Immunoreactive cells with anti-carp α-GTH, Anti-carp β-GTH, and anti-tilapia-GTH sera, in the proximal pars distalis of Carp and Tilapia pituitaries.

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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: $A > B \geq F \geq S$. 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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163. *Immunocytochemical Localization of Two GnRHs in Brain and Pituitary of the African Catfish, Clarias gariepinus.* M. A. ZANDBERGEN, J. JANSSEN-DOMMERHOLT, P. L. T. LIEM, J. PEUTE,[1] AND H.J.TH. GOOS, Research Group for Comparative Endocrinology, Department of Experimental Zoology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

In extracts of brain–pituitary tissue from the African catfish, two GnRHs were detected, i.e., catfish-I-GnRH which is unique compared with identified GnRHs from several other vertebrates and catfish-II-GnRH which cannot be distinguished from chicken-II-GnRH (*Gen. Comp. Endocrinol.* 75, 427–436, 1989). The present study used two antisera: (a) anti-lamprey GnRH which cross-reacts with catfish-I-GnRH, but not with chicken-II GnRH; and (b) anti-chicken-II GnRH which cross-reacts with catfish-II-GnRH, but not with catfish-I-GnRH. Binding studies in brain and pituitary extracts with these antisera indicate that chicken-II GnRH is not able to displace the binding with anti-lamprey GnRH and vice versa. In addition, anti-lamprey GnRH does not cross-react with paraformaldehyde-fixed chicken-II GnRH on a nitrocellulose blot. Light microscopical immunocytochemistry reveals the presence of fibers immunoreactive to both antisera and predominantly located in the ventral hypothalamus; fiber endings are observed in the proximal pars distalis of the pituitary gland and in the ependymal area of the nucleus lateralis tuberis pars medialis. Immunoreactive cell bodies are located in the ventral diencephalon, rostral to the nucelus praeopticus, whereas in the latter no immunoreactive cells are observed. At the electron microscopical level, application of both antisera demonstrates the colocalization of catfish-I- and catfish-II-GnRH in neurosecretory fibers in the gonadotropic region of the pars distalis. [1]Supported by NATO Grant RG.0454/88.

164. *Gonadotropin-Releasing Hormone Immunoreactivity in the Brain and Pituitary of the Three-Spined Stickleback, Gasterosteus aculeatus.* E. ANDERSSON,* B. BORG,* N. M. SHERWOOD,† AND H.J.TH. GOOS,‡ *Department of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden; †Department of Biology, University of Victoria, Victoria, B.C., Canada V8W 2Y2; and ‡Department of Experimental Zoology, University of Utrecht, Padualaan 8, Utrecht, The Netherlands.

In teleosts the release of gonadotropic hormone (GTH) is stimulated by gonadotropin-releasing hormone (GnRH). Brains from sexually mature male stickleback were fixed by perfusion and the distribution of GnRH immunoreactivity was studied by the peroxidase–antiperoxidase (PAP) technique on cryosections. Four different GnRH antisera were used: two antisera against salmon GnRH, one against chicken II GnRH, and one against mammalian GnRH (LHRH). Immunoreactive neurons were found in the hypothalamic preoptic nucleus and nucleus lateralis tuberis with numerous immunoreactive fibers entering the pituitary and reaching into the proximal pars distalis where the GTH cells are located. Also, in the olfactory bulbs, immunoreactive neurons were found. Furthermore, scattered immunoreactive fibers were found throughout the brain and in the retina. There was no difference in the distribution of neurons and fibers immunoreacting with the different antisera.

165. *Immunoreactive Cells with Anti-carp α-GTH, Anti-carp β-GTH, and Anti-tilapia-GTH Sera, in the Proximal Pars Distalis of Carp and Tilapia Pituitaries.* M. ABRAHAM,* E. BURZAWA-GERARD,† AND P. Y. LE BAIL,‡ *The Hebrew University of Jerusalem, Jerusalem, Israel; †Laboratoire de Physiologie générale et comparée, Paris, France; and ‡Laboratoire de Physiologie des Poissons, Rennes, France.

The proximal pars distalis of the pituitary in carp contains four cell types: two cell types reacting both with polyclonal anti-carp α-GTH and anti-carp β-GTH, one cell type reacting with polyclonal anti-salmon STH, and a fourth cell type not reacting with any of these three antisera. The two cell types which react to both anti-carp α-GTH and anti-carp β-GTH sera are the large globular type containing GTH II cell, and another cell type provisionally termed "NIRGA" (nonidentified—reacting with GTH antiserum). The NIRGA cells might be thyrotrophs, GTH I cells, or functional phases of GTH II cells. In the tilapia pituitary, three cell types were distinguished: GTH II cells, NIRGA cells, and somatotrophs. The secretory granules and globules of the tilapia GTH II cells are strongly stained with polyclonal anti-tilapia GTH serum. The secretory granules of the NIRGA cells of tilapia are weakly stained with the same immunoserum, while the rod-like structures from the NIRGA cells strongly react. There is a pronounced difference in the immunoreaction between granules and globules of the carp GTH II cells. While the former contain both α and β subunits in equal amounts, the globules have more β than α subunits. No exocytotic figures were observed either in the GTH II cells or in the NIRGA cells of carp or tilapia.