ASSESSING PHENOL COMPOUNDS FOR THYROID DISRUPTING ACTIVITY USING A COMBINATION OF IN VIVO AND IN VITRO ASSAYS IN *XENOPUS LAEVIS*

Yumiko Kudo[1], Kiyoshi Yamauchi[2]
[1]Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan and [2]Department of Biology, Faculty of Sience, Shizuoka University, Shizuoka 422-8529, Japan

Several environmental contaminations cause various abnormalities and disturbance in the endocrine system. We investigated the effects of phenol compounds containing halogenated phenols on thyroid hormone signaling pathway in *Xenopus laevis in vivo* and *in vitro*. In competitive 3,3',5-[^{125}I]triiodothyroinine (T_3) binding to *xenopus* thyroid hormone receptor (xTR) and to *xenopus* transthyretin (xTTR), 3,3',5-trichlorobisphenol A (TCBPA) and 2,4,6-triiodophenol (TIP) were potent competitors, while, *o-t*-butylphenol (BP) and 2-isopropylphenol (IPP) were impotent or weaker competitor. BPA and TIP acted as T_3-antagonists in *Xenopus* tadpole metamorphosis assay in the presence of T_3, judging from morphological and T_3-dependent transcriptional changes: interocular distance and xTRβ transcript. Interestingly, BP and IPP, with which [^{125}I]T3 binding to xTR did not compete, showed T_3-antagonist activity, suggesting that these chemicals interfered with thyroid hormone signaling pathway at some step(s) besides T3 binding to xTR.

MECHANISMS UNDERLYING ANP-MEDIATED INHIBITION OF DRINKING IN EEL

Takehiro Tsukada, Susumu Hyodo, Yoshio Takei
Department of Physiology, Ocean Research Institute, the University of Tokyo, Nakano-ku, Tokyo 164-8639, Japan

Drinking is essential for survival in hyperosmotic environment for seawater (SW) fish to cope with dehydration. Since atrial natriuretic peptide (ANP) strongly inhibits drinking and decreases plasma Na concentration in SW-adapted eels, it is considered to promote seawater adaptation in fish. In the present study, we attempted to analyze the mechanisms underlying ANP-mediated inhibition of drinking in the brain of SW eels. Initially, relative expression of ANP receptors (NPR-A) was grossly evaluated in the a few brain areas by RT-PCR for screening of its presence, and then an immunohistochemical technique was used to determine the distribution of NPR-A in more detail in the brain. The results showed that immunopositive cells were detected in glossopharyngeal vagal motor complex and area postrema (AP), which are known to be the sites involved in the regulation of drinking. The endothelial cells of blood vessels were also stained in the whole brain area. Since AP was the only site stained by trypan blue injected into the circulation, this circumventricular structure may be the site of action of ANP from blood, resulting in inhibition of drinking.

OSMOREGULATION IN ELEPHANT FISH, *CALLORHYNCHUS MILLII* (HOLOCEPHALI)

Susumu Hyodo[1], Justin D. Bell[2], John A. Donald[2], Yoshio Takei[1], Tes Toop[2]
[1]Laboratory of Physiology, Ocean Research Institute, University of Tokyo, Nakano-ku, Tokyo 164-8639, Japan and [2]Deakin University, Geelong, 3217 Victoria, Australia

Osmoregulatory mechanisms in holocephalan fishes are unknown except that they conduct urea-based osmoregulation as in elasmobranchs. We therefore examined changes in plasma parameters of elephant fish, *Callorhynchus millii*, after gradual transfer to concentrated (120%) or diluted (80%) seawater (SW). In control fish, plasma Na and urea concentrations were about 310mM and 450mM, respectively. The plasma urea concentration of elephant fish was considerably higher than that reported for chimaeras, another holocephalan species. After transfer to 120% SW, the plasma Na concentration markedly increased, while a decrease in plasma urea concentration was observed following transfer to 80% SW. The elephant fish do not have a discrete rectal gland. Instead, approximately 10 tubular structures are located in the wall of post-valvular intestine. Each tubular structure contains a putative salt-secreting component composed of a single-layered columnar epithelium, which was stained with anti-Na^+, K^+-ATPase serum. These results suggest that the tubular structures in the posterior intestine represent a primitive form of the rectal gland in elephant fish.

ARGININE VASOTOCIN STIMULATES PHOSPHORYLATION OF AQUAPORIN-H2 IN THE FROG URINARY BLADDER

Takahiro Hasegawa, Masakazu Suzuki, Shigeyasu Tanaka
Department of Biology, Faculty of Science, Shizuoka University, Oya 836, Shizuoka 422-8529, Japan

Water reabsorption from the urine in the amphibian urinary bladder is regulated by arginine vasotocin (AVT). It is believed that the high water permeation is induced by increasing the amount of water channel protein, called aquaporin (AQP), on the luminal membrane. Our recent studies have shown that AQP-h2 protein, identified from the pelvic ventral skin of the tree frog (*Hyla japonica*), is expressed in the granular cells of the urinary bladder and that the vesicles bearing AQP-h2 translocate from cytoplasmic pools to the luminal membrane in response to AVT. In the present study, we generated a specific antibody against phosphorylated AQP-h2 (p-AQP-h2), and examined the role of phosphorylated AQP-h2 on the translocation in the frog urinary bladder. After isolated bladders were incubated with AVT (10^{-8}M) for a short period of time (0 to 15 min), the specimens were subject to Western blot analysis and immunocytochemistry. p-AQP-h2 was detected after 2 min of incubation, localized on the luminal membrane. These findings indicate that phosphorylation of AQP-h2 plays an important role in the translocation of vesicles bearing AQP-h2 in the granular cells of the amphibian urinary bladder.

APOPTOSIS AND CELL PROLIFERATION IN THE ANTERIOR INTESTINE OF AN AMPHIBIOUS, EURYHALINE MUDSKIPPER

Kenji Narita[1], Hideya Takahashi[1], Akiyoshi Takahashi[2], Tatsuya Sakamoto[1]
[1]Ushimado Marine Biological Station, Okayama Univeristy, Okayama 701-4303, Ja pan and [2]School of Fisheries Sciences, Kitasato Univeristy, Ofunato, Iwate 022-0101, Japan

In the seawater-adapted euryhaline fishes, to replace water lost by drinking seawater, the permeability of gut is generally greater than that of the freshwater-adapted fish. We examined apoptosis and cell proliferation in the anterior intestine of mudskipper transferred from isotonic 30% seawater to fresh water or to seawater for 1 day and 7 days, as well as those kept out of water for 1 day. The apoptosis (DNA laddering) were induced during seawater acclimation. TUNEL staining detected a large number of apoptotic cells over the intestinal epithelium of seawater fish. DNA synthesis ([^{3}H]thymidine incorporation) in the freshwater fish were greater than those in seawater fish. Labeling with antibodies to proliferating cell nuclear antigen indicate that proliferating cells were randomly distributed in the intestinal epithelium of freshwater fish whereas the seawater proliferating cells were in the troughs of the intestinal folds. There were no significant changes in fish kept out of water. During adaptation to different salinities, the modification in cell renewal system may play an important role in regulation of the gut permeability.

BODY FLUID REGULATION IN FOUR ANURAN SPECIES INHABITING DIFFERENT NATURAL HABITATS: FLUCTUATION OF BLOOD COMPONENTS UNDER VARIOUS OSMOTIC CONDITIONS

Tomoko Nishio, Mayumi Katayama, Norifumi Konno, Kouhei Matsuda, Minoru Uchiyama
Department of Biology, Faculty of Science, Toyama University, 3190 Gofuku, Toyama 930-8555, Japan

Four anuran species, *Bufo marinus*, *Rana nigromaculata*, *Rhacophorus arboreus*, and *Xenopus laevis* were examined in this study. Animals were acclimated under various osmotic conditions for 5-7 days. Hematocrit value (Ht) as a possible indicator of plasma volume, plasma osmolality, and concentrations of components (Na^+, Cl^-, K^+ and urea) were also measured. In dehydration, plasma concentrations of aldosterone (ALDO) and all components measured were increased in all anurans species examined. In animals maintained in 300 mOsm NaCl solution, plasma osmolality, Na^+, Cl^-, and urea concentrations were significantly increased, and Ht and plasma ALDO were significantly decreased. In *B. marinus* maintained in tap water, plasma osmolality, and concentrations of Na^+ and ALDO were significantly decreased. In *Rha. arboreus* acclimated to tap water, plasma Na^+ and ALDO concentrations were insignificantly changed in comparison with those of control. Body weight was increased but not significant. Together with the present study, it is considered that the adaptability of anurans to various osmotic conditions may be influenced by their natural habitats.

NEPHRONE STRUCTURES AND IMMUNOLOCARIZATION OF Na^+, K^+- AND H^+-ATPase IN THE KIDNEY OF TWO SQUAMATA SPECIES

Ryousuke Kikuchi[1], Norifumi Konno[1], Kouhei Matsuda[1], Hideki Yoshizawa[2], Minoru Uchiyama[1]
[1]Department of Biology, Faculty of Science, Toyama University, Toyama, Toyama 930-8555, Japan and [2]Department of Biology, Matumoto Dental University, Shiojiri, Nagano 399-0704, Japan

In this study, we examined nephron structure and localization of two ion pumps (Na^+, K^+- and H^+-ATPase) in the metanephric kidney of two Squamata species, Japanese grass lizard (*Takydromus tachydromoides*) and green anole lizard (*Anolis carolinensis*) according to the morphological and immunohistochemical studies with the light- and the electron-microscopy. In those species, the kidney was composed of some lobes. It was demonstrated that those metanephric nephrons, which lack Henle's loop, were consisted of the glomerulus, neck segment, proximal tubule, intermediated segment, narrow part of the distal tubule, wide part of the distal tubule, and collecting duct. In the immunohistochemical study, high Na^+, K^+-ATPase immunoreactivities were observed in the basolateral cell membranes of the distal portion of nephron. Positive H^+-ATPase immunoreactivities were observed in the intercalated cells of the collecting duct. The present results suggest that the distal portion of nephron in the lizards is important segments on ion transport of sodium and proton. Species, sexual and seasonal differences are also reported in the kidney of the present species.