Development and validation of a radioimmunoassay for studying plasma levels of gonadotropin II (GTH-II) in striped bass (Morone saxatilis)

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NEUROENDOCRINOLOGY 2

C09 TESTOSTERONE, G_NRH_A, AND DOMPERIDONE AFFECT THE HYPOPHYSEAL-GONADAL AXIS IN IMMATURE BLACK CARP

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In previous experiments testosterone (T) given chronically to immature black carp *(Mylopharyngodon piceus)* increased cGtH content, bcGtH IIβ mRNA levels and augmented the response of pituitary cells to GnRHs. However, the steroid had no effect on the circulating level of the GtH, possibly indicating dopaminergic (DA) inhibition or lack of GnRH. In order to examine these possibilities, T and GnRHa were administered to 3 y old fish in slow release microspheres either separately, together or combined with domperidone (DOM). The T treatment was followed by increased cGtH content and mRNA levels, and elevated *in vitro* release of cGtH in response to GnRH. However, when T was given together with GnRH + DOM, cGtH content and the response to GnRH were augmented further. Plasma cGtH was elevated initially in all fish treated with T, but returned to control levels in all but those which were also exposed to GnRH. Oocyte diameter in T-injected fish was smaller than in GnRH-injected or control fish. However, only these ovaries secreted E2 in response to hCG. It is concluded that a possible long-term suppression of DA inhibition facilitated the stimulation by T of cGtH synthesis and release and the response to GnRHs. The increased levels of cGtH in the circulation possibly facilitated the formation of GtH receptors in ovarian follicles.

C10 *IN VIVO* AND *IN VITRO* EFFECTS OF SEX STEROIDS ON GONADOTROPIN (GtH-II) SYNTHESIS IN THE EUROPEAN EEL, *ANGUILLA ANGUILLA*

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A positive feedback of sex steroids on GtH-II synthesis in immature fish is an important step in the initiation of puberty. In the female silver eel, *in vivo* experiments indicate that endogenous gonadal hormones, produced during experimental sexual maturation, stimulate βGtH-II mRNA (dot blot) and GtH-II (RIA) pituitary levels. Treatment by estradiol (E2), but not by testosterone (T), stimulates βGtH-II mRNA and GtH-II pituitary levels ; the effects of E2 are potentiated by T but not by non-aromatizable androgens (DHT, 5α androstanediol). Thus, *in vivo* data suggest that the positive feedback on GtH-II synthesis is E2-dependent. In contrast, *in vitro* (pituitary cell culture) experiments reveal an androgen-specific control of GtH-II synthesis : T (or non-aromatizable androgens), but not E2, dose-dependently stimulates βGtH-II mRNA and GtH-II cell content. Moreover, E2 dose-dependently supresses the *in vitro* effects of androgens, providing a new cell model of E2 and T antagonism. Use of anti-estrogens *in vitro* suggests that E2 may act through two different pathways, stimulatory and inhibitory. These data suggest multiple ways of interactions between sex steroid receptors and likely other transcriptional factors, in the control of eel GtH-II expression and emphasize the importance of combining *in vivo* and *in vitro* approaches.

C11 INFLUENCE OF NUTRITIONAL AND SEXUAL STATUS OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* ON THE PERMISSIVE EFFECT OF INSULIN-LIKE GROWTH FACTOR-I ON *IN VITRO* GROWTH HORMONE RESPONSE TO GONADOTROPIN RELEASING-HORMONE

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Recently, we have observed the *in vitro* permissive effect of a pretreatment with IGF-I on the GH response to GnRH of pituitary cells collected from immature and at the beginning of gametogenesis fish, but not in mature males and females. However, these experiments do not allow to define if these differences of response are due to sexual status since preovulated and prespermiating fish are spontaneously fasting.

The aim of the present work was to determine the respective influences of nutrition and sexual status on the induction of this permissive effect of IGF-I. Cell cultures were performed with pituitaries collected from normally fed or starved immature fish as well as from spermiating males. In normal conditions of culture, increasing doses of sGnRH have no action on GH release, whatever the sexual and nutritional status of the fish. On the other hand, when pituitary cells were pre-incubated with human IGF-I (10^{-8} M) for 48 hours they became responsive to sGnRH (10^{-8} M to 10^{-6} M) in the subsequent 24 hour incubation period when they were collected from normally fed or starved immature animals, while no response was detected in spermiating fish. These results indicate that the permissive effect of IGF-I on *in vitro* GnRH-induced GH release seems more related to sexual than to nutritional status of the fish.

C12 RECRUITMENT OF LHRH NEURONS AND INCREASED ACCESS OF LHRH TERMINALS TO PORTAL CAPILLARIES : INTEGRAL MECHANISMS FOR LH SURGE INDUCTION

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LHRH neurons display a remarkable plasticity associated with the spontaneous or steroid-induced LH surge. We propose that underlying the apparently diffuse population of scattered LHRH neurons, is a spatio-temporal organization of functional subgroups. Dynamic changes in detectability of LHRH occurs in some subgroups of the population in association with the LH surge. Similar subgroups are identified by localization of nuclear Fos expression. These studies suggest that there is a progressive recruitment of LHRH neurons contributing to the increased LHRH secretion required for LH surge induction. In addition, these studies suggest a hierarchy of importance of specific LHRH subgroups to the generation of an LH surge. In parallel with these alterations in LHRH neuronal perikarya, changes occur in LHRH terminals. LHRH is depleted from specific regions of the median eminence, in association with LH surges. Confocal microscopy combined with dual label immunocytochemistry, demonstrates that LHRH terminals of rats, guinea pigs and humans are prevented from reaching portal capillaries by the end-feet of tanycytes, specialized ependymal cells. Studies in rats reveal that this glial obstruction between LHRH terminals and portal capillaries is removed on the day of proestrous, prior to and during the preovulatory LH surge. These findings document the plasticity retained by LHRH neurons and terminals of adult mammals. NSF IBN-9310267, HD 19803 to J.C.K and HD 19174 to B.S.R.

C13 GALANIN MODULATES CARDIOVASCULAR FUNCTIONS AFTER CENTRAL AND PERIPHERAL INJECTIONS IN THE CONSCIOUS TROUT *ONCORHYNCHUS MYKISS*

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Trout galanin (tGAL) is a 29-amino acid neuropeptide, whose N-terminal sequence (residues 1-14) has been fully conserved through the evolution. Despite its widespread distribution within the central and peripheral nervous system of fish, little is known about the functional significance of GAL. The present study was undertaken to investigate the cardiovascular effects (mean arterial blood pressure, P_{DA} ; heart rate, HR ; cardiac output, Q ; systemic vascular resistance, Rs) of intracerebroventricular (ICV) and intraarterial (IA) injections of tGAL (0.1, 0.5 and 1 nmol/kg) in the