IDENTIFICATION AND INTRACELLULAR DISTRIBUTION OF AROMATASE IN EEL (ANGUILLA ROSTRATA) PITUITARY

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Both testosterone (T) and estradiol (E) are hormones in their own right, acting via separate receptor mechanisms. In addition, T is the immediate and obligatory precursor of E. The enzyme which catalyzes this transformation (aromatase) is not limited to E-secreting tissues like the gonads but is also present in close proximity to E-target sites in the brain and pituitary. Since the latter are cytologically and functionally heterogeneous, it is important to determine whether aromatase is co-localized with E receptors in the same cell or whether E formed in one cell type serves as a parahormone, regulating functions of adjacent, aromatase-negative cells. In experiments reported here we used the eel (Anguilla rostrata) to study the cellular location of aromatase. As in other teleosts, different hormone secretory cells are segregated topographically, within the gland, permitting separation by microdissection. Additionally, since exposure of fish to salt- vs freshwater (SW vs FW) or treatment with hormones alters activity of certain cell types in a predicted manner, correlated changes in aromatase would provide additional evidence for its location.

Adult female eels collected in brackish water were obtained from a commercial supplier and maintained 4-6 wks in running SW or 2 wks in running FW. SW eels were injected intraperitoneally 3 times weekly (7-10 injections) with methyl-T (1 µg/g body weight); methyl-5α-dihydrotestosterone (DHT, 1 µg/g); E (1 µg/g); or vehicle alone (CON, sesame oil). An additional group received a suspension of carp pituitary powder (GtH, 10 µg/g in normal saline). Three animals from each treatment group were sacrificed for biochemical analyses and pituitaries divided under a binocular dissecting microscope as follows: rostral pars distalis (RPD, containing lactotropes>>corticotropes>thyrotropes); proximal pars distalis (PPD, containing somatotropes>gonadotropes); neurointermediate lobe (NIL, containing melanotropes and numerous terminals of the magnocellular and parvicellular neurosecretory systems). Hypothalamus-preoptic area (HPOA), telencephalon (TEL), cerebellum (CB), ovary, whole pituitary and muscle served as control tissues. Aromatase was assayed in tissue homogenates by conversion of [^{3}H]T to [^{3}H]E in the presence of an NADPH generating system according to previously described methods (Callard et al., Gen. Comp. Endocrinol. 43:243, 1981). E was isolated after ether extraction, thin layer chromatography and phenolic partition. The authenticity of the final product was verified in samples of each tissue-type by reverse isotope dilution and recrystallization to constant specific activity. Other parameters measured were body weight, gonadal weight, body color and eye length. Pituitaries from additional eels in each treatment group were taken for analysis by standard light microscopic methods.

Comparison of uninjected SW controls shown in panels A-D (fig. 1) indicates that aromatase activity was uniformly distributed in the 3 major pituitary divisions and not significantly different from adjacent brain (HPOA); however, levels of activity in these neuroendocrine tissues (1-3 pmol/mg protein) were much higher than in TEL (0.2 pmol/mg protein), CB (non-detectable); ovary (0.1 pmol/mg protein) and muscle (non-detectable). Injection of eels with T increased mean aromatase 3-8 -fold in HPOA, PPD and NIL, al-

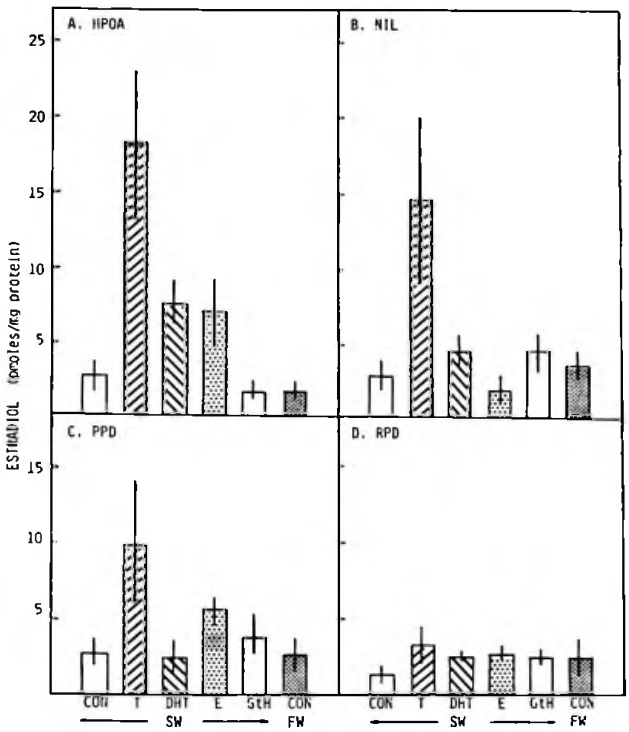

Figure 1. Distribution of aromatase in eel pituitary and brain. (A) HPOA, hypothalamus-preoptic area; (B) NIL, neurointermediate lobe; (C) PPD, proximal pars distalis; (D) RPD, rostral pars distalis. See text for treatment details. CON, uninjected; T, testosterone; DHT, 5α-dihydrotestosterone; E, estradiol; GtH, gonadotropin; SW, saltwater; FW, freshwater; Values = mean ± sem.

though the response was significant only in HPOA and NIL. DHT or E, active metabolites of T, were partially effective in mimicking T in HPOA but changes were not significant. Other treatments were ineffective. Morphological studies are incomplete.

These data provide the first identification of aromatase in brain and pituitary of eels and show that this species resembles other teleosts in having levels 100-1000 times higher than the same tissues in mammals and other vertebrates (Callard, Amer. Zool. 23:607, 1983). Also, ovarian levels are low relative to neuroendocrine tissues, suggesting that brain and pituitary may contribute significantly to the circulating E pool. Earlier studies in sculpin (Olivereau and Callard, Gen. Comp. Endocrinol. 58:280, 1985) and tilapia (unpublished) show that aromatase is concentrated in regions rich in somatotropes. Aromatase has also been identified in growth hormone/prolactin-secreting cell strains derived from rats, whereas corticotropes and gonadotropes were aromatase-negative under the same conditions (Callard et al., Endocrinology 113:152, 1983). By contrast, the uniform distribution of enzyme in the eel pituitary indicates it may be localized in several different secretory cell types. The possibility that a portion of the activity measured in pituitary is derived from neuronal endings cannot be ruled out since neurosecretory processes invade the pars distalis as well as the NIL. Although cytological observations are incomplete, T, E and DHT are known to induce hypertrophy of gonadotropes and synthesis of GtH in eels and other teleosts through actions on the brain and pituitary. Thus, sex steroid effects on aromatase in the HPOA, NIL and PPD may involve neurosecretory neurons, their terminals or the gonadotropes themselves. Neither FW exposure nor E injection, treatments known to induce hypertrophy of lactotropes and to increase prolactin secretion, had an effect on aromatase in the RPD of eels, further supporting the conclusion that several different cell types are involved and that the enzyme may be differentially regulated in each. This investigation was supported by NSF PCM82-03248.