VITELLOGENESIS: A QUANTITATIVE BIOMARKER FOR ENDOCRINE DISRUPTION IN WILD LIVING FISH?

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Abstract

The two dominant species of the Hessian part of the river Rhine, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*), differ markedly in the temporal and spatial compartmentalization of vitellogenesis and in the biochemical properties of the yolk precursor molecule (vitellogenin), occurring in blood. Therefore, an intimate knowledge of the natural process of vitellogenesis in female fish is an indispensable prerequisite for the use of the paradoxical hepatic induction of vitellogenin (VTG) in male fish as an indicator of xenoestrogenic exposure.

Introduction

Perch (Fig. 1a) and roach (Fig. 2a) differ in reproductive physiology, nutritional type and migrational behaviour, therefore, they may respond differently to xenobiotic exposure. For many years, HLUG has directed research efforts to determine if the paradoxical induction of VTG in male individuals of both species can be used as an indicator for xenoestrogenic burden.

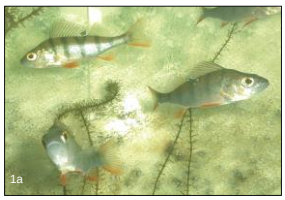


Fig. 1a: Perch; Fig. 1b: Immunological identification of VTG in electrophoretically separated serum proteins of perch, kDa molecular weight, VTG vitellogenin, BS perch serum from ethinylestradiol treated juvenile and adult males and mature females

Materials and Methods

In laboratory experiments, oestrogen-dependent proteins were induced in both species by exposure to synthetic hormones, and their presence was demonstrated in blood by SDS-PAGE separation of serum proteins (Fig. 2d). For further determination of VTG, antisera to yolk protein of mature female carp (*Cyprinus carpio*) and to typical female proteins of hormone treated perch have been used for immunological identification (Allner et al., 1999; Hennies et al., 2002).
Immuno-histological techniques were applied to identify VTG-producing cells in liver tissue, and to study structural changes of oocytes, related to the uptake of exogenous yolk protein.

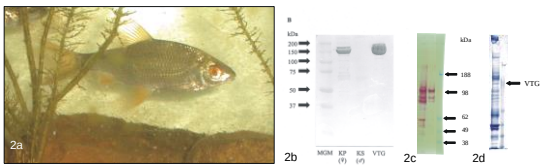


Fig. 2a: Roach, Fig. 2b: Immunological identification of VTG in electrophoretically separated serum proteins of carp, kDa molecular weight, MGM molecular weight standards, KP female serum, KS male serum
Fig. 2c: Immunological identification of VTG (4,7ng/µl; 3,2ng/µl) in electrophoretically separated serum proteins of roach, kDa molecular weight
Fig. 2d: SDS-PAGE separation of serum proteins

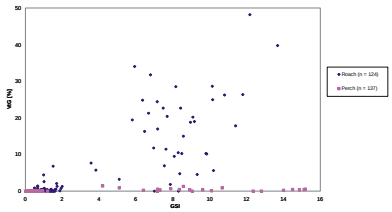


Fig. 3: Relative share of VTG of the entire plasma proteins of wild living females of perch and roach in relation to the gonadosomatic index (gonadal weight/body weight*100)

Results and Discussion

Perch VTG is unstable and transforms quickly into many metabolites, even in aprotinin-stabilised serum (Fig 1b). In contrast, VTG of cyprinidiformes decays to a lower extend (Fig. 2b, Fig. 2c).
The comparison of the percentage of VTG of the entire plasma proteins in mature females of both species shows higher titres of VTG in roach than in perch (Fig. 3). Furthermore, VTG is detectable in maturing roach in blood. In perch, VTG appears in blood only during the final stages of oogenesis (Germinal Vesicle Breakdown). VTG could be clearly identified through immuno-histology in roach liver (Fig. 4d), whereas female perch liver did not provide clear evidence for specific binding (Fig. 5c).

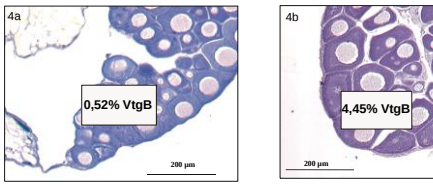


Fig. 4a-c: Cross section of the ovary of roach with different VTG content in blood (VtgB)

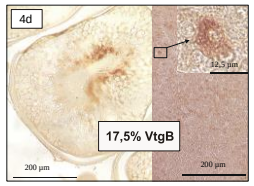
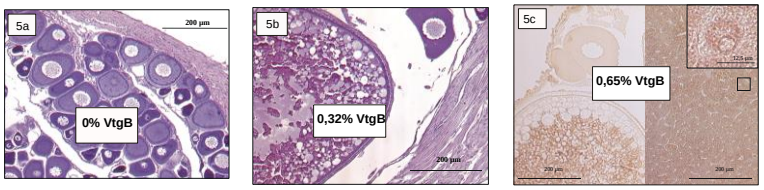


Fig. 4d: Immuno-histochememical presentation of VTG in the ovary and in liver tissue

Fig. 5a-d: Cross section of the ovary of roach with different VTG content in blood (VtgB)
Fig. 5d: Immuno-histochememical presentation of VTG in the ovary and in liver tissue



Conclusion

Biosynthesis of VTG differs fundamentally in roach and perch. Since the VTG concentration in the blood of even maturing female perch is very low, and since VTG disintegrates very quickly, the paradoxical induction of VTG in male perch is not a suitable quantitative biomarker for xenoestrogen burden. Consequently, there is no biomarker estabished to monitor the endocrine disruption by xenobiotics in the large group of perciform teleosts.
Immunological identification of SDS-PAGE separated proteins provided evidence for the existence of several immuno-reactive bands. In order to avoid overestimation of VTG concentrations in immuno-assays it is indispensable to know the origin and the function of the different proteins showing cross-reactivity. Especially regarding the interpretation of background levels it is important to ensure that no unspecific binding e.g. to other phospholipoproteins takes place.

Literatur

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