Specific R.I.A. for Gth1 and Gth2 in the rainbow trout Oncorhynchus mykiss gonadoropins blood plasma levels during the spawning period

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means of in situ hybridization and immunocytochemistry. Both techniques demonstrated the presence of Islet-1 in some nuclei of the ventral telecephalon, the ventral preoptic area, and the mediobasal hypothalamus. No particular peptidergic phenotype could be correlated to the Islet-1 phenotype. The presence of Islet-1 in brain regions known for participating in the neuroendocrine control of pituitary functions in fish suggests not only a role for this transcription factor in the development but also in the regulation of some functions in the endocine mature brain.
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P210 CYCLIC ALLATOSTATIN-LIKE PEPTIDE DETECTION IN THE BRAIN-RETROCEREBRAL COMPLEX OF THE EARWIG *LABIDURA RIPARIA*, RELATED TO THE REPRODUCTIVE CYCLE

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A polyclonal antibody against allatostatin-3 of *Blatella germanica* (BLAST-3) (a gift from X. Bellès, Barcelona) is used to immunolocalize allatostatin- like peptides in the brain-retrocerebral complex of *Labidura riparia* adult females. Immunoreactive cells are strongly stained in *pars intercerebralis* and mainly in *pars lateralis*. In the deutocerebron, one cell is stained at the root of each antennal nerve. During the reproductive cycle, these cells and their axons show immunoreactivity at previtellogenic, ovulation and ovarian arrest periods. During vitellogenesis, immunoreactivity is restricted to only four pericaryons in the *pars intercerebralis*. When young vitellogenic females are injected with 20-hydroxyecdysone (20E), which inhibits vitellogenesis, full immunoreactivity reappears, suggesting sensibility of these cells to 20E as is logically expected for a negativre feedback loop.
The results show that BLAST-3-like material is produced cyclically in *Labidura* in correlation with low levels of JH and the lack of vitellogenesis. They suggest that allatostatin or allatostatin-like neuropeptides might be involved in the inhibition of JH synthesis. This study contributes also to provide information on the degree of homology of allatostatins across various insects.

P211 TO THE INVESTIGATION OF THE AMYGDALA NEUROENDOCRINE FUNCTION (THE ECOLOGICAL ASPECT)

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On the basis of an extensive material there were for the first time determined zones of sex dimorphism (ZSD) of amygdala (Kalimullina, 1986). The response of neurons on the insulin deficit was investigated in the ZSD. The work was curried out at white male-rats (Wistar line). Registration of reactive changes of neurons and their karyovolumetrical characteristics was recorded. The results showed that the following regularities are present : 1) neurons of all zones of SD are sensitive to deficit of insulin, which indicates to the importance of critical period of brain's sex differentiation in the formation of neuroendocrine centers in organism ontogenesis ; 2) the topography of amygdala's zones shows the possibility of the direct (or indirect, through hypothalamus) influence of amygdala on visceral centers of the brain's stem ; 3) the majority of amygdala's zones, which react to alloxan diabetes, have direct anatomic connections with the main or accessory olfactory bulbs.

P212 CONTROL OF GONADOTROPHIN RELEASE IN TROUT ESTROGEN RECEPTOR-CONTAINING DOPAMINERGIC NEURONS AND TYROSINE HYDROXYLASE EXPRESSION

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Central dopaminergic (DA) pathways in fish are involved in a negative feedback of estradiol (E_2) on gonadotrophin II (GtH_2) release. The inhibition of catecholamine synthesis in vitellogenic female rainbow trout increases plasma GtH_2 and decreases DA levels in the pituitary and in some brain regions such as the telencephalon and the preoptic area. Double immunocytochemical stainings demonstrated that some DA neurons in the preoptic area contain E_2 receptors. The gene of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine synthesis could be a target gene of E_2. We understood the cloning of TH in the trout using a brain expression library. The coding sequence has been obtained and exhibits high similarities with TH in others vertebrates. The estrogenic regulation of brain TH expression is currently analysed by *in situ* hybridization.
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P213 SPECIFIC R.I.A. FOR GTH1 AND GTH2 IN THE RAINBOW TROUT *ONCORHYNCHUS MIKYSS*, GONADOTROPINS BLOOD PLASMA LEVELS DURING THE SPAWNING PERIOD

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GTH1 and GTH2 have been purified from rainbow trout pituitary glands using immobilized metal ion affinity chromatography. After separation of their α and β sub-units, antibodies have been raised against GTH1-β and GTH2-β. In the GTH1 assay, using the stable form of GTH1 as labelled hormone, the cross-reactivity with GTH2 was 9-10 % for the D50 dose, whereas it was around 50 % using the native form including stable and dissociable GTH1. The sensitivity of the assay is 0.2 to 0.4 ng/ml, those of the GTH2 assay was 0.1 to 0.2 ng/ml. In this assay there was no cross-reactivity at all with GTH1. In both assays, rainbow trout growth hormone (GH) and prolactine did not compete. GTH1 and GTH2 blood plasma levels have been determined from 15 days before ovulation until 15 days after, by sampling every 2 days, the egg stage being determined at each sampling by individual egg stripping. GTH2 was undetectable until the appearance of the first lipid dropplets, when it rose transciently to 21,25 ± 4,19 ng/ml. GTH1 levels which had decreased at the end of the vitellogenesis, also increased during this period, starting 8 days before ovulation and peaking at the same time as GTH2 at 10,3 ± 2,02 ng/ml. After ovulation, both hormones levels decreased but did not return to basal. GTH1 increased from days + 6 to + 8, peaking at day + 10 at 19,39 ± 3,31 ng. Similarly GTH2 levels also increased, but later peaking at day 14 at 18,84 ± 4,53 ng. Hormone levels were not returned to basal at day + 16.

P217 γ-AMINOBUTYRIC ACID (GABA) STIMULATES GONADOTROPIN-II (GTH-II) RELEASE IN AFRICAN CATFISH

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GABAergic nerve fibres are found in close proximity to gonadotrophs in the pars distalis of the catfish pituitary, suggesting that GABA may be important for the regulation of GTH-II release. Previous work demonstrated that GABA did not directly stimulate GTH-II release from pituitary fragments *in vitro*. Here, male African catfish at different stages of pubertal development were injected i.p. with GABA (300 mg/kg) and a sGnRH analog (sGnRHa ; 5 µg/kg) and blood was collected 1.5 h later for determination of plasma GTH-II. Dopamine inhibits GTH-II release in fish, and to study the involvement of this catecholamine, groups of catfish were also pretreated with the dopamine antagonist sulpiride (1 µmole/kg) 5 h before injection of GABA or sGnRHa. In 5 mo old adolescent fish (first spermatozoa) and 10 mo old males (end of puberty), GABA and sGnRHa respectively stimulated a 2- and 2.5-fold increase in GTH-II concentrations. In 12 mo old fully adult fish, GABA and sGnRHa respectively stimulated a 5-fold and 3-fold increase in GTH-II. At all ages, sulpiride did not alter the GTH-II response to GABA suggesting that dopamine does not modulate GABA action during puberty. In contrast, sulpiride did potentiate sGnRHa-stimulted GTH-II release. These data demonstrate that GABA has a