epithelium when the animals become adults. However, the development of such irreversible vaginal changes is inhibited by concurrent vitamin A (VA) treatment. Neonatal exposure to keratinocyte growth factor (KGF), which is a paracrine mediator of epithelial-mesenchymal interactions, also induces the persistent proliferation and cornification of the vaginal epithelium in adult mice. This study was designed to examine whether concurrent administration of VA inhibits the development of the irreversible vaginal changes in mice exposed neonatally to KGF. The results showed that neonatal KGF treatment produced irreversible proliferation and cornification of the vaginal epithelium in ovariectomized adult mice, whereas neonatal treatment with KGF plus VA did not induce such vaginal changes. Also, the number of layers and thickness of the vaginal epithelium were increased in mice neonatally treated with KGF as compared with the control, and the increase was not seen in mice neonatally treated with KGF plus VA.

DOPAMINE β-HYDROXYLASE-LIKE PROTEINS IN THE MOUSE MAMMARY GLAND

Manabu Matsuda[1,2], Nelson D. Horseman[2]
[1]Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan and [2]Department of Molecular and Cellular Physiology, University of Cincinnati Medical School, Cincinnati, OH 45267, USA

Suppression subtractive hybridization cloning was undertaken to identify the genes that were differentially expressed in the mammary tissues from PRL-deficient mice compared with hyperprolactinemic and progesterone-treated mice. Of the several genes identified as being PRL-dependent, one clone encoded cDNA fragments for mouse monooxygenase X (mMOX), a gene related to dopamine β-hydroxylase (DBH) which catalyzes noradrenaline and octopamine biosynthesis from dopamine and thylamine. mMOX mRNA localized exclusively in the secretory epithelium in the mammary glands, although the expressions of the gene product in both prolactin-nonstimulated mammary tissue and cleared fat pad were not completely negative. Thus, prolactin appeared to induce differentiation of mMOX-positive mammary buds, and the gene might be useful for a molecular marker of the epithelial cell differentiation during mammary lobulo-alveolar development. In addition, expression of DBH was also evident in the mammary tissue and was decreased by prolactin. Another DBH-like protein (a putative molecule from the genomic database) was exclusively expressed in the thymus but not in the mammary tissue.

EFFECTS OF NEONATAL TREATMENT OF MICE WITH ESTROGEN AND VITAMIN A ON THE EXPRESSION OF EPIDIDYMAL ESTROGEN RECEPTOR α

Maho Ogoshi, Keiko Nakahashi, Manabu Matsuda, Miho Kyokuwa, Takao Mori
Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan

In the male murine, the expression pattern of estrogen receptor (ER) in the developing epididymis is modified by the neonatal exposure to estrogens. On the other hand, we have previously demonstrated that abundance of vitamin A during the so-called 'critical period' inhibited some harmful effects of the neonatal estrogen treatment on both female (e.g., ovary-independent proliferation of the vaginal epithelium) and male mice (i.e., decrease in the number of sperm). In the present study, the effect of concurrent administration of vitamin A acetate on the effect of neonatal estradiol-17β treatment on the epididymal expression of ERα was examined in mice. Injection of estradiol-17β for 5 days after birth resulted in an increase in the ERα expression level in the vas deferens adjacent to the cauda epididymis at 18 days of age when compared to the intact control mice, while no significant difference was observed in the epididymal ERα expression between the intact control and the group of mice injected neonatally with both vitamin A acetate and estradiol-17β. Thus, vitamin A treatment appeared to inhibit the outcome of neonatal estrogen-effect in the developing mouse epididymis.

CHANGES IN EXPRESSION OF ErbBs AND THEIR LIGANDS IN NEONATALLY DES-EXPOSED MOUSE VAGINA

Shinichi Miyagawa[1,2,3], Yoshinao Katsu[2,3], Hajime Watanabe[2,3], Taisen Iguchi[1,2,3]
[1]School of Life Science, The Graduate University for Advanced Studies, Okazaki, Aichi 444-8585, Japan, [2]Center for Integrative Bioscience, Okazaki National Research Institutes, Okazaki, Aichi 444-8585, Japan, and [3]Core Research for Evolutional Science and Technology, Kawaguchi, Saitama 332-0012, Japan

Neonatal treatment of mice with estrogens, including diethylstilbestrol (DES), induces estrogen-independent persistent proliferation and cornification of vaginal epithelium, resulting in tumor later in life. However, the molecular mechanisms of these changes have not been elucidated so far. ErbBs, tyrosine kinase receptors and their ligands, epidermal growth factor family, are involved in the development in the reproductive tracts and their aberrant expression frequently occurs in cancer. In order to clarify the correlation between estrogen-independent cell proliferation and erbBs signaling, we examined the expression profile of erbBs and their ligands in neonatally DES-exposed mouse vagina. Female mice were injected (s.c.) with 3 micro gram per day DES or oil vehicle alone for 5 days beginning on the day of birth (day 0), and mRNA expression was examined the following day (day 5). Some mice were ovariectomized on day 46 and sacrificed on day 60. We found that some EGF family ligands were highly expressed in neonatally DES-exposed mouse vagina, showing estrogen-independent persistent proliferation and cornification.

GLOBAL GENE EXPRESSION IN UTERUS AND VAGINA OF NEONATAL MICE EXPOSED TO DIETHYLSTILBESTROL

Atsuko Suzuki[1,3], Hajime Watanabe[1,3], Hiroshi Handa[2], Taisen Iguchi[1,3,4]
[1]Center for Integrative Bioscience, Okazaki NRI, Okazaki, [2]Tokyo Institute of Technology, Yokohama,[3]CREST, JST, and [4]Granduate University for Advanced Studies

Mice exposed neonatally to diethylstilbestrol (DES) show disorganized uterine myometrial layers, uterine adenocarcinoma and stratification and cornification of vaginal epithelium. These reproductive abnormalities are induced by estrogens when exposed within a critical window. The molecular mechanism of these abnormalities, however, remains unknown. Thus, we examined global gene expression in uterus and vagina of 0, 5 and 60-day-old mice 6h after DES injection by microarray analysis. Number of induced or repressed genes by DES revealed 10-fold less in neonatal mouse uterus than those of adults. In vagina, number of genes exhibited expression change by DES in neonatal mouse vagina was also smaller than those of adults. In 5-day-old mice, number of genes expression by DES were ca. 1/5 of genes of adult vagina. Thus, gene response to DES in uterus and vagina are increased with age. Genes affected in neonatal period may be related to abnormalities in reproductive tracts.

BIOSYNTHESIS AND ACTION OF ESTROGEN IN THE DEVELOPING PURKINJE NEURON

H. Sakamoto, Y. Mezaki, K. Ukena, K.Tsutsui
Laboratory of Brain Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739-8521, and CREST, JST, Japan

We have demonstrated recently that Purkinje neurons of the rat cerebellum actively synthesize progesterone *de novo* from cholesterol only during neonatal life. We have further found that progesterone promotes both dendritic growth and synaptogenesis in Purkinje neurons through intranuclear receptor-mediated mechanisms. On the other hand, recent studies have shown that estrogen receptor β is expressed in the rat Purkinje neuron. Therefore, it is possible that not only progesterone but also estrogen may be involved in the formation of cerebellar cortex. With these findings as a background, in this study, we first investigated the expression of aromatase (P450arom), which forms estradiol-17β, in the rat cerebellum using RT-PCR. P450arom mRNA was expressed in the cerebellum during neonatal life and its expression was localized in Purkinje neurons. We then examined the effect of estradiol-17β on Purkinje neuron morphology during neonatal life. Both *in vitro* and *in vivo* studies showed that estradiol-17β promoted the growth of Purkinje dendrites. Taken together, it is possible that both estradiol-17β and progesterone may be essential for the formation of cerebellar cortex.

EFFECTS OF ESTROGENIC CHEMICALS ON DEVELOPMENT OF *XENOPUS LAEVIS*

K. Sone[1,3], M. Hinago[1,3], A. Kitayama[2], J. Morokuma[2], N. Ueno[2], H. Watanabe[1,3], T. Iguchi[1,3]
[1]Center for Integrative Bioscience, Okazaki National Research Institutes, Okazaki, Aichi 444-8585 Japan, [2]Division of Morphogenesis, National Institute for Basic Biology, Okazaki, Aichi 444-8585 Japan, and [3]CREST/JST

Many chemicals released into the environment have capacities to disrupt the systems of normal development. In this work, we investigated the influence of bisphenol A (BPA) and nonylphenol (NP) on *Xenopus laevis* at embryonic and larval stages, as a model animal in aquatic environment. Embryos were exposed to eight different concentrations of BPA (0.3–6.8 mg/L) or NP (0.2–11.0 mg/L) from stages 6 to 45. Reduced length, tail flexure, microcephaly, rudimental gut coiling and edema were observed at 4.4 mg/L BPA and 4.6 mg/L NP, 4 days of exposure. Hypersensitive stages of *X. laevis* embryos to these chemicals were clarified by exposing at 5 different periods, BPA affected only earlier stage and NP markedly affected at later stage. To analyze the functional mechanism of BPA and NP in induction of morphological changes, we adapted a DNA array technology. We identified several genes induced or repressed by these chemicals during frog development.

ESTROGEN-INDUCED BLOOD VESSEL MALFORMATION, BLOOD CLOTTING,AND SEX REVERSAL IN TRANSGENIC MEDAKA

Toshiyuki Kawamura[1], Ryota Hori-e[1], Ichiro Yamashita[1], Masato Kinoshita[2]
[1]Center for Gene Science, Hiroshima University, Kagamiyama 1-4-2, Higashi-Hiroshima 729-8527, [2]Department of Fisheries, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

Estrogens play key roles in vertebrate physiology. The vasculature, like the reproductive tissues, bone, liver, and brain, is an important target of estrogen's action. On the contrary, recent studies have found that hormone replacement therapy (HRT) is associated with increased risk of venous thromboemboism. We established three transgenic medaka fish lines overexpressing the medaka estrogen receptor under the constitutive medaka β-actin promoter. The transgenic embryos became hypersensitive to estrogens, and failed to develop yolk veins while blood clots formed in the blood island. These results indicate that activation of estrogen receptor caused the estrogen-induced developmental defects. On the contrary, the transgenic fish underwent the genetically determined gonadal differentiation (testis or ovary) and showed the same sex-reversal rates as those of wild-type non-transgenic fish in the treatments with estrogen and androgen. These results present invaluable data to