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Role of Protein and Steroid Hormones in Vitellogenesis in the Catfish, *Heteropneustes fossilis*

P NATH and B I SUNDARARAJ FNA
Department of Zoology, University of Delhi, Delhi 110 007, India

Investigations on hormonal regulation of vitellogenesis in the catfish, *Heteropneustes fossilis*, have been briefly reviewed. During vitellogenesis, vitellogenin, a common precursor of egg yolk proteins, is synthesized in the liver and transported by blood to the ovary, where it is deposited as yolk. Radioactive phosphorus incorporation studies in catfish indicate that the label is first incorporated into phosphoprotein fraction of the liver which is eventually released into circulation. Studies using gonadectomized female catfish reveal that ovary is necessary for the gonadotropin to act and promote estrogen secretion which in turn induces synthesis of vitellogenin. Among the steroids (progesterone, testosterone, cortisol and estrogens) tested, estrogens (estrone, estradiol and estriol) induce vitellogenin formation without promoting its incorporation into oocytes. In the hypophysectomized female catfish, only piscine (salmon and carp) gonadotropins induce complete vitellogenesis leading to formation of yolky oocytes, whereas mammalian gonadotropins (FSH and LH) bring about vitellogenin synthesis but do not promote its incorporation into oocytes. The mammalian gonadotropins, unlike piscine gonadotropins, seem to have retained only the capacity to induce vitellogenin synthesis but have lost the ability to promote vitellogenin incorporation into oocytes.

Introduction

In fishes, as in other oviparous vertebrates, the developing embryos obtain their nutritional requirements from the yolk present in the eggs. The process of deposition of yolk in the developing oocytes is termed as vitellogenesis, which in most vertebrates is a seasonal or cyclical phenomenon. The key product in vitellogenesis is a multicomponent lipophosphoprotein called vitellogenin which is synthesized in the liver and transported by blood to the ovary, where it is cleaved into the final egg yolk proteins (phosvitin and lipovitellin) and deposited as yolk granules or platelets (Tata 1976, Wallace 1978). The chemistry of vitellogenin and egg yolk proteins has been intensively studied for the last forty years. Vitellogenin has a molecular weight of about 450,000-600,000 (Wallace 1970, Redshaw & Follett 1971, Ansari et al. 1971, Deeley et al. 1975, Tata 1976), although the precise

chemical entities that constitute yolk vary widely from species to species. Of the two egg yolk proteins, phosvitin accounts for nearly all the phosphoprotein phosphorus and lipovitellin for almost all the lipid associated with the vitellogenin molecule. Flickinger & Rounds (1956) have demonstrated by isotopic and serological means that radioactive phosphate is first incorporated into the liver as a protein-bound component, it then appears in the plasma and is finally located in the egg yolk proteins. Thus, yolk proteins of egg-laying vertebrates are extraovarian in origin and are transported by the circulatory system from the liver (as vitellogenin) and deposited in the developing oocytes (as phosvitin and lipovitellin).

Much of the earlier work on vitellogenesis has been done on birds and amphibians (see reviews by Wallace & Bergink 1974, Bergink et al. 1974, Follett & Redshaw 1974, Tata 1976, Wallace 1978), whereas information on the mechanism of vitellogenesis in fishes is meagre (Donaldson 1973, Dodd 1975, Fontaine Y A 1975, Fontaine M 1976, Wallace 1978). So far, little is known about the nature and biosynthesis of vitellogenin and the mechanism of yolk deposition in the commercially important fishes of India. The present paper summarizes the work on the hormonal control of vitellogenesis in the catfish, *Heteropneustes fossilis* (Bloch).

Identification of Yolk Precursor Proteins

During the spawning period, the ovary of the catfish, *H. fossilis*, is enlarged and contains numerous yolky oocytes. The serum from such females, when subjected to gel chromatography on Ultrogel AcA 34, shows a characteristic high molecular weight protein in the second fraction. Of the various serum proteins the second fraction is the only one that contains alkali-labile phosphorus and, therefore, represents vitellogenin (see Emmersen & Petersen 1976). This protein is absent in the serum of normal male or hypophysectomized female catfish but appears when these fishes are treated with estrogen (Nath 1978, Sundararaj & Nath 1978). Similar results have been obtained in coho salmon *Oncorhynchus kisutch* (Markert & Vanstone 1971), in *Platichtys flesus* (Emmersen & Petersen 1976). and in *Salmo gairdneri* (Hara & Hirai 1978). Even in amphibians, a new protein component is present in the serum of estrogenized or gonadotropin-treated females (see Wallace 1978).

Vitellogenin Synthesis

In most oviparous vertebrates, vitellogenin is synthesized in the liver under the influence of estrogen and is then secreted into blood. It is eventually deposited in the ooplasm of developing oocytes (Wallace 1978). Investigations conducted on the catfish indicate that incorporation of ^{32}P labelled NaH_2PO_4 into the phosphoprotein fractions of the liver of estradiol-treated female catfish was four times greater than that of control and levels of labelled vitellogenin increased in the blood coincident with declining levels of labelled phosphoprotein in the liver. Clearly, decrease in the labelled phosphoprotein in the liver is associated with an increase in serum vitellogenin level suggesting its release from the liver into circulation (Nath 1978, Nath & Sundararaj 1978). In this regard, Beuving and Gruber (1971) have reported that there is no storage of vitellogenin in the liver and it is secreted into the blood stream immediately after its synthesis. Our study indicates that liver is the site of vitellogenin synthesis as is the case in other oviparous vertebrates (see Wallace 1978).

Vitellogenin Formation in Gonadectomized Catfish

Our experiments have shown that ovariectomy during the prespawning or the spawning

season results in a rapid decrease in the level of circulating vitellogenin. This decrease can be offset by just two injections of estradiol (E_2). Interestingly, salmon gonadotropin (SG-G100), which induces complete vitellogenesis in the hypophysectomized female catfish (Sundararaj et al. 1972), is unable to do so in ovariectomized catfish. Obviously, the integrity of the ovary is essential for gonadotropin-mediated vitellogenin synthesis in the liver. These observations clearly indicate that the ovarian tissue in response to gonadotropin synthesizes estrogen which acts on the liver to promote vitellogenin synthesis (Nath 1978).

Action of Steroids on Vitellogenesis

In the catfish, synthesis of lipophosphoprotein is induced following treatment with estradiol (E_2) in both sexes (Nath 1978, Sundararaj & Nath 1978). This response to E_2 is similar to that observed in other fishes (Bailey 1957, Emmersen & Petersen 1976, Wallace 1978, Hara & Hirai 1978), amphibians (Follett & Redshaw 1974, Wallace 1978), and birds (Bergink et al. 1974, Gruber et al. 1976). This indicates that in oviparous vertebrates E_2 induces *de novo* synthesis of egg yolk proteins.

Regardless of the duration of treatment, estradiol is the most potent steroid in inducing vitellogenin synthesis in the catfish (Nath 1978). In short-term treatment, where only three estrogen injections (1000 IU/day) were given on three consecutive days, vitellogenin levels reached a peak six days after the first injection and gradually declined thereafter. In long-term treatment with various steroids (estrogens, testosterone, progesterone and cortisol : 1 or 10 μg/day), where hypophysectomized females were treated for 20 days, the estrogens (estrone, estradiol and estriol) appeared to be the only steroids to have induced vitellogenin formation. However, yolky oocytes were not formed. Among the estrogens, estradiol is the most potent in inducing vitellogenin formation (Sundararaj & Nath 1978). This again emphasizes the fact that estrogen, like mammalian gonadotropins, induces the formation of vitellogenin but does not promote its incorporation into oocytes. Upadhyay et al. (1978) also reported that estradiol-17β failed to induce vitellogenesis in juvenile rainbow trout, *Salmo gairdneri*.

Effect of Mammalian and Piscine Gonadotropins on Vitellogenesis

In the hypophysectomized catfish, vitellogenin formation is induced by using mammalian gonadotropins (luteinizing hormone: LH and follicle-stimulating hormone: FSH). It is logical to assume that they act on the follicular cells of oocytes to bring about estrogen elaboration which in turn stimulates the liver to produce vitellogenin. Histological examination of ovaries of LH-treated catfish reveals a slight hypertrophy of the follicular cells possibly suggesting estrogen production.

An interesting aspect of mammalian gonadotropins in our study is that, while they are very effective in promoting vitellogenin synthesis in the hypophysectomized catfish, they lack the ability to promote incorporation of vitellogenin into oocytes. In earlier studies on the catfish (Sundararaj & Anand 1972, Anand et al. 1975), ovine LH and human chorionic gonadotropin (hCG) with or without growth hormone (STH) were totally ineffective in inducing formation of yolky oocytes in the hypophysectomized catfish. Recently, van Ree (1977) has also shown that ovine LH is relatively ineffective in maintaining vitellogenesis in ovarian fragments from *Brachydanio rerio* under *in vitro* conditions. It appears, therefore, that mammalian gonadotropins do not act as complete gonadotropin in fishes (Nath 1978).

In contrast to mammalian gonadotropins, those from piscine sources such as salmon

gonadotropin (SG-G100) and carp (c-GTH) not only induce synthesis of vitellogenin but also bring about its incorporation into oocytes (Nath 1978, Sundararaj & Nath 1978). Remacle et al. (1976) reported that c-GTH maintains *in vitro* partially vitellogenic oocytes in ovarian fragments from *Carassius auratus*. van Ree (1977) stated that c-GTH is much more potent than ovine LH in maintaining follicular development and in stimulating enzymes for steroid production. Further, a process of extravesicular vitellogenesis is observed better in c-GTH-enriched cultures, than in cultures with ovine LH. Therefore, c-GTH is potent in stimulating the uptake of vitellogenic substances from the tissue culture medium, a property not shared by ovine LH. Wallace & Bergink (1974) have reported that gonadotropin is necessary for the removal of vitellogenin from the blood stream, and for promoting its uptake from circulation into vitellogenic oocytes by a reverse micropinocytotic process (Dumont 1972). These results indicate that incorporation of vitellogenin into oocytes is under the direct control of gonadotropin. In contrast to this, Upadhyay et al. (1978) have suggested in their studies on experimentally induced vitellogenesis in juvenile rainbow trout, *Salmo gairdneri*, with purified salmon gonadotropin, that gonadotropin is only necessary for the induction of endogenous yolk in the oocyte cytoplasm (first phase of vitellogenesis) and that some other pituitary hormone(s) is involved in the process of incorporation of vitellogenin into the oocyte (second phase of vitellogenesis).

Concluding Remarks

In females, environmental factors, such as photoperiod and temperature (see Vasal & Sundararaj 1976, Sundararaj & Vasal 1976) activate (through pathways presently unknown) the hypothalamo-hypophyseal complex to secrete gonadotropin which is responsible for the conversion of non-yolky primary oocytes into yolky oocytes. The present study indicates that mammalian gonadotropins can only induce formation of vitellogenin without promoting its incorporation into oocytes. Even long-term treatment with LH or hCG with or without STH does not result in the formation of yolky oocytes in the hypophysectomized catfish (Anand et al. 1975). Piscine gonadotropins, on the other hand, induce complete vitellogenesis leading to formation of yolky oocytes. Therefore, mammalian gonadotropins resemble piscine gonadotropins in having retained only the capacity to bring about vitellogenin formation through estrogen elaboration but differ from them in having lost the capacity to bring about vitellogenin incorporation into oocytes.

Mammalian gonadotropins have, however, the capacity to maintain yolky oocytes in the ovary of hypophysectomized gravid catfish. (Sundararaj & Anand 1972). Administration of estradiol-17β to hypophysectomized gravid catfish maintains, albeit partially, the yolky oocytes indicating that mammalian gonadotropins may be maintaining yolky oocytes in the ovary of hypophysectomized gravid catfish through estrogen elaboration (Anand & Sundararaj 1974). The inability of mammalian gonadotropins to promote incorporation of vitellogenin into oocytes is perhaps understandable. Eutherian mammals, being viviparous, have dispensed with the necessity to store yolk in the eggs for embryonic development. As a consequence, during the course of evolution, their gonadotropin molecule appears to have lost its ability to incorporate vitellogenin into oocytes.

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