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An Enzyme-Histochemical Study Concerning the Localization of Steroid Glucuronide Production in the Reproductive Organs of African Catfish, *Clarias gariepinus*

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ABSTRACT

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In ovaries of the African catfish, *Clarias gariepinus*, uridine diphosphoglucose dehydrogenase (UDPGD) and 3β-hydroxysteroid dehydrogenase (3β-HSD) appear in the granulosa of postovulatory follicles. In testes, both enzymes are present in interstitial cells. The combined presence of UDPGD and 3β-HSD points to the formation of steroid glucuronides at these sites. Interstitial cells between the tubules of the seminal vesicle also show 3β-HSD activity, both in captive fish and fish from nature. However, in these interstitial cells UDPGD activity was restricted to a few cells in the seminal vesicle of fish from nature. The latter enzyme was furthermore demonstrated in the epithelium of the seminal vesicle tubules, its activity being weaker in captive fish. Thus, in the seminal vesicle, glucuronidation of steroids may take place in interstitial cells, and it cannot be excluded that this process can also take place in the epithelium of the tubules.

INTRODUCTION

Colombo et al. (1980, 1982) demonstrated that etiocholanolone glucuronide formed by the mesorchial gland of male *Gobius jozo* serves as a sex pheromone for female conspecifics. Van den Hurk and Lambert (1983) observed that steroid glucuronide-containing fractions of postovulatory ovaries of *Brachydanio rerio*, as well as a mixture of testosterone glucuronide and oestradiol-17β glucuronide, attract male conspecifics. The formation of these latter steroid conjugates was demonstrated after in vitro incubation with pieces of postovulatory

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zebrafish ovaries in the presence of ^{3}H-labeled precursor steroids (Lambert et al., 1986). Steroid glucuronides formed in gonads thus may act as pheromones in fish.

In our research group the formation of steroid glucuronides and their role in the reproduction of the African catfish, *Clarias gariepinus*, are under investigation. Biochemical studies have indicated that, in this fish species, ovaries (Lambert and Van Den Hurk, 1982), testes (Schoonen and Lambert, 1986a) and seminal vesicles (Schoonen and Lambert, 1986b) are able to produce steroid glucuronides. Nothing is known, however, about the sites of steroid glucuronidation in the reproductive organs of this and other fish species.

The aim of the present enzyme-histochemical study was to locate the synthesis of steroid glucuronides in the ovary, the testis and the seminal vesicle of the African catfish. Therefore, the localization of uridine diphosphoglucose dehydrogenase (UDPGD) was investigated. This enzyme catalyses the transformation of UDP-glucose into UDP-glucuronic acid (Dutton, 1980). This glucuronic acid is a substrate in the glucuronidation of hydroxylated compounds, including steroid hormones. The localization of UDPGD was compared with that of 3β-hydroxysteroid dehydrogenase (3β-HSD), a key enzyme in the formation of biologically active steroid hormones.

MATERIALS AND METHODS

African catfish, *Clarias gariepinus* (ca. 300 g), were reared from eggs to maturity in aquaria. The fish were kept in a copper-free recirculating system at 25°C and under a photoperiod corresponding to the time of year in The Netherlands. Females were considered sexually mature if they possessed post-vitellogenic eggs with a diameter of 1 mm. Such females are able to produce viable eggs following induced ovulation.

A group of five reared mature females was injected intraperitoneally with (5 mg/kg body weight) pimozide, a gift from Janssen Pharmaceuticals Ltd. (Beerse, Belgium) and (0.05 mg/kg) Des Gly^{10}[D-Ala^{6}]LHRH ethylamide (LHRHa; Sigma, St. Louis, MO) to induce ovulation. Pimozide and LHRHa were suspended in a vehicle consisting of 0.8% NaCl, 0.1% sodium metabisulphite and 0.25% bovine serum albumin (BSA, fraction V, Sigma). A control group of five females was injected with an equivalent volume of pimozide/LHRHa-vehicle. The female catfish were killed by decapitation at 12 h after injection. Another group of five mature non-spawning male African catfish (ca. 2 kg) from the Hula Nature Reserve in Northern Isreael, caught during the breeding season (May 1984), was obtained from Ing. W.J.A.R. Viveen (Dutch–Israeli *Clarias* Project, Tiberias). These males and five of the reared males were killed by decapitation.

Central parts of gonads and distal parts of the finger-like seminal vesicle lobes were used for enzyme-histochemical studies. These studies were carried

out on 10-μm thick sections of tissues that were immediately frozen with CO_2 after killing the fish, and cut with a cryostat microtome (Minotome TH, Damon/IEC Division) at $-25°$C. 3β-hydroxysteroid dehydrogenase (3β-HSD) was demonstrated according to the method of Van Den Hurk (1973), with epiandrosterone (Merck-Darmstadt, Germany) as a substrate and an incubation temperature of 30° C. Alternate sections were stained with Mayer's haemalum-eosin (Burck, 1981) for more detailed morphological information. Demonstration of uridine diphosphoglucose dehydrogenase (UDPGD) was based on the methods of Balogh and Cohen (1961) and Jacobsen and Jørgensen (1973), using 0.82 m*M* uridine-5′-diphosphoglucose sodium salt (Boehringer-Mannheim, Germany), 2.15 m*M* EDTA (Merck-Darmstadt, Germany), 0.24 m*M* Nitro blue tetrazolium salt (BDH Chemicals Ltd. England), 0.75 m*M* NAD (Boehringer-Mannheim, Germany) and 5% polyvinyl alcohol (M.W. $\pm$125 000; BDH Chemicals Ltd, England) in 0.02 *M* Tris-HCl buffer pH 8.3. Incubation was carried out for 1 h at 30° C. In pilot experiments, the chosen concentrations of ingredients, pH, incubation time and incubation temperature appeared to give optimal staining intensities in the catfish tissues studied. Sections were also incubated in solutions lacking substrate or NAD to control the specificity of the reaction. In addition, uridine-5′-diphosphoxylose (0.1 m*M*) was used as an inhibitor of the UDPGD reaction.

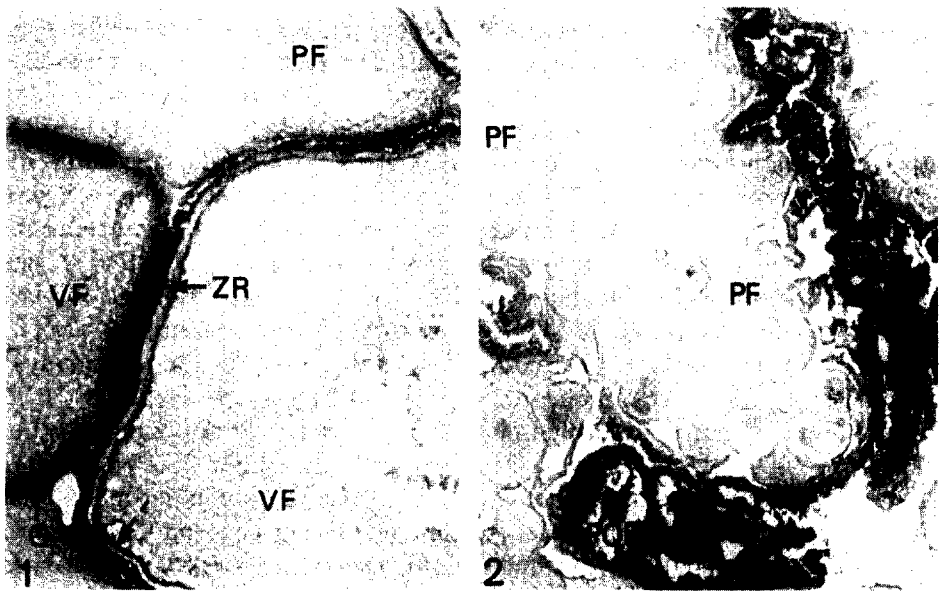
RESULTS

Ovary

Ovaries of pimozide/LHRHa-treated and control catfish generally showed a moderate to strong UDPGD activity in the granulosa around small vitellogenic oocytes (Fig. 1) and in the peripheral ooplasm of these and larger oocytes. A strong UDPGD reaction was also observed in the granulosa of postovulatory follicles (POFs; Fig. 2) that were only present in pimozide/LHRHa-treated fish. Strong 3β-HSD activity was demonstrated in special theca cells and interstitial cells of all female catfish investigated, as has previously been illustrated (Van Den Hurk and Richter, 1980). Pimozide/LHRHa-treated fish, in addition, showed a weak to moderate 3β-HSD activity in the granulosa of POFs.

Testis

Male fish had testes with a generally weak but prominent UDPGD activity in interstitial cells (Fig. 3). 3β-HSD was also localized in interstitial cells only, the enzyme activity being strong in both captive fish and fish taken from nature (Fig. 4).



Figs. 1-6. UDPGD and 3β-HSD activity in the ovary, testis and seminal vesicle of the African catfish. Abbreviations: E, epithelium of seminal vesicle tubule; G, granulosa; IC, interstitial cell; PF, previtellogenic follicle; POF, postovulatory follicle; ST, seminiferous tubule; SVT, seminal vesicle tubule; VF, vitellogenic follicle; ZR, zona radiata.
Fig. 1. Ovary with UDPGD activity in the granulosa of a vitellogenic follicle and its peripheral ooplasm (dotted arrow). $\times$120.
Fig. 2. Ovary showing UDPGD activity in POFs. $\times$88.

Seminal vesicle

UDPGD was localized mainly in the epithelium of the seminal vesicle tubules (Fig. 5). The enzyme activity was stronger in fish from their natural habitats than in captive fish. Fish from nature in addition showed a weak or moderate UDPGD activity in a few interstitial cells distributed in the stroma between the seminal vesicle tubules. 3β-HSD was demonstrated in interstitial cells only (Fig. 6). 3β-HSD activity was stronger in fish from nature as compared with captive fish. Sections of ovaries, testes and seminal vesicles that were incubated in substrate-free or NAD-free media remained unstained. Uridine-5'-diphosphoxylose completely inhibited the UDPGD reaction.

DISCUSSION

UDPGD is involved in the formation of polysaccharides or glucuronides that may be coupled to numerous xenobiotic and endogenous hydroxylated compounds (Dutton, 1980). β-glucuronides constitute the principal excretory derivates of bilirubin and steroids such as oestrone, oestradiol-17β, androsterone and etiocholanolone (Halliman, 1981). In fish, these steroid conjugates are of importance in the biological inactivation of steroids (Kime, 1980) and

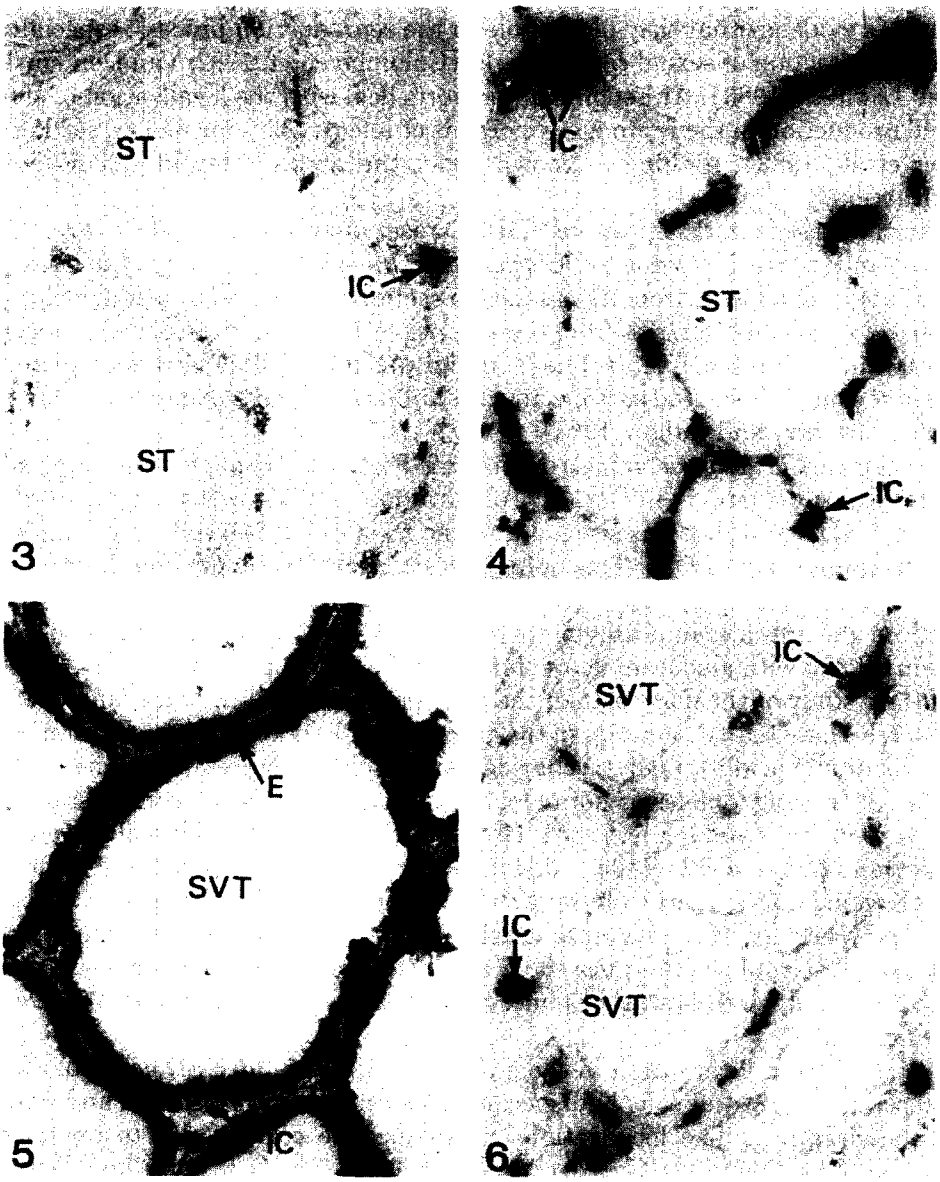


Fig. 3. Testis with UDPGD activity in interstitial cells. × 120.
Fig. 4. Testis with 3β-HSD activity in interstitial cells. × 120.
Fig. 5. Seminal vesicle lobe with UDPGD activity in the epithelium of the tubules and in interstitial cells. × 120.
Fig. 6. Seminal vesicle lobe with 3β-HSD activity in interstitial cells. × 120.

there is evidence that they play a role in the reproduction process, especially in the attraction of sexual counterparts (Colombo et al., 1982; Van Den Hurk and Lambert, 1983). Although many reports deal with the localization of steroid production in the reproductive organs of teleost fish (for review, see Fostier et al., 1983), nothing is known about the sites of steroid glucuronide formation.

In the present work, a positive UDPGD reaction was found in the ovary, the testis and the seminal vesicle of *Clarias gariepinus*. The specificity of this reaction not only appears from its absence in sections incubated in media lacking substrate or NAD, but also from the absence of formazan deposits after incubation in the presence of uridine-5′-diphosphoxylose. This latter compound has previously been used as a specific inhibitor of UDPGD (Neufeld and Hall, 1965; McGarry and Gahan, 1985).

In female *Clarias gariepinus*, a distinct UDPGD activity appears in the granulosa of postovulatory follicles. This observation together with the appearance of 3β-HSD activity at this site point to the formation of steroid glucuronides in the granulosa of POFs. This opinion is strengthened by previous biochemical findings (Lambert and Van Den Hurk, 1982) showing the formation of steroid glucuronides in postovulatory catfish ovaries. UDPGD has previously been related with glucuronides of steroids by Jacobson and Jørgensen (1973) in their study of the pars recta of the proximal tubules of the rat kidney. It is unlikely that UDPGD activity in the granulosa of POFs reflects incorporation of glucuronic acid into polysaccharides, since this structure, unlike the granulosa of growing follicles, is not involved in the production of such substances (Van Den Hurk and Peute, 1985). However, the UDPGD activity observed in the granulosa of small vitellogenic follicles and in the pheripheral ooplasm of vitellogenic oocytes probably reflects incorporation of UDP-glucuronic acid into polysaccharides or glucuronidation of non-steroidal compounds, since formation of steroid glucuronides could not be demonstrated biochemically in a previous study with ovaries of non-ovulated females (Lambert and Van Den Hurk, 1982).

Apart from a localization in the granulosa of POFs, 3β-HSD could be visualised in interstitial cells and special theca cells of both ovulated and non-ovulated catfish. The localization of this enzyme corresponds to previous observations (Van Den Hurk and Richter, 1980; Van Den Hurk and Peute, 1985), and is indicative of the sites of steroid hormone production. In testes of *Clarias gariepinus* both 3β-HSD and UDPGD were demonstrated in interstitial cells only, indicating these cells as sources of steroid glucuronides. Recently, production of steroid glucuronides in the testes of African catfish was demonstrated biochemically by Schoonen and Lambert (1986a).

In the seminal vesicle, 3β-HSD activity appeared to be restricted to interstitial cells. This observation confirms findings of Nayyar and Sundararaj (1969) with the Indian catfish, *Heteropneustes fossilis*. UDPGD could be dem-

onstrated in a few interstitial cells in fish from nature only. The combined presence of UDPGD and 3β-HSD activity in these interstitial cells of fish from nature points to a formation of steroid glucuronides. Biochemical studies have revealed that fish from nature form a mixture of five different steroid glucuronides (Schoonen and Lambert, 1986b), whereas captive fish are able to synthesize only two of these conjugates (Schoonen, personal communication, 1986). The production of steroid glucuronides thus is limited in captive fish, which might be the reason for the inadequacy of the enzyme-histochemical method used to demonstrate UDPGD activity in the interstitial cells of these animals.

UDPGD activity was furthermore clearly present in the epithelium of the seminal vesicle tubules, especially in fish caught in their natural habitats. 3β-HSD activity, however, was absent at this site. The seminal vesicle epithelium has been demonstrated to be involved in the production of a fluid containing polysaccharides (Nawar, 1959). Consequently, the presence of UDPGD in this epithelium might be indicative of the formation of these latter substances. On the other hand, it cannot be totally excluded that the site of steroid glucuronidation in the seminal vesicle may differ from the site of steroid formation, which would thus mean that UDPGD activity in the epithelium of the tubules reflects glucuronidation of steroids released by the interstitial cells.

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