residures 403-417 of the rat ETAR modified by the multiple antigen peptide complex system. By Western blot analysis of rat kendyes, affinity-purified anti-ETAR antibody detected about 47.3 and 64.5 kDa bands. By light microscopy, intense ETAR-like immunoreactivity was seen in the basal side of the distal tubules and collecting ducts. In addition to the basal side staining, immunoractive puncta were scattered in the cells of the renal butules and collecting ducts, except in the Henle's loop. By electron microscopy using the pre-embedding method, immunopositive signals were seen on the basolateral cytoplasmic membrane of the interdigitations and basal infolding of the distal tubules and collecting ducts. In contrast, only a part of the basolateral cytoplasmic membrane of the interdigitations and basal infolding were immunopositive in the proximal tubules. These results suggest that endothelin's action on the basolateral membrane is through ETAR, especially on the distal tubules and collecting ducts.

RECONSTRUCTED MOUSE VAGINAL EPITHELIUM IN 3D-COCULTURE

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17β-estradiol (E_2) regulates proliferation, stratification and cornification of mouse vaginal epithelium *in vivo*. These dramatic morphological changes attract biologists to study the mechanism of E_2 action. Because of complex hormonal and tissue-to-tissue interaction *in vivo*, it is difficult to understand the specific and direct effects of E_2 on the vagina. We have established vaginal clonal epithelial and fibroblastic cell lines from $p53^{-/-}$ prepubertal female mice. All of them have estrogen receptor-α protein. In the present study, one epithelial cell line (SV-4b6b) and two fibroblastic cell lines (SV-4b4b2 and SV-6c4a1b) were chosen and subjected to 3D co-culture. SV-4b4b2 and SV-6c4a1b cells were embedded in type I collagen gel, and SV-4b6b cells were seeded on the gel, and cultured with or without E_2 at air-liquid interphase for 3 weeks. Under these conditions, SV-4b6b cells developed a stratified epithelium, and E_2 obviously induced epithelial cornification. Accumulation of basement membrane was clearly observed. The present study showed that newly established vaginal cell lines had ability to reconstruct vaginal structure *in vitro*.

MOLECULAR CLONING OF A cDNA ENCODING AN MIH-LIKE PEPTIDE OF THEKKURUMA PRAWN *PENAEUS JAPONICUS*

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The crustacean molt-inhibiting hormone (MIH) is released from the X-organ sinus gland complex and suppresses ecdysteroid synthesis by the Y-organ. MIH of the kuruma prawn *Penaeus japonicus* has been characterized and its cDNA cloned. Recently, a partial amino acid sequence of a MIH-like peptide of *P. japonicus* has been reported. In the present study, we have isolated a cDNA encoding the MIH-like peptide. A cDNA fragment was isolated using RT-PCR with two degenerate oligonucleotide primers designed based on the amino acid sequence of the MIH-like peptide. Subsequently, a full length of cDNA was amplified by 5'- and 3'-RACE. The MIH-like peptide precursor cDNA had 804 bp comprising a 5'-untranslated region (45 bp), an open reading frame (306 bp), a stop codon (TAA), and a 3'-untranslated region (450 bp). Conceptually translated peptide consisted of a putative signal peptide (23 residues) and an MIH-like peptide (79 residues). The amino acid sequence of the MIH-like peptide was similer to that of *P. japonicus* MIH (68% identity). The MIH-like peptide was also homologous to *Metapenaeus ensis* gonad-inhibiting hormone and *M. ensis* MIH (66 and 67% identity, respectively).

MOLECULAR CLONING OF cDNAs ENCODING ANDROGENIC GLAND HORMONE PRECUSORS FROM THREE SPECIES OF THE TERRESTRIAL ISOPOD

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In crustaceans, male sexual characters are induced by androgenic gland hormone (AGH), which is produced by the male-specific androgenic gland. cDNAs encoding AGH precursors have recently been cloned from three species of the terrestrial isopod *Armadillidium vulgare*, *Porcellio scaber* and *P. dilatatus*. In order to clarify the molecular diversity of AGH, we further tried to clone cDNAs encoding AGH precursors from three other terrestrial isopod species, *A. nasatum*, *P. laevis* and *Alloniscus balssi*. cDNA fragments encoding AGHs of the three species were amplified by RT-PCR with degenerate oligonucleotide primers designed based on the amino acid sequences of the three known AGHs. Subsequently, the full-length cDNAs were amplified by 5'- and 3'-RACE. All the three AGH precursors consisted of a signal peptide, B chain, C peptide and A chain, the organization of which were the same as that of the three already-known AGHs. The amino acid sequences of A and B chains, which comprise a mature peptide of AGH, are highly conserved among the six species (about 80% identity), while that of C peptide showed less identity (about 50%).

IDENTIFICATION AND BIOLOGICAL ACTIVITY OF A FROG GH-RELEASING PEPTIDE-RELATED PEPTIDE

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Previously we identified in the bullfrog hypothalamus a novel peptide with a C-terminal -Leu-Pro-Leu-Arg-Phe-NH_2 sequence. This peptide having GH-releasing activity was designated as fGRP. Analysis of cDNA encoding fGRP indicated a possible generation of three other fGRP-related peptides (fGRP-RPs) with their C-termini of -Leu-Pro-Leu/Gln-Arg-Phe-NH_2 from the precursor molecule. Mass spectrometric analyses confirmed that these three fGRP-RPs were derived from the precursor in the hypothalamus. It was also revealed that one of the fGRP-RPs possesses a prolactin-releasing activity.

EXISTENCE OF MULTIPLE C-TYPE NATRIURETIC PEPTIDE GENE FAMILY IN TELEOSTS

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The natriuretic peptide (NP) family is a group of peptides involved in body fluid regulation in vertebrates, and comprises four major members, atrial NP (ANP), B-type NP (BNP), C-type NP (CNP) and ventricular NP (VNP). Among them, CNP is characterized by the absence of the "tail" segment extending from the carboxy terminus of the ring. It has been isolated from a variety of vertebrate species from elasmobranches to mammals. The information on the function of CNP is, however, still insufficient at present. In this study, we attempted to isolate cDNAs encoding CNP from medaka (*Oryzias latipes*) as a first step in elucidating the functions of CNP. We successfully obtained two types of cDNA clones from the brain, each of which encodes a different type of CNP. By the reverse transcription-PCR analyses, it was found that two CNP genes are expressed mainly in the brain. However, the two genes exhibited different patterns of expression in peripheral tissues, which suggests functional differentiation between them. We also identified genes orthologous to each CNP in the genome of the puffer fish, *Fugu rubripes*.

FOURTH NATRIURETIC PEPTIDE IDENTIFIED IN THE STURGEON, *ACIPENSER TRANSMONTANUS*

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We have cloned all natriuretic peptide (NP) cDNAs from a chondrostean fish (sturgeon, *Acipenser transmontanus*) to examine the molecular evolution of NP family in vertebrates. PCR reaction was performed from the brain, atrium and ventricle, which were main tissues of NP production, with four degenerate primers that can amplify all types of NPs. ANP cDNA was cloned from the atrium, VNP from the ventricle, and CNP from the brain as expected from the results of teleostean fish. Furthermore, an additional NP cDNA was cloned from the ventricle despite only three NPs have been identified in teleostean fish. The novel NP has three structual characteristics common to BNP, which has been regarded as a tetlapod-specific homologue of VNP: (1) it has a BNP-specific processing site (2) highly homologous residues in the coding region, and (3) plural AUUUA repeats in 3' untranslated region of its mRNA. These results suggest that the NP system, which has been thought to function with ANP, VNP and CNP in teleost and ANP, BNP and CNP in tetrapods, is in fact composed of four types of NPs in all vertebrate species including teleosts and mammals.

DEVELOPMENT OF NOVEL AND SENSITIVE ENZYME-IMMUNOASSAY FOR PACAP: COMPARISON OF BRAIN PACAP CONCENTRATIONS AMONG SOME VERTEBRATE SPECIES

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A novel and sensitive enzyme-immunoassay (EIA) desjgnated as avidin-biotin complex detectable EIA (ABCDEIA) for PACAP was developed by using biotin-labeled PACAP/avidin/biotin-conjugated enzyme complex. Some vertebrate PACAPs with 27, 38 and 44 amino acid residues cross-reacted in an ABCDEIA with biotin-labeled PACAP38 or 44. On the other hand, an ABCDEIA with biotinylated PACAP27 detected only PACAP27, recognizing neither PACAP38 nor 44. The concentrations of PACAP38 in the whole brain of the macaque, rat and mouse were 8.15-14.91 pmol/g wet weight, but the concentrations of PACAP27 were almost null. The concentrations of PACAP in the bullfrog, stargazer and stingray brains were 141.57 pmol/g wet weight as PACAP38, 432.77 pmol/g as PACAP38 and 71.45 pmol/g as PACAP44, respectively. These values were 5-30 times as high as those in the mammalian brains. No PACAP27 was detected in the frog and fish brains.