Effect of shortened photoperiod on the annual blood levels of GTH1 and GTH2 in rainbow trout (Oncorhynchus mykiss)

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stimulatory effect on GTH-II release that increases with testicular development and sex steroid production in catfish. Pituitary responsiveness to GABA was partially correlated with sGnRHa action suggesting that the stimulatory effect of GABA may be mediated by activation of the GnRH system. The lack of effect of sulpiride indicates that GABA action is independent of the inhibitory dopaminergic system in the male African catfish. Supported by the Wellcome Trust (UK).

P215 DEVELOPMENT AND VALIDATION OF A RADIOIMMUNOASSAY FOR STUDYING PLASMA LEVELS OF GONADOTROPIN II (GTH-II) IN STRIPED BASS *(MORONE SAXATILIS)*

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Striped bass is a commercially important farmed fish and an emerging model for the study of reproductive endocrinology. An ELISA for striped bass (stb) GtH-II (Mananos *et al.*, 1996) was found to be affected by plasma interference. Therefore, a radioimmunoassay (RIA) for stbGtH-II was developed and validated for plasma measurements. Intact GtH-II purified from pituitaries of hybrid striped bass (*Morone saxatilis* x *Morone chrysops*) was used for the labeled ligand and the standard, and the antibody was raised against β subunit of this hormone (Mananos *et al.*, 1996). The sensitivity of this assay was 0.4 ng/ml. Serial dilution of striped bass plasma and of pituitary extracts of different *Morone* sp. were parallel to the standard curve. An stbGtH-II internal standard placed into plasma of immature fish (with no detectable levels of GtH-II) was measured faithfully. These results indicate no plasma interference in this assay. The RIA was used to study the *in vivo* effect of GnRH on GtH-II release in striped bass. Injection of female striped bass and hybrid striped bass with [D-Ala^6,Pro^9-NEt]-LHRH (50 µg/kg) induced a similar rapid increase in the circulating GtH-II level, which was maintained for 24 hours. Female striped bass were injected with two doses of the three native forms of GnRH present in striped bass : seabream (sb) GnRH, salmon (s) GnRH and chicken (c) GnRH-II. All three forms induced a dose-dependent GtH-II secretion, with a potency order of cGnRH-II > sGnRH > sbGnRH. Therefore, the stbGtH-II RIA is an additional reliable tool for the study of hormonal control of reproduction in striped bass and other *Morone* species.

P216 EFFECT OF SHORTENED PHOTOPERIOD ON THE ANNUAL BLOOD LEVELS OF GTH1 AND GTH2 IN RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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3 groups of a precocious winter strain of rainbow trout were reared at constant temperature (8.5 °C) in spring water on a spawner diet. Fish from group N were submitted to a simulated natural 12 month photoperiodic cycle for spawning in early winter. Fish from group S9 were submitted to a shortened photoperiodic cycle by reducing light hours/day, 2 months after spawning, from 16 to 8 h over 7 months, resulting in late summer spawning. Fish from group S6 were treated in the same way over 6 months, 2 weeks after spawning, resulting in late spring spawning. Twice a month samples were analysed for GTH1 and GTH2 using specific RIA.
N fish GTH1 profile exhibits 3 successive slight increases and decreases followed by a final sharp peak. The first, at daylight/12 hours, peaks one month before the very slow oocyte development phase (VSD). The second corresponds to the slow development (SD) and the third the rapid development (RD) phases. The last occurs during maturation, peaking at 30 ng/ml. In S9 fish, the four increases in GTH1 persist but the first during the VSD phase. The second peak during the SD phase is almost doubled. In S6 fish only 2 peaks occur : one very high (65 ng/ml) during the vitellogenesis, the second during the final phase and the ovulation.
GTH2 profiles exhibit a unique peak during ovulation for each group. Hormone levels diminish along with the cycle length.

P217 DEVELOPMENT OF A HOMOLOGOUS RIA FOR GTH II OF THE MEDITERRANEAN YELLOWTAIL, *SERIOLA DUMERILII*

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Highly purified M. yellowtail GTH II and its β-subunit were used to raise antisera in rabbit. Anti-α,βGTH II, but not anti-α,βGTH II, cross-reacted with TSH cells. Hence, the latter antiserum was used to set up a GTH II RIA system. The GTH II β-subunit was iodinated with [^{125}I] using the chloramine T method. Intact GTH II was used to compete with the radiolabeled GTH II β-subunit for the antigen binding sites. At a final dilution of 1 :70,000 the antiserum bound approximately 30 % of the labeled β-subunit (10,000 cpm). The sensitivity of the RIA was 0.25 ng GTH II β-subunit/ml and the standard curve ranged from 0.25 to 50 ng/ml. To validate the assay, GTH II levels were measured in serum samples collected from adult fish that were sampled before and at different times after a single injection of salmon GnRH (50 µg/kg). A significant increase in the GTH II blood levels was recorded as compared to pre-injection levels in the sGnRH treated fish at 1, 2, 4, and 8 h after injection, while control levels were attained 24 h after sGnRH injection. Moreover, serial dilutions of pituitary extracts led to a displacement of radiolabeled GTH II β-subunit that was parallel to the standard curve. We conclude that the assay system is suitable to quantify circulating and pituitary GTH II levels.

P218 COMPARATIVE EFFECTS OF ACETYLCHOLINE ON α-MSH RELEASE FROM FROG AND RAT PARS INTERMEDIA

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The involvement of acetylcholine (ACh) in the control of melanotrope cell activity has been demonstrated in various species. In the present study, we have investigated the effect of muscarinic agonists on frog and rat pituitary melanotrophs. Muscarine caused a dose-dependent stimulation of α-MSH release from perifused frog neurointermediate lobes (NIL), and this effect was mediated by M_1 muscarinic receptors. Muscarine provoked an increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) in cultured frog melanotrophs. The effect of muscarine was totally suppressed when Ca^{2+} was omitted in the incubation medium. However, the calcium channel blockers nifedipine (10^{-5} M) and ω-conotoxine (10^{-6} M) did not affect the muscarine-induced α-MSH secretion. Muscarine stimulated IP_3 formation in frog NIL. Pretreatement of frog NIL with PMA (10^{-6} M, 24 h) markedly reduced the muscarine-induced α-MSH secretion. The tyrosine kinase inhibitor ST 638 (10^{-4} M) also reduced the effect of muscarine on α-MSH release and $[Ca^{2+}]_i$. In the rat NIL, ACh exerted an inhibitory effect on α-MSH release. The action of ACh was mimicked by muscarine and blocked by atropine, but it was not suppressed by the M_1 muscarinic antagonist pirenzepine. Incubation of rat NIL with pertussis toxin suppressed the effect of muscarine on α-MSH release. Besides, muscarine reduced the cAMP content of rat melanotrope cells. These data demonstrate that ACh controls the activity of pituitary melanotrophs in both frog and rat through activation of muscarinic receptors. However, the transduction pathways and the final responses in the two models are totally different.
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P219 MORPHOLOGICAL CHANGES INDUCED BY BACKGROUND ADAPTATION IN THE FROG PARS INTERMEDIA*

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