THE DEVELOPMENT OF THE THYROID GLAND IN TELEOSTEAN EMBRYOS: HISTORICAL PERSPECTIVES

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Abstract

The paper briefly reviews historical and recent work dealing with the early ontogeny of the thyroid primordium in teleostean fish embryos, with particular emphasis on salmonid species. The recent work describing the appearance of the thyroid primordium as a tubular structure, the bifurcation of the primordium at its anterior and posterior margins to form a complex scaffold-like complex is described, as is the delay in the appearance of thyroid follicles , becoming evident only in the late embryo or early juvenile stages. Also, methods that allow the determination of the time of onset of synthesis and secretion of thyroid hormones by embryos are examined, as is the evidence of the first presence of thyroid hormone receptors prior to the appearance of the thyroid tissue in the embryo, and the impact of treatment of embryos with exogenous thyroid hormone, or exposure of the embryos via yolk thyroid hormones of maternal origin on developmental events.

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Introduction

The complex interrelated events associated with the early developmental biology of vertebrates represent one of the most challenging research areas in biology, and during the last decade, there has been an increasing interest in understanding the factors, including the endocrine factors, that regulate early developmental biology. The thyroid hormones (TH s) are known to play an essential role in early vertebrate ontogeny, and are particularly important for the normal development of the central and peripheral nervous system, heart, liver, intestinal tract and skeletal and smooth muscle (reviewed by Hsu and Brent, 1999). Work in teleost fishes has been less intense, although ongoing work in several laboratories is examining the expression of T_3-receptor (TR) genes during early development (e.g., Yamano and Miwa, 1998; Llewellyn et al., 1999), and attempting to determine the timing of the functional maturation of the hypothalamus-pituitary gland-thyroid tissue (HPT) axis (Raine and Leatherland, 1999, 2000). Of particular interest in this regard is consideration of the role played by the thyroid hormones that are present in the yolk of fish eggs and embryos. This source of hormone represent a relatively uncontrolled exposure of the embryo to thyroid hormone during critical developmental periods (reviewed by Leatherland, 1994), and the significance of the yolk hormones is still not well understood.

In this paper, we review briefly early and current work dealing with the role of thyroid hormones in early development of teleostean fishes, with particular emphasis on trout and salmon species. Of special emphasis is the early ontogeny of the thyroid tissue, the timing of the onset of thyroid hormone synthesis and secretion by the embryo, the effect of exogenous and maternal sources of TH on the early development of the embryo, and the timing of the appearance of TR's in the embryo.

Early ontogeny of thyroid tissue in fish embryos

Since the late 19th Century (Maurer, 1886), there have been only 3 papers published that deal with the early ontogeny of the thyroid tissue of teleost fishes [Atlantic salmon, *Salmo salar* (Hoar, 1939), fathead minnow, *Pimephales promelas* (Wabuke-Bunoti and Firling, 1983), and rainbow trout, *Oncorhynchus mykiss* (Raine and Leatherland, 2000)]. In fact, there has been only a handful of studies related to thyroid ontogeny in any of the vertebrate classes. There is general agreement that the thyroid primordium originates as an evagination of the ventral pharyngeal floor, but there is no consensus as to how the thyroid follicles form. In the most recent work on rainbow trout, serial sectioning revealed that the primordium develops into a tubular structure and distinct follicles do not appear until relatively late in embryogenesis (40 days post-fertilization in trout raised at $8^{o}C$). When they are formed, the follicles are produced by the pinching off of bifurcated anterior and posterior branches of the tubular primordium. Even in juvenile trout, when the yolk has been fully absorbed, a tubular thyroid structure is present in addition to the follicles (J.C. Raine, A. Takemura and J.F. Leatherland, unpublished data). Thus the assessment of thyroid function in fish embryos and early juveniles, based solely on the measurements of thyroid tissue histological characteristics, such as the number of follicles, might be inappropriate.

Timing of the onset of thyroid hormone synthesis and secretion

The formation of the THs (thyrogenesis) involves the synthesis of thyroglobulin by the thyrocytes, the exocytosis of this protein into the follicular lumen of the thyroid tissue, and the extracellular iodination, oxidation and glycosylation of the protein, within the lumen. The release of the hormones involves pinocytosis of thyroglobulin by the thyrocytes and the proteolytic release, within cytoplasmic vesicles, of iodinated thyronine compounds that comprise elements of thyroglobulin structure. Because of the complexity of thyrogenesis and TH release or secretion, details of these events have only recently been described in the adult mammal thyroid gland, and relatively little is known of the processes in vertebrate embryos. There are very few studies of these events in fish embryos.

The timing of the onset of hormone production in fish embryos has been difficult to assess, particularly during the period before histologically distinct thyroid tissue is seen. The first incorporation of radioiodide into the embryo has been used by some authors as an indication of thyrogenesis (e.g., Greenblatt et al., 1989), although evidence suggests that much of this iodide becomes associated with the yolk, and not with the pharyngeal thyroid tissue (see discussion in Raine and Leatherland, 2000). Recently (Raine and Leatherland, 1999, 2000), we took a different approach to the problem, using immunohistochemistry to examine the period of thyroid development from first appearance of a primordium until the formation of follicular thyroid tissue in rainbow trout. We found temporal changes in the distribution of immunostaining (IS) of the thyroid tissue with polyclonal antibodies raised against L-thyroxine (T_4) and triiodo-L-thyronine (T_3), and noted that IS was first seen at the periphery of the tubular primordium/follicle lumen. We proposed that this IS response was probably associated with the oxidative iodination of the thyroglobulin and secondary oxidation to form T_4 and T_3 that continue to be covalently bound within the thyroglobulin molecule. A second type of IS staining pattern was seen in embryos sampled a few days later, when IS was found both at the periphery of the follicles and also in association with some of the thyrocytes. The presence of and IS response in the thyrocytes was thought to represent thyroglobulin vesicles in which proteolysis of partial exposure of the THs was present for ligation with antibodies. These stages of thyroid function in rainbow trout embryos were referred to as Phases 1 and 2, respectively (Raine and Leatherland, 2000), and provide the first evidence of the timing of TH synthesis and release by fish thyroid tissue.

The interpretation of the physiological meaning of these phases is based on the assumption that the IS is, in fact, related to synthesis and secretory events. To test this hypothesis, we examined the pattern of IS in the thyroid tissue of two species, medaka (*Oryzias latipes*) [sexually immature adults] and rainbow trout [juvenile fish weighing approximately 12 g], following treatment of the fish with bovine TSH. For these studies we used two methods, the Vectastain horseradish-peroxidase method employing light microscopy, and an immunofluorescent method using confocal microscopy. In case of the rainbow trout, the fish were fed with T_4 or T_3 for several days prior to challenging with TSH in order to lower endogenous TSH production and thereby reduce or eliminate the secretion or release of endogenous TH. In the case of the medaka, the fish were sampled in March when the thyroid tissue is at a relatively low activity level. In both species, a progressive pattern of IS was found with an increase in staining response in the peripheral zones of the follicle lumen, particularly in the region of colloid vesicles, and then the appearance of IS in the cytoplasm of some thyrocytes (Raine, 1997; J.C. Raine, A. Takemura and J.F. Leatherland, unpublished data). These observations suggest that the temporal changes in the pattern of IS in the embryos are related to changes in the activity of thyroid tissue function, and lend support to the hypothesis that Phase 1 represents a period of thyroglobulin synthesis, associated with oxidative iodination and condensation, and Phase 2 involves the proteolysis of thyroglobulin to release the covalently-bound hormone.

For rainbow trout embryos, the onset of Phase 1 activity was coincident with the appearance of immunostainable (with anti-hTSH) pituitary thyrotrophs. Moreover, between Phases 1 and 2 there was a marked proliferation in the number of thyrotrophs (Raine and Leatherland, 2000). This suggests that the initiation of TH synthesis in the trout is linked to the initiation of activity of the HPT axis. Thus, biotic and abiotic factors that influence the maturation of the HPT axis could potentially influence the timing of first synthesis and secretion of the embryo's own TH.

Effect of exogenous and maternal sources of TH on early development of the embryo

i. Exogenous hormone

The effects of immersion of teleostean embryos and juvenile stages in solutions of TH are well reported (literature reviewed by Reddy and Lam, 1992; Leatherland, 1994; Brown and Kim, 1995). Such studies result in an increased rate of development of early embryos, including a more rapid absorption of the yolk sac. Some reports also note that these TH-enhanced rates of development reduce the viability of the embryos and early juvenile stages, and deformity and mortality rates are high. Although these studies are clearly toxicological or pharmacological in nature, they do provide two important pieces of information. They show that the TH's can influence developmental events in fish embryos, both in terms of the rate of development, and the outcome of the developmental processes, and that the TR's are present very early in development, in most species before the appearance of the embryo's own thyroid tissue. Recent work dealing with the expression of TR genes in fishes supports this finding (see below).

The biological significance of this early presence of the TR and the responsiveness of embryonic tissues to TH's remains illusive. However, it may have marked significance in consideration of the actions of environmental toxicants, particularly the environmental endocrine disruptors (reviewed by Leatherland, 1998).

ii. Yolk hormone

Since the first reports of the presence of THs in the yolk of teleostean fish embryos (Kobuke et al., 1987; Tagawa and Hirano, 1987), there have been several studies of the changing patterns of yolk thyroid hormone content in a broad range of teleostean fish species (reviewed by Leatherland, 1994). In general, the pattern of change suggested a progressive decrease in 'whole body' TH content. However, although it was assumed that the TH measurements reflected the content of the whole organism (tissue, body fluids and thyroid), the patterns of change were not what would be expected if this were true. There was, for example, no evidence of an elevation of whole body TH content associated with the appearance of colloid in the thyroid tissue. Also, for two species studied in our laboratory (Arctic charr, *Salvelinus alpinus,* and rainbow trout), and for coho (*Oncorhynchus kistuch*) and chinook salmon (*Oncorhynchus tshawytscha*) (Greenblatt et al., 1989), sporadically high 'whole body' TH values were found in individual fish. In an attempt to explain that apparent paradox, we postulated that the 'whole body' TH measurements might not be measuring the covalently bound TH in the thyroglobulin and tested the hypothesis by digesting homogenates of the pharyngeal tissue with trypsin with the purpose of releasing covalently bound TH from thyroglobulin, thus making the molecules available for ligation with antibodies in T_4 and T_3 RIAs. After 1 hour digestion, there was a 100 fold increase in assayable T_4, and a 2-3 fold increase in assayable T_3, providing evidence in support of our argument that the extraction processes used had not, *ipso facto*, extracted the TH contained within the thyroglobulin. Thus, the 'whole body' TH measurements made in embryos and early juvenile stages of fishes to this time probably do not accurately reflect the TH contained within the thyroid tissue compartment.

In light of the effects of TH-administration on fish embryo development (see above), and the early appearance of TRs in fish embryos, the importance of the yolk hormones on the development of the embryo prior to the onset of thyroidogenesis by the embryo has been of considerable interest. Surprisingly, several studies failed to demonstrate an effect of altered yolk TH content on embryonic events (reviewed by Leatherland, 1994), suggesting that the yolk hormones may not be involved. However, it is possible that the yolk hormones exposure is regulated by the embryo by altering the rates of TH metabolized and excretion, and thus experimental variation in the yolk TH content may not change the levels of exposure of the embryonic tissues to TH. It is likely that the differences in responses of embryos to TH challenge by immersion in a solution of hormone and by altering levels of TH in the yolk are related to ability of the embryo to control the circulating TH levels. Following immersion, TH will enter the blood via the gills, and the levels may exceed the ability of the embryos to metabolize and excrete excess hormone. In contrast, the transfer of hormone from the yolk into the somatic tissues of the embryos will likely be at a rate well within the ability of the embryo to maintain TH homeostasis.

Timing of the appearance of TRs and potential sites of action of THs

As discussed above, indirect evidence based on the responsiveness of embryonic tissues to TH challenge suggests an early appearance of the TRs in fish, probably before the thyroid tissue of the embryo has commenced thyrogenesis. Recent studies of TR gene expression in fishes and in *Xenopus laevis* support this evidence (Banker et al., 1991; Essner et al., 1997; Yamano and Miwa, 1998; Llewellyn et al., 1999), and, as discussed above, it is possible that the yolk THs, with the levels of exposure to the TRs regulated by TH metabolism and excretion, play a role in early developmental events. In addition to the biological importance of this process related to the control of early development, and the environmental toxicological relevance are considerable. Factors that influence the delivery of THs to developing tissues (by impacting the transport of hormones in the blood or across membranes) or compromise the binding of T_3 to the TR proteins can potentially disrupt embryonic development at key windows of opportunity and bring about irreversible changes (Porterfield, 1994).

Relatively little is known about the specific roles of thyroid hormones during early vertebrate development. Studies of the effects of hypothyroidism on early development suggest a marked influence of the THs in neurological development of neonatal mammals (reviewed by Porterfield and Hendrich, 1993; Cao et al., 1994), and recent studies of TR knockout mice have revealed new information of TH actions in mammalian embryos (reviewed by Hsu and Brent, 1998). Mice with TR gene inactivation exhibit very different responses compared with mice that have TR gene inactivation. The knockout mice show sensory and neurological impairment, growth arrest, delayed small intestinal maturation, cardiac dysfunction and thermoregulatory problems. Although the central and pleiotropic role of the THs in metamorphosis of amphibian tadpoles is well established, at this time, there is little specific information related to TH functions in fishes. With the increased understanding of the polymorphic nature of TRs, and an appreciation of the fact that not all actions of the THs are effected via genomic receptor sites (e.g., Davis and Davis, 1996), it is anticipated that the cellular roles of THs in developmental processes will now begin to be defined.

Conclusion

The currently available evidence suggests that the THs can influence early developmental processes in teleostean fishes, and that TR protein is expressed in fish embryos in advance of the appearance of thyroid tissue in the embryo and considerably in advance of the onset of thyroidogenesis. In salmonid fishes the thyroid primordium is a tubular structure that does not take the form of a follicular glandular tissue until relatively late in embryonic development, and even in juvenile fish, the tubular primordium may still be present. Therefore, the use of criteria such as follicle counts as a means of assessing thyroid activity in early developmental stages of some species may not be appropriate. The onset of TH synthesis and release in salmonid fishes, as determined using immunohistological methods, occurs concurrently with the proliferation of pituitary thyrotrophs, and begins before the appearance of thyroid follicles. Relatively little is known about the cellular and tissue actions of the THs during early development of fish embryos.

References

Banker, D.E., J. Bigler and R.N. Eisenman. 1991. The thyroid hormone receptor gene (c-*erb*A) is expressed in advance of thyroid gland maturation during early embryonic development of *Xenopus laevis*. Mol. Cell. Biol. 11: 5079-5089.

Brown, C.L. and B.G. Kim. 1995. Combined application of cortisol and triiodothyronine in the culture of larval marine finfish. Aquaculture 135: 79-86.

Cao, X.-Y., X.-M. Jiang, Z.-H. Dou, A.R. Murdon,M.-L. Zhang, K. O'Donnell, T. Ma, K. Amette, N. DeLong and G. DeLong. 1994. Timing of ulnerability of the brain to iodine deficiency in endemic cretinism. New England J. Med. 331: 1739-1744.

Davis, P.J. and F.B. Davis. 1996. Nongenomic actions of thyroid hormones. Thyroid 6: 497-504.

Essner, J.J., J.J. Breuer, R.D. Essner, S.C. Fahrenkrug and P.B. Hackett Jr. 1997. The zebrafish thyroid hormone receptor 1 is expressed during early embryogenesis and can function in transcriptional repression. Differentiation 62: 107-177.

Greenblatt, M., C.L. Brown, M. Lee, S. Dauder and H.A. Bern. 1989. Changes in thyroid hormone levels in eggs and larvae and in iodide uptake by eggs of coho and chinook salmon, *Oncorhynchus kisutch* and *O. tschawytscha*. Fish Physiol Biochem 6: 261-278.

Hsu, J.-H. and G.A. Brent. 1998. Thyroid hormone receptor gene knockouts. Trends Endocrinol. Metabol. 9: 103-112.

Hoar, W.S. 1939. The thyroid gland of the Atlantic salmon. J. Morphol. 65: 257-295.

Kobuke, L., J.L. Specker and H.A. Bern. 1987. Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch*. J. Exp. Zool. 242: 89-94.

Leatherland, J.F. 1994. Reflections on the thyroidology of fishes: from molecules to humankind. Guelph Ichthyol. Rev. 2: 1-67.

Leatherland, J.F. 1998. Changes in thyroid hormone economy following consumption of environmentally contaminated Great Lakes fish. Toxicol. Indust. Health 14: 41-57.

Llewellyn, L., M.A. Nowell, V.P. Ramsurn, T. Wigham, G.E. Sweeney, B. Kristjánsson and Ó. Halldórsson. 1999. Molecular cloning and developmental expression of the halibut thyroid hormone receptor-. J Fish Biol (Suppl A) 55: 148-155.

Maurer, F. 1886. Schilddrüse und Thymus der Teleostier. Morphol. Jahrbuch. 11: 129-175.

Porterfield, S.P. 1994. The vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ. Health Perspect. 102:125-130.

Porterfield, S.P. and C.E. Hendrich. 1993. The role of thyroid hormones in prenatal and neonatal neurological development: current perspectives. Endocr. Rev. 14: 94-106.

Raine, J.C. 1998. Ontogeny of Thyroid Function in Rainbow Trout, *Oncorhynchus mykiss*. M.Sc. Thesis, University of Guelph, Guelph, Canada, 124p.

Raine, J.C. and J.F. Leatherland. 1999. Ontogeny of thyroid tissue and tissue thyroid hormone clearance in rainbow trout embryos reared at two temperatures. Fish Physiol. Biochem. 20: 209-217.

Raine, J.C. and J.F. Leatherland. 2000. Morphological and functional development of the thyroid tissue in rainbow trout (*Oncorhynchus mykiss*) embryos. Cell Tiss. Res. (In press).

Reddy, P.K. and T.J. Lam. 1992. Role of thyroid hormones in tilapia larvae (*Oreochromis mossambicus*): I. Effects of the hormones and an antithyroid drug on yolk absorption, growth and development. Fish Physiol. Biochem. 9: 473-485.

Tagawa, M. and T. Hirano. 1987. Presence of thyroxine in eggs and changes in its content during early development of chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 68: 129-135.

Yamano, K. and S. Miwa 1998. Differential gene expression of thyroid hormone receptor and in fish development. Gen Comp Endocrinol 109: 75-85.

Wabuke-Bunoti, N. and C.E. Firling. 1983. The pre-hatching development of the thyroid gland of the fathead minnow, *Pimephales promelas* (Rafinesque). Gen. Comp. Endocrinol. 49: 320-331.

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