PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 486–489 (1985)

RAPID COMMUNICATIONS

IMMUNOREACTIVE TYROSINE HYDROXYLASE IN THE BRAIN AND PITUITARY GLAND OF THE PLATYFISH[1]

LESLIE R HALPERN-SEBOLD, HENRIETTA MARGOLIS-KAZAN, MARTIN P SCHREIBMAN[2] and TONG H JOH

Biology Department, Brooklyn College, Brooklyn, New York 11210 and Department of Neurology, Cornell University Medical College, New York, New York 10021

Abstract. Immunoreactive tyrosine hydroxylase (ir-TH), the rate-limiting enzyme for the synthesis of dopamine and other catecholamines, was localized in the brain and pituitary gland of sexually mature platyfish (Xiphophorus maculatus). This is the first report of ir-TH in the nucleus olfactoretinalis, an LHRH-containing nucleus in the brain which plays an important role in the development and functioning of the reproductive system in platyfish. Ir-TH was also localized in the nucleus preopticus and paraventricular organ. In the pituitary gland ir-TH is found in the prolactin cells and in some fish, in some of the gonadotropin-containing cells of the pars intermedia, but not in the gonadotrops of the pars distalis.

The localization of ir-TH in brain centers and pituitary cells associated with reproductive system regulation is discussed in the context of the interaction of monamines, neuropeptides and pituitary hormones during the maturation and operation of the brain-pituitary-gonadal axis.

Considerable attention has been devoted to localizing monoaminergic neurotransmitters and to studying their role in modulating neuroendocrine processes in vertebrates. In mammals, dopamine and serotonin (5-hydroxytryptamine) have been shown to regulate the activity of gonadotropin releasing hormone (LHRH) and the gonadotropins (GTH) of the anterior pituitary (1-3).

In teleosts, both aminergic and peptidergic neurons directly innervate pituitary gonadotrops (4). Immunocytochemical (ICC) methods have been used to localize LHRH (4-6) and serotonin (7-9), while the distribution of dopamine has generally been analyzed by formaldehyde induced fluorescence techniques (10). Few studies, however, have utilized antibodies to dopamine itself, or to the enzymes necessary for catecholamine synthesis (9,11).

The platyfish, Xiphophorus maculatus, a small freshwater teleost, is a useful model with which to study genetic and neuroendocrine-regulated processes. In platyfish, the age at sexual maturation is determined by a specific gene (P), and developmental changes in the brain-pituitary-gonadal (BPG) axis occur at specific chronological ages which are determined by P allele constitution (12). Our success in identifying various pituitary hormones (13), neuropeptides (5,7,14), and neurotransmitters (7) has enabled us to develop calendar/maps for the distribution of these substances at various stages during the sexual development and aging of platyfish. In order to study catecholamine distribution, we employed ICC methods to localize immunoreactive tyrosine hydroxylase (ir-TH), an enzyme

[1]Supported by the NIA (AGO 1938) and the City University of New York (PSC-CUNY).

[2]All correspondence should be addressed to M.P. Schreibman, Department of Biology, Brooklyn College, Brooklyn, New York 11210.

0037-9727/85 $1.50

necessary for the synthesis of dopamine, norepinephrine and epinephrine, in young, sexually mature fish.

Materials and Methods. One-year old, sexually mature male (n=5) and female (n=5) platyfish of known genetic constitution (Jamapa stock; P alleles for early maturation) were studied. Animals were sacrificed by decapitation, and heads were immersed in Bouin's fluid containing acetic acid (under vaccuum for 24 hours), decalcified ("Decal", Omega Chem. Co.), dehydrated in ethanol and butanol and embedded in paraffin. Five um thick sagittal sections were mounted serially on gelatin-coated slides.

The indirect immunoperoxidase anti-peroxidase (PAP) method (15) as modified for our material (13) and utilizing antiserum against rat TH (16) at dilutions of 1:250-1:500, was employed. Control procedures included the absorption of the final dilution of antiserum with synthetic LHRH (Peninsula) or bovine serum albumin, and the substitution of normal rabbit serum or other antisera for the primary antiserum. The TH antiserum used in this study has been previously characterized and run with homologous (TH) antigen controls (16).

Results. The anatomical terms used in the descriptions below are according to Peter et al. (17) and Münz et al. (6). Observations are similar for both male and female platyfish.

In the forebrain, ir-TH is localized in perikarya and processes of the nucleus olfactoretinalis (NOR) (fig. 1). Ir-perikarya are fusiform in shape and give rise to thick processes which extend dorsocaudally and ventro-caudally. Ir-TH perikarya are also present in the pars magnocellularis and pars parvocellularis of the nucleus preopticus (NPO) (figs. 2,3). These neurons are fusiform or multipolar in shape and have processes which are difficult to trace. Ir-TH is present in perikarya in the wall of the third ventricle and the nucleus recessus lateralis (NRL) and nucleus recessus posterioris (NRP) of the paraventricular organ (PVO; fig. 4), however, immunoreactive staining is considerably less intense than seen in the NPO and NOR. In the pituitary gland, ir-TH is localized in the prolactin cells of the rostral pars distalis (RPD) and in some fish, ir-cells are also localized in the pars intermedia (PI; fig. 4).

Discussion. In this report, we have demonstrated that in platyfish, ir-TH is localized in the NOR, NPO, PVO and pituitary gland (RPD and PI), regions which play important roles in the development and maintenance of the reproductive system in teleosts (4). Ir-TH has been localized in various centers of the brain in several teleosts (9; Joh, unpublished), however, this is the first report in which ir-TH has been demonstrated within perikarya and processes of the NOR of fishes.

It is generally believed that in vertebrates LHRH and monoamines act in concert to regulate GTH synthesis and release by both stimulatory and inhibitory actions (1-4, 18). In platyfish, the appearance of LHRH in the NOR, nucleus preopticus periventricularis (NPP; an area in the anterior preoptic region), and nucleus lateralis tuberis (NLT) in sequence according to a specific timetable of maturation, precedes, and is essential for, the development of gonadotrops and subsequently the gonads (19). Therefore, the presence of ir-TH in the NOR and the preoptic region (NPO) strongly suggests a functional relationship between catecholamines and LHRH during reproductive system development and operation. It may be that catecholamines, acting alone or together with other neurotransmitter substances, serve to regulate the time of sexual maturation.

In goldfish, a gonadotropin-release-inhibitory factor (GRIF) is believed to originate from the anterior preoptic region (18) and it has been suggested that dopamine is the agent responsible for the GRIF action (20). Our ability to localize ir-TH in the NPO lends support to these hypotheses and suggests that similar mechanisms may operate in platyfish.

The presence of ir-TH in some ir-GTH containing cells of the PI but not in the vCPD of sexually mature fish supports our contention that these two GTH producing areas play different roles in reproductive physiology. Based on temporal immunocytochemical and physiological studies (19,21) we have suggested that PI cells play their primary role in immature fish from

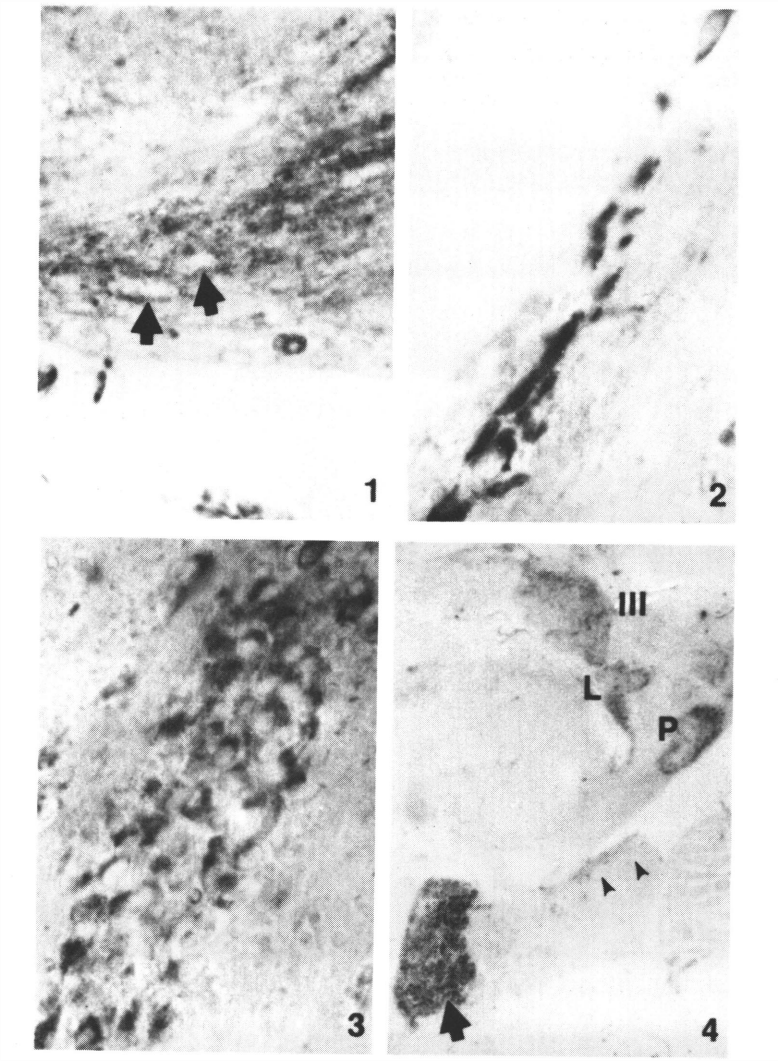

Figure 1. Sagittal section through the NOR showing ir-perikarya (arrow) and processes. 595X.
Figure 2. Ir-cells of the pars magnocellularis of the NPO. 610X.
Figure 3. Ir-cells of the pars parvocellularis of the NPO. 695X.
Figure 4. Brain: ir-cells of the third ventricle (III), NRL (L), and NRP (P). Pituitary: ir-prolactin cells (arrow) and paler ir-cells in the dorsal pars intermedia (arrowheads). 120X.
Note: In all figures anterior is to the left and ir-TH appears black.

birth to puberty and that the CPD gonadotrops are most essential in sexually mature platyfish. A study of ir-TH localization from birth to puberty in the light of our developmental studies on LHRH (19,21) may clarify the specific interrelationships which exist between neurotransmitter and neuropeptide during brain-pituitary-gonadal development.

1. Barraclough CA, Wise PM. The role of catecholamines in the regulation of pituitary LH and FSH secretion. Endocrine Rev Vol 3:91-119, 1982.

2. Barraclough CA, Wise PM, Selmanoff MK. A role for hypothalamic catecholamines in the regulation of gonadotropin secretion. Rec Prog Horm Res 40:487-529, 1984.
3. Walker RF. Quantitative and temporal aspects of serotonin's facilitory action on phasic secretion of luteinizing hormone in female rats. Neuroendocrinol 36:468-474, 1983.
4. Peter RE. Evolution of neurohormonal regulation of reproduction in lower vertebrates. Amer Zool 23:685-695, 1983.
5. Schreibman MP, Halpern LR, Goos HJTh, Margolis-Kazan H. Identification of luteinizing hormone-releasing hormone (LH-RH) in the brain and pituitary gland of a fish by immunocytochemistry. J Exper Zool 210:153-160, 1979.
6. Münz H, Stumpf WE, Jennes L. LHRH systems in the brain of platyfish. Brain Research 221:1-13, 1981.
7. Margolis-Kazan H, Schreibman MP, Halpern-Sebold LR. Immunoreactive serotonin and somatostatin in the brain and pituitary of the platyfish. Amer Zool 23:883, 1983.
8. Kah O, Chambolle P. Serotonin in the brain of the goldfish, Carassius auratus. Cell Tissue Res 234:319-333, 1983.
9. Yoshida M, Nagatsu I, Kawakami-Kondo Y, Karassawa N, Spatz M, Nagatsu T. Monoaminergic neurons in the brain of goldfish as observed by immunohistochemical techniques. Experentia 39:1171-1174, 1983.
10. Parent A, Dube L, Braford MR, Northcutt RG. The organization of monoamine-containing neurons in the brain of the sunfish (Lepomis gibbosus) as revealed by fluorescence microscopy. J Comp Neur 182:495-516, 1978.
11. Geffard M, Kah O, Chambolle P, Le Moal M, Delaage M. Premiere application immunocytochimique d'un anticorps anti-dopamine a l'étude du systeme neurveux central. CR Acad Sci 295:797-802, 1982.
12. Schreibman MP, Kallman KD. The genetic control of the pituitary-gonadal axis in the platyfish, Xiphophorus maculatus. J Exper Zool 200:277-294, 1977.
13. Margolis-Kazan H, Schreibman MP. Cross-reactivity between human and fish pituitary hormones as demonstrated by immunocytochemistry. Cell Tissue Res 221:257-267, 1981.
14. Schreibman MP, Halpern LR. The demonstration of neurophysin and arginine vasotocin by immunocytochemical methods in the brain and pituitary gland of the platyfish, Xiphophorus maculatus. Gen Comp Endocrinol 40: 1-7, 1979.
15. Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG. The unlabeled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315-333, 1970.
16. Joh TH, Gregham G, Reis DJC. Immunochemical demonstration of increased tyrosine hydroxylase protein in sympathetic ganglia and adrenal medulla elicited by reserpine. PNAS 70: 2767-2771, 1973.
17. Peter RE, Macey MJ, Gill VE. A stereotaxic atlas and technique for forebrain nuclei of the killifish, Fundulus heteroclitus. J Comp Neurol 159:103-128, 1975.
18. Peter RE, Nahorniak CS, Chang JP, Crim, LW. Gonadotropin release from the pars distalis of goldfish, Carassius auratus, transplanted beside the brain or into the brain ventricles: Additional evidence for gonadotropin-release-inhibitory factor. Gen Comp Endocrinol 55: 337-346, 1984.
19. Halpern-Sebold LR, Schreibman MP. Ontogeny of centers containing luteinizing hormone-releasing hormone in the brain of platyfish (Xiphophorus maculatus) as determined by immunocytochemistry. Cell Tissue Res 229: 75-84, 1983.
20. Chang JP, Peter RE. Effects of dopamine on gonadotropin release in female goldfish, Carassius auratus. Neuroendocrinol 36:351-357, 1983.
21. Schreibman, MP, Margolis-Kazan, H, Halpern-Sebold LR. Immunoreactive gonadotropin and luteinizing hormone releasing hormone in the pituitary gland of neonatal platyfish. Gen Comp Endocrinol 47:385-391, 1982.

Received December 3, 1984.
P.S.E.B.M. 1985, Vol. 178.
Accepted January 8, 1985.