reversal potential for the apFaNaC current was more positive than +40 mV, and was sensitive to extracellular Na^+ concentration, suggesting that the channel is Na^+ selective.

IDENTIFICATION OF NEUROPEPTIDES IN A PROSOBRANCH GASTROPOD, *THAIS CLAVIGERA*

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In the rock shell, *Thais clavigera* (prosobranch gastropod), a musculinization of females, called 'imposex' is induced by organotin compounds, such as TBT and/or TPT in seawater, at approximately 1 ng/L. As the first step to investigate the involvement of neuropeptide(s) in this phenomenon, we started a peptide-isolating project on the animal. A peptidic extract was prepared from the 500 pieces of the ganglia and soft bodies (without digestive and reproductive organs, kidney, hypobranchial gland, ctenidium and osphradium), and fractionated with HPLC. A part of each fraction was screened by the dot-blot assay using some anti-neuropeptide antibodies or bioassay. As the results, we purified more than 10 of immnopositive or bioactive substances, and 6 of them were identified as the peptides by the amino-acid sequencing and mass spectrometry. The identified peptides included the 4 kinds of novel peptides, such as GGNG-peptide related peptides and GLWamide-like peptide. Elucidation of the localization and physiological functions of those peptides are in progress. This work is financially supported by the grant-in-aid from the Ministry of Environment, Japan, to T. H.

ANALYSIS OF METAL BINDING ACTIVITY OF VANADIUM-BINDING PROTEINS (VANABINS) FROM AN ASCIDIAN *ASCIDIA SYDNEIENSIS SAMEA*

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We have previously identified several vanadium-binding proteins (vanabins) expressed abundantly in the cytoplasm of vanadium-accumulating cells, vanadocytes, of a vanadium-rich ascidian *Ascidia sydneiensis samea*. We have cloned cDNAs for the two vanabins, vanabin1 and vanabin2, and examined their metal binding ability using recombinant proteins. Vanabin1 and vanabin2 can bind 10 or 20 vanadium ions, respectively, at +4 oxidation state (VO^{2+}) at a dissociation constant of around 2 X 10^{-5} M. In this study, we found that by an EPR study the coordination environment of vanabin2 against VO^{2+} ions is N_2O_2 type, and amino residue of lysines contribute to the coordination. In addition, by a metal-chelating column method, we found that vanabins can bind to copper (II) and iron (III) ions.

ANALYSIS OF METAL-RELATED GENES IN THE VANADIUM-RICH ASCIDIAN, *ASCIDIA SYDNEIENSIS SAMEA*

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Several ascidian species are known to accumulate high levels of vanadium in their vanadocytes. The highest level observed in *Ascidia gemmata* corresponds to about 10^7 times the levels in seawater. To investigate the phenomenon, we carried out an expressed sequence tag analysis (EST) of blood cells of *A. sydneiensis samea*, in which 13 mM vanadium is accumulated. We obtained randomly selected 1,300 ESTs from the vandocytes and whole blood cells cDNA libraries. In this study, 62 metal-related genes were identified. In particular, ferritin H-subunit, which is known as an iron storage protein, and novel vanabins were found. Vanabins have been extracted from A. sydneiensis samea blood cells, cloned and identified as low molecular weight vanadium-binding proteins. Vanadium binding ability of these proteins was confirmed by immobilized metal affinity chromatography and gel filtration column chromatography.

REGULATION OF BODY FLUID AND THE STRUCTURE OF KIDNEYS IN JAPANESE ARBOREAL FROG, *RHACOPHORUS ARBOREUS*

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We observed that the kidney of Japanese arboreal frogs is large in size and is constituted of many small and thin nephrons (73rd Annual Meeting of ZSJ). The structure of their kidney seems to indicate the adaptation to arboreal life. The aim of the present study is to elucidate the renal function in the body fluid regulation of Japanese arboreal frog, *R. arboreus*. The frogs were kept in various experimental conditions (normal, dry, hydration and 300mOsm NaCl solution) for 5 days. Body weight, serum osmolality, ions concentrations, urea concentration, hematocrit value and aldosterone level were measured. In dehydrated frogs decreased in body weight, each serum value was significantly increased. On the other hand, no significant change was observed in hydrated frogs. In high saline treated frogs, serum Na, urea concentrations and osmolality were significantly higher than their environment. Their body weights were significantly increased and hematocrit value was significantly decreased. In the kidney of frogs used the above acclimated study, we studied immunohistochemically on ion transporters and ion pumps expressed in the nephron constituting cells.

WATER FLOW-REGULATION IN THE GOLDFISH AND NEWT BY C-RFA PEPTIDE-PRETREATMENT

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C-RFa (Carassius RFamide) is a bioactive peptide isolated from the brain of the Japanese crucian carp. The fact that the sequence of C-RFa is significantly homologous (65%) with that of mammalian prolactin (PRL)-releasing peptide (PrRP) suggests that C-RFa is a fish-counterpart of mammalian PrRP. Pretreatment of C-RFa (intraperitoneal injection) in goldfish decreased the water influx of the branchial tissues while anti-C-RFa antibody increased it, suggesting that C-RFa regulates water movement through the gills via PRL action. We also found that the thickness of the scale's mucus layer in the goldfish was increased by C-RFa-pretreatment. Though the homologue to C-RFa for Urodela has not been isolated or characterized, we were also intrigued to test the effect of C-RFa on that skin which may function as an obstacle to water intrusion through the body surface. Pretreatment with anti-C-RFa-antibody did significantly affect the size and the development of glandular elements in the skin of the Japanese newt (*Cynops pyrrhogaster*). These results suggest that counterparts of mammalian PrRP for fish and newt may play an important role for the regulation of water flux through the integument.

PHYSIOLOGICAL ROLE OF ENDOGENOUS ANP EVALUATED BY IMMUNONEUTRALIZATION

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Atrial natriuretic peptide (ANP) has been recognized as a hormone involved in body fluid regulation in vertebrates. In marine teleost, ANP is suggested to promote seawater (SW) adaptation because it suppresses plasma Na+ concentration in SW by inhibiting drinking of ambient SW and subsequent absorption of NaCl by the intestine when administered exogenously into SW eels. To evaluate the role of endogenous ANP in SW adaptation, the present study was undertaken to remove circulating ANP secreted mainly from the heart using specific antisera against ANP in SW eels. The antisera cross-react with both ANP and ventricular NP (VNP) that has spectra of actions similar to ANP. The removal of endogenous ANP and VNP consistently produced opposite effects that occurred after ANP infusion; the immunoneutralization increased drinking rate and plasma Na+ concentration in SW eels compared with controls injected with normal rabbit sera. Therefore, circulating ANP plays a pivotal role in hyponatremic regulation of eels in SW via suppression of drinking and, probably, of intestinal NaCl absorption.

IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES OF THE BRAIN NUCLEI ASSOCIATED WITH DRINKING BEHAVIOR IN THE EEL ACCLIMATED TO SEA WATER

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Immunohistochemical and Electrophysiological Studies of the Brain Loci Associated with Drinking Behavior in the Eel Acclimated to Sea WaterMasaaki Ando, Yuzo Matsunaga and Sunao Ito (Lab. of Integrative Physiol., Fac. of Integrated Arts & Sci., Hiroshima Univ. Higashi-Hiroshima 739-8521, JapanSea water eels are the most suitable model for analyzing drinking behavior in vertebrates. We have identified three nuclei (magnocellular preoptic nucleus (PM), anterior tuberal nucleus (NAT) and area postrema (AP)) lacking the blood-brain barrier, which can therefore respond to systemic dipsogens or antidipsogens. On the other hand, swallowing results from relaxation of the upper esophageal sphincter (UES) muscle. The UES was innervated by the glossopharyngeal-vagal motor complex (GVC) in the medulla oblongata. The PM was immunoreactive to ANG II and AVT antibodies. The AP was tyrosine hydroxylase immunoreactive and the GVC was choline acetyltransferase immunoreactive. The neuronal activity of the GVC was inhibited by adrenaline in a concentration dependent manner. From distribution of somata and fibers, a neuronal network for controlling drinking behavior was proposed.