMIUM ON OSTEOCLASTS AND OSTEOBLASTS IN THE SCALES OF GOLDFISH

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The direct effects of bisphenol-A and cadmium on osteoclasts and osteoblasts were examined using a culture system of goldfish scales. Tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP) were used as markers of osteoclasts and osteoblasts, respectively. Bisphenol-A and cadmium significantly suppressed both TRAP and ALP activities. Cadmium (even at 10^{-13} M) significantly suppressed TRAP activity, suggesting that this system may be utilized as an acute biosensor for cadmium. From an analysis of a reverse transcription-PCR in the *in vitro*-cultured scales, it was demonstrated that the *insulin-like growth factor-1* mRNA expression was decreased by a bisphenol-A treatment. In cadmium-treated scale, mRNA expression of the *estrogen receptor* and *insulin-like growth factor-1* decreased

compared with the control. Furthermore, we found that bisphenol-A and cadmium were induced a hypocalcemia and disrupted calcium homeostasis in a respective *in vivo* experiment. Therefore, teleosts scale is a good model for evaluating the influence of endocrine disrupters and heavy metals on bone cells and calcium metabolism.

EFFECTS OF ESTRADIOL ADMINISTERED THROUGH DIET ON CIRCULATING VITELLOGENIN AND REPRODUCTIVE ORGANS IN MALE JAPANESE QUAIL

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To establish a method for administrating endocrine disruptor chemicals through diet, estradiol-17β was mixed with powdered quail food at the concentrations of 0.01, 0.1, 1, 10, 100, 1000 ppm and served to male Japanese quail for a week in one group and 4 weeks in the other group. Average food consumption was constant at about 15 g/day for each bird. Circulating vitellogenin significantly increased after a week in a group served with estradiol 1ppm mixed diet and groups with more higher concentrations. Combined testicular weight and the area of the cloacal protrusion decreased significantly at the highest dose of estradiol. The four week experiment indicated that 2 weeks were required to attain the plateau in the lower dose groups. The highest dosage, i.e. 1000 ppm, was rather toxic and survival rate was 33.3 % by the end of the 4 week experiment. To assess an effect of phytoestrogens on vitellogenin assay, phytoestrogen low diet was used to compare with normal diet which contain dizein, genistein and so on. The results indicated that these phytoestrogens did not induce circulating vitellogenin at the concentrations contained in the soybean and alfalfa contained diet.

COMPETITIVE INTERACTIONS OF CHLORINATED PHENOLS WITH THYROID HORMONE BINDING TO THYROID-HORMONE-BINDING PROTEINS.

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We investigated the effects of bisphenol A, nonylphenol and their seven chlorinated derivatives on thyroid hormone (T3) to purified chicken and bullfrog transthyretin (cTTR and bTTR) and to the ligand-binding domains of chicken and bullfrog thyroid hormone receptor (cTR LBD and bTR LBD). The concentrations at which the chlorinated derivatives displaced radioactive T3 from TTR were 100-1000 times less than those of their parent molecules. The interaction of the chlorinated derivatives with the cTR and the bTR LBDs were weaker than those of the chlorinated derivatives with cTTR and bTTR. Chlorinated derivatives with a greater degree of chlorination were more efficient competitors of T3 binding to TTR and TR. A structure-activity relationship between the phenol compounds and TTR (TTR assay) and TR (TR assay) was established. Structures with chlorine in either ortho positions or both ortho positions, with respect to the hydroxy group, were more efficient competitors.

ENDCRINE DISRUPTING CHEMICALS PROMOTE PURKINJE DENDRITIC GROWTH DURING CEREBELLAR CORTICAL FORMATION

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Recently, we have demonstrated that in the rat Purkinje neurons produce actively estradiol as a neurosteroid during neonatal life, when cerebellar cortical formation occurs dramatically. We have further demonstrated that estrogen promotes Purkinje dendritic growth via its receptor in this neuron in the neonate. On the other hand, endocrine disrupting chemicals, such as octylphenol, bisphenol A and nonylphenol are thought to mimic the action of estrogen. In this study, possible actions of endcrine disrupting chemicals on the growth of Purkinje neurons during neonatal life were examined using newborn rats. octylphenol and bisphenol A promoted the dendritic growth of Purkinje neurons, unlike their somata. However nonylphenol did not induce any significant changes in Purkinje morphorogy. The effect was blocked by estrogen receptor antagonist tamoxifen. Taken together, these results suggest that the endcrine disrupting chemicals, octylphenol and bisphenol A promote Purkinje dendritic growth via estrogen receptor localized in this neuron during neonatal life, like estrogen.

MATERNAL THYROID STATUS INFLUENCES DEVELOPMENT OF THE SEXUALLY DIMORPHIC NUCLEUS IN RATS

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Thyroid hormone (TH) is essential for brain development. Although TH-dependent stage of brain development has been considered to be perinatal period, recent observations indicate the importance of maternal thyroid status before the onset of fetal TH synthesis. To clarify the TH-dependent stage of early brain development, pregnant rats were given a single injection of propylthiouracil (PTU) and the behavior of their offspring was analyzed by the open field test. The male offspring of the dam injected PTU on 7.5 days or 13.5 days gestation behaved dissimilar from the control male offspring. They moved here and there actively and showed rearing frequently. The behavior was similar to it of female offspring. The volume of SDN-POA in the male offspring was reduced compared with controls. These results suggest that fetal hypothyroidism depresses the development of SDN-POA in male fetuses.

IDENTIFICATION OF A RECEPTOR INTERACTED AN OXITOCIN-LIKE PEPTIDE, ANNETOCIN, FROM EARTHWORM *EISENIA FOETIDA*

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Oxytocin is a neuropeptide or hormone that regulates reproduction. Previously, we also identified an oxytocin-like peptide from earthworm, annetocin, which induces egg-laying behavier. In this presentation, we report a receptor interacting with the annetocin. By using degenerate oligonucleotide primers deduced from the conserved regions of the mammalian oxytocin/vasopressin receptors, a cDNA encoding a homologous receptor was characterized. To examine that the homologous receptor responds with the annetocin actually, we used oocyte expressional system. Annetocin leads to a signal transduction at receptor expressed oocyte in addition to annetocin, indicating that the putative receptor is an annetocin receptor. In addition, we performed RT-PCR experiment to decide the receptor expressing tissue. The receptor was expressed in clitellum strongly, but little in head region including cerebral ganglion and subpharyngeal ganglion. On the other hand, expression of annetocin is in subpharyngeal ganglion but not in clitellum. These results indicate that the annetocin is produced in the subpharyngeal ganglion and carried to peripheral tissue in clitellum expressing the receptor.

CLONING OF *OCTOPUS* CEPHALOTOCIN RECEPTOR, A MEMBER OF RECEPTORS OF OXYTOCIN/VASOPRESSIN SUPERFAMILY

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Members of oxytocin/vasopressin (OT/VP) superfamily widely distribute in invertebrates as well as vertebrates, and play key roles as signaling molecules in the regulation of reproduction and osmoregulation. We have revealed that the common octopus *Octopus vulgaris*, has two members of the superfamily, octopressin (OP) and cephalotocin (CT), as in vertebrates, an observation made for the first time in invertebrates. Since OP and CT have discriminative biological properties, the presence of specific receptors has been proposed. cDNA of an orphan receptor cloned from the Octopus brain encoded a polypeptide of 397 amino acids that displays sequences characteristic of G-protein coupled receptors. The orphan receptor showed high homology to receptors of OT/VP superfamily and seemed to conserve the agonist-binding pocket common to the OT- and VP-receptors. *Xenopus* oocytes that express the orphan receptor responded to the application of CT by an induction of membrane chloride currents coupled to the inositol phosphate/calcium pathway. Expression of CTR mRNA was detected in the central and the peripheral nervous systems, the ovary and the oviduct, and the pancreas.

BINDING SELECTIVITY OF TACHYKININ-RELATED PEPTIDES FOR THEIR RECEPTORS IN *DROSOPHILA MELANOGASTER*

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Diverse invertebrate tachykinin-related peptides have been so far identified from various species. Previously, we revealed that six *Urechis* tachykinin-related peptides displayed equivalent activation on their receptor, unlike vertebrate tachykinins which showed distinct binding selectivity to respective receptor subtypes. To confirm the generality of such properties of tachykinin-related peptides, we examined the binding affinity of five *Drosophila* tachykinin-related peptides for two cognate receptor candidates expressed in *Xenopus* oocytes.(1) Satake H. et al . (2003) Zool. Sci., 20, 533-549(2) Kawada T. et al.. (2002) Eur. J. Biochem. 269, 4238-4246