Recombinant fish pituitary hormones GH and PRL: Purification and characterization of biological properties

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1. *The Steroid Hormone of Sunlight Soltriol (Vitamin D): Receptors in Vertebrate Phylogeny and Role in Seasonal Regulation of Life.* W. E. STUMPF AND H. J. BIDMON, Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, North Carolina 27599.

In the past, vitamin D has been regarded as "the calcium homeostatic steroid hormone." However, autoradiographic studies with tritiated dihydroxycholecalciferol in rodents have demonstrated specific nuclear binding (receptors) in endocrine, neural, cardiovascular, glandular, reproductive, and immunological tissues, as well as in skin (*Histochemistry* 89, 209). Vitamin D-soltriol has been shown to affect pituitary TSH cells and pancreatic β cells to elevate TSH and insulin blood levels. Extensive distribution of soltriol target cells has now been demonstrated in representatives of all vertebrate groups in the central nervous system and pituitary and in both female and male reproductive organs. Vitamin D-soltriol cannot be understood in the narrow sense of systemic and cellular calcium regulation alone; it is a steroid hormone of sunlight that influences seasonal adaptation of all vital functions that include development, growth and motor activities, sexual maturation and reproduction, immune responses, and cardiovascular and endocrine-autonomic regulation.

2. *Comparative Studies of Corticosteroid Receptor Binding in Lower Vertebrates.* A. GERHARD AND W. HANKE, Zoological Institute II, Kaiserstr. 12, D-7500 Karlsruhe, Federal Republic of Germany.

The binding of different natural and synthetic corticosteroids to glucocorticoid receptors was investigated in several species of lower vertebrates, *Myxine glutinosa* (Cyclostomata), *Cyprinus carpio, Oreochromis mossambicus* (Teleostei), *Ambystoma mexicanum,* and *Xenopus laevis* (Amphibia). The dissociation constants (K_D) and the maximal concentration of binding sites (N_{max}) were determined by Scatchard plot. The aim of the study was to answer the following questions: 1. Is there an evolution of receptor specificity? 2. Is there any obvious difference between a "mineralocorticoid" and a "glucocorticoid" receptor? The naturally occurring corticosteroids, aldosterone (A), corticosterone (B), cortisol (F) and 11-deoxycortisol (S) were bound by gill as well as by liver of the Atlantic hagfish, while the synthetic compounds dexamethasone (d) and triamcinolone acetonide (TA) were not. No differences in K_D and N_{max} between gill and liver were observed. The binding hierarchy was: $A > B \geqslant F \geqslant S$. The binding affinities of the hormones in the two teleost in both organs were identical $TA > D > F$. In carp organs binding of F was similar to that of S. Comparing both species, carp in general showed a higher N_{max} than tilapia. In the liver of amphibians the order of binding was $TA > D > B > F$. Binding of A was found in *Axolotl* liver and binding of S in *Xenopus* liver. In conclusion, the receptor does not seem to discriminate between the four natural corticosteroids in hagfish. The teleost and amphibian receptor showed an affinity for the

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severe obesity syndrome and disturbances in fertility. Feeding behaviour is regulated by the CNS. The central appetite regulatory system appears to be arranged in a cascade, with an interaction of a gradually increasing number of neuropeptides and neurotransmitters. The hypothalamus appears to act as a neuroendocrine transducer in this complex process. In addition to well-known experimental models such as hypothalamic lesion techniques and dietary and genetic obesity, viral infection can also induce an obesity syndrome. Presuming that BDV-induced obesity is based upon a central nervous process, it was of interest to know whether correlative alterations in different brain peptides could be identified. Adult female Lewis rats (4–5 weeks) were infected intracerebrally with BDV, maintained on food and water ad libitum, and killed at intervals by means of perfusion (Bouin's fixative). Immunostaining was performed on paraffin-embedded sections (7 μm) using the PAP technique with antisera raised against gonadotropin-releasing hormone (GnRH), neuropeptide Y (NPY), neurotensin, vasopressin, serotonin, and somatostatin. Initial results demonstrate distinct differences in the immunoreactivity of GnRH, vasopressin, neurotensin, serotonin, and somatostatin systems between BDV-infected rats and control rats. Decrease of numbers, distributions, and staining intensities of cell bodies and fibers projecting to the median eminence and organum vasculosum laminae terminalis were noted.

232. *Recombinant Fish Pituitary Hormones GH and PRL: Purification and Characterization of Biological Properties.* F. RENTIER-DELRUE,* D. SWENNEN,* J. A. MARTIAL,* G. FLIK,† S. J. WENDELAAR BONGA,† P. PRUNET,‡ P. Y. LEBAIL,‡, S. DROT,§ A. LAMPROY,‖ A. PONCIN,‖ C. DENIS,‖ S. LEBECQUE,‖ F. LIEFFRIG,‖ AND J. SMAL,‖ *Laboratoire de Génie génétique, Université de Liège, B6 Sart Tilman, 4000 Liège, Belgium; †Department of Zoology, University of Nijmegen, Nijmegen, the Netherlands; ‡Laboratoire de physiologie des poissons, INRA, Rennes, 35042 France; §F.U.L., 6900 Mirwart, Belgium; and ‖Eurogentec S.A., Sart Tilman, 4000 Liège, Belgium.

In teleost fish, the primary role of growth hormone (GH) is to promote growth and development while prolactin (PRL) is involved in many functions such as water and electrolyte balance, growth, and reproduction. We have reported previously the cloning and characterization of trout and tilapia GH and PRL cDNAs (*DNA* 8, 109–117, 261–270, 271–278, 1989; 8, 119–125, 1989). These cDNAs were inserted into bacterial expression vectors. The expressed recombinant proteins were produced in 5-liter fermentors and purified to high homogeneity by HPLC with a yield ranging from 10 to 30 mg/liter of *E. coli* culture. Biological activities were characterized *in vitro* and *in vivo*. The recombinant trout GH (rtGH) potency was equivalent to that of native chinook salmon (*Oncorynchus tschawytscha*) as assessed by radioimmunoassay and radioreceptorassay using trout liver membrane. Administration of rtGH to yearling trouts (0.1 μg/g body wt twice a week) by i.p. injection dramatically increased growth rate after 6 weeks of treatment: 260 and 231% over the control group in weight and length gain, respectively. Recombinant tilapia PRLs (rtiPRL-I and -II) were tested on tilapia (*Oreochromis mossambicus*). Both rtiPRLs bound specifically to kidney membranes from freshwater tilapia. They stimulated the epithelial growth of the skin and increased the mucus production within 1 day after injection (0.25 μg/g body weight). Both rtiPRLs also increased the plasma calcium concentration in a way similar to PRL produced by grafted pituitary glands. (This research was supported in part by a grant "FIRST" of the Région Wallonne, Belgium.)

233. *Study of a Direct Effect of Neuropeptide Y (NPY) on the Pituitary of the Chicken (Gallus domesticus).* J. MERCKAERT AND F. VANDESANDE, Zoological Institute, Laboratory of Neuroendocrinology and Immunological Biotechnology, Naamsestraat 59, 3000 Leuven, Belgium.

Selection for growth almost always impairs reproductive capacity. This is clearly seen for leghorn and broiler chickens. In practice, broilers are submitted to a feeding restriction during a critical period of their development. One of the neuropeptides which may play a role in the antagonism between growth and reproduction is neuropeptide Y (NPY). NPY injected centrally into rats stimulates feeding behaviour but at the same time it can stimulate or inhibit LH release. NPY also affects the release of ACTH, PRL, and TSH *in vivo*. *In vitro* experiments on isolated rat pituitaries showed that NPY directly stimulates release of LH, FSH, and GH from the pituitary. Do such effects also occur in chickens? Using autoradiography, binding sites for NPY on dispersed, immunocytochemically stained pituitary cells are sought and influences of NPY on the secretory activity of the different cell types are examined using an *in vitro* perifusion system and radioimmunoassay.

234. *The Role of Cyclic Nucleotide-Dependent Protein Kinases in the Mode of Action of Crustacean Molt Inhibiting Hormone.* U. VON GLISCYNSKI AND D. SEDLMEIER, Institut für Zoophysiologie, Endenicher Allee 11-33, D-5300 Bonn 1, Federal Republic of Germany.

Molting in crustaceans is initiated by increasing ecdysteroid in hemolymph. Synthesis of ecdysteroids in the Y-organ seems to be regulated negatively by molt inhibiting hormone (MIH), a peptide from the sinus gland.