IGFs structure, function and regulation in fish

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more elevated in LD melanotropes. In addition, the cell subsets showed a differential response to TRH and dopamine, two classical *pars intermedia* regulators. Although TRH stimulated hormone release in both subpopulations, a more pronounced activation of LD melanotropes was observed. With regard to dopamine, the neurotransmitter inhibited αMSH secretion in LD cells, but it had no effect in HD ones. In conclusion, our results suggest that the intermediate lobe is composed of two cell subsets representing different functional states, which could correspond to different phases of a putative cell secretory cycle.

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S10 ANALYSIS OF HORMONE BIOSYNTHESIS AT THE SUBCELLULAR LEVEL : THE PARS INTERMEDIA OF *BUFO MARINUS*

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In the intermediate pituitary of the anuran amphibian, *Bufo marinus*, the N-acetylation of ACTH(1-13)NH_2 to yield α-MSH occurs as a co-secretory processing event ; whereas the N- acetylation of β-endorphin occurs as a posttranslational processing event (14). In order to understand how these two N-acetylation reactions are segregated, *B. marinus* intermediate pituitary cells were analyzed by immunogold labeling electron microscopy, and using an ultracentrifugation procedure. The results of these studies support the following hypotheses. The proteolytic cleavage of ACTH(1-39) to yield ACTH(1-13)NH_2 is a late processing event occurring in secretory granules. The cleavage of β-LPH to yield non-acetylated β-endorphin is an early processing event which may occur in the ER or the Golgi. Thus, there is a spatial and temporal separation of the posttranslational processing events associated with the β-LPH portion and ACTH portion of the POMC biosynthetic pathway in amphibian intermediate pituitary cells.

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S11 DIFFERENTIAL ACTION OF SECRETOINHIBITORS ON MELANOTROPE CELLS OF *XENOPUS LAEVIS*

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The melanotrope cell of *Xenopus laevis* is innervated synaptically by nerve terminals containing the coexisting transmitters GABA, in electron-lucent vesicles, and dopamine and Neuropeptide Y (NPY), colocalized in dense core vesicles. All these transmitters inhibit α-MSH secretion, GABA through both $GABA_A$ and $GABA_B$ receptors, dopamine through a D_2 receptor and NPY through a Y_1 receptor. We are conducting studies aimed at finding a functional rationale for the multiple secreto-inhibitors. To this end we have tested the hypothesis that the inhibitory mechanisms have differential effects on the sensitivity of melanotropes to cyclic-AMP-dependent mechanisms. We have found that the under $GABA_B$ inhibited conditions secretion is fully restored by cyclic-AMP ; secretion is only partially restored under either $GABA_A$ or NPY inhibition ; there is almost no restoration under dopamine inhibition (see also abstracts of Buzzi *et al.* and Lieste *et al.*). Moreover, we have found that the $GABA_A$ and $GABA_B$ receptors can be differentially activated, low concentrations of GABA preferentially activating the $GABA_B$ receptor mechanism (see abstract of Buzzi *et al.*). We have also found differential effects of secreto-inhibitors on α-MSH biosynthesis ; 3 day treatment with $GABA_A$ or $GABA_B$ receptor agonists had little effect on biosynthesis whereas both dopamine and NPY had strong inhibitory action on gene expression (see abstract of Dotman *et al.*). We conclude that the GABAergic mechanisms are for short-term inhibition of melanotrope cell function whereas dopamine and NPY are for long-term inhibition. We are currently examining if differential release of transmitter substances from the electron-lucent and dense core vesicles is possible, which would allow for differential activation of the GABAergic *versus* D_2/Y_1 mechanisms.

S12 THE NEUROACTIVE STEROID PREGNANOLONE EXERTS MULTIPLE MODULATORY EFFECTS ON $GABA_A$ RECEPTORS

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The effects of the neuroactive steroid pregnanolone (5β-pregnane-3α-ol-20-one) on the native $GABA_A$ receptor present in cultured frog melanotrophs were investigated by using the patch-clamp technique in the whole-cell configuration. In the current-clamp mode, bath application of pregnanolone (10^{-8} to 10^{-6} M) prolonged the GABA-induced inhibition of spontaneous action potentials. In the voltage-clamp mode, pregnanolone (10^{-6} M) reversibly enhanced the GABA-evoked current (10^{-7} to 10^{-5} M). Conversely, high doses of pregnanolone (3×10^{-5} M) markedly inhibited the GABA-evoked current. Pregnanolone potentiation of GABA-induced responses was accompanied by an increase of current and conductance desensitization rates. The presence of pregnanolone (10^{-5} M) in the patch pipette did not modify GABA-evoked currents potentiation provoked by bath application of the steroid (10^{-6} M). Epipregnanolone (a stereoisomer of pregnanolone) had no effect on the $GABA_A$ current. The cage convulsant TBPS reduced the GABA-evoked current, and the inhibitory effect of TBPS was totally reversed by pregnanolone. In contrast, the central type benzodiazepine antagonist flumazenil did not impair the potentiating effect of pregnanolone. It is concluded that pregnanolone exerts a complex modulatory effect on $GABA_A$ receptors. At moderate concentrations, pregnanolone potentiates the effect of GABA ; at higher concentrations, pregnanolone exerts a direct inhibitory action on the $GABA_A$ receptor.

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INSULIN AND IGF

S13 IGFs STRUCTURE, FUNCTION AND REGULATION IN FISH

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The aim of this presentation is to underline the original characteristics of fish IGFs. Divergence between IGF-I and IGF-II occurred before the separation of teleost and tetrapode but after that one of agnathe and gnathostome. The aminoacid sequence of binding sites to type I receptor and to binding proteins are identical in mammals and fish mature IGF-I. However, notable differences are observed in type 2 receptor site of IGF-II. Human and fish IGF-I have a similar bioactivity on fish cells. Recombinant fish IGF-II has a low bioactivity as well as a low binding capacity to human type 2 receptor and to mammal and fish type I receptor, may be due to a bad refolding. In fish, IGF-I acts on general growth, reproduction and osmoregulation, in part via cell multiplications. The synthesis and secretion regulation of IGF-I and IFG-II appears actually confusing. However, contrary to mammals, both of them seems to be stimulated by growth hormone. The pituitary direct pathway of GH inhibition by IGF-I appears dominant in fish, in opposition to what is observed in mammals. Further work is required to confirm fish IGF specificity.

S14 IGF-I RECEPTORS IN SKELETAL AND CARDIAC MUSCLES OF FISH

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Insulin receptors have been studied in a variety of vertebrate species. However, information regarding IGF-I receptors and functions in ecto-