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Distribution of retinoids and 3,4-didehydroretinoids in the goldfish

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Abstract

Using high-performance liquid chromatography, the quantity of retinol, retinal, and retinyl esters as well as their 3,4-didehydro derivatives were measured in several body compartments and in the remainder of the body of 11 goldfish. Eighty-six percent of these retinoids and 3,4-didehydroretinoids existed as retinyl and 3,4-didehydroretinyl *esters*, 10% as retinal and 3,4-didehydroretinal, and 4% as retinol and 3,4-didehydroretinol. Most (94%) of these retinoids and 3,4-didehydroretinoids were found in ocular tissues and high ratios of 3,4-didehydroretinoids/retinoids were observed in most tissue extracts. These data suggest that in the goldfish ocular tissues are important locations of retinoid and 3,4-didehydroretinoid metabolism, which favors the formation and (or) storage of 3,4-didehydroretinoids.

Introduction

Rhodopsin and porphyropsin are visual pigments located in the retina of many lower vertebrates (for reviews, see Bridges 1972; Beatty 1975; Knowles and Dartnall 1977). These visual pigments are derived from the respective conjugation of 11-*cis* retinal and 11-*cis* 3,4-didehydroretinal to a species-specific opsin (Wald 1938, 1939; Dartnall 1957; Tsin and Santos 1985). Previous studies on fishes and amphibians showed that light and temperature treatments altered the rhodopsin/porphyropsin ratios in certain species (Dartnall et al. 1961; Allen and McFarland 1973; Tsin 1979; Tsin and Beatty 1979, 1980; for reviews, see Bridges 1972; Beatty 1975; Knowles and Dartnall 1977). However, the mechanism underlying this change of visual pigment composition is not known.

It is possible that the relative availability of both retinoids and 3,4-didehydroretinoids determines the rate of rhodopsin and porphyropsin formation, thereby regulating the proportions of these visual pigments. In studying possible mechanisms regulating visual pigment ratios, experiments have been carried out on the *in vivo* conversion of retinol (vitamin A₁) to 3,4-didehydroretinol (vitamin A₂) (Tsin et al. 1985) in tadpoles and goldfish. It was shown that these species *can* derive 3,4-didehydroretinol from exogenous (radio-labelled) retinol but at a very slow rate. However, it was not possible to fully identify the location of this conversion because a detailed description of retinoids and 3,4-didehydroretinoids in body compartments was not available. In the present study, therefore,

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we have conducted a preliminary survey to provide data on the distribution of retinoids and 3,4-didehydroretinoids in this species.

Methods

Goldfish (*Carassius auratus*; mean body weight, 7.6 gm) were obtained from Grassy fork Fisheries (Martinsville, Indiana) and held at room temperature and in room light before the experiment. Fish were dark adapted overnight before decapitation and eye removal (under dim red light; Wratten series II filter). The retina and the underlying retinal pigment epithelium (RPE) were removed separately from the eye cup. The liver and the gastrointestinal (GI) tract were subsequently dissected from the fish. Left and right retinas of a fish were pooled and sonicated in 2% digitonin to extract visual pigments. The method of pigment extraction and analysis followed standard procedures (see Tsin and Beatty 1979).

Other body compartments (liver, GI tract, RPE, eye cup, and the remainder of the fish) were ground separately in anhydrous sodium sulfate and extracted for retinoids (with acetone). The acetone extract was evaporated to dryness by nitrogen, and the residue was redissolved in a small volume of 0.5% dioxane in *n*-hexane and introduced onto a column packed with 5% water-deactivated alumina (1 × 2 cm). This column was then eluted with 10 mL of 0.5% dioxane in *n*-hexane to collect retinyl and 3,4-didehydroretinyl esters. Retinal and 3,4-didehydroretinal were subsequently eluted from this alumina column using 10 mL of 3% dioxane in *n*-hexane before retinol and 3,4-didehydroretinol were eluted with 10 mL of 10% dioxane in *n*-hexane. These ester, aldehyde, and alcohol fractions were separately analyzed by isocratic high-performance liquid chromatography (HPLC). Retinyl and 3,4-didehydroretinyl esters were saponified in methanolic KOH (Tsin et al. 1984) prior to HPLC analysis. The recovery from the saponification exceeded 85%. In the present study, no detectable amount of aldehydes was observed in extracts from these body compartments (see Table 1).

The HPLC analyses of retinoids and 3,4-didehydroretinoids have also been reported in detail earlier (Tsin et al. 1984; Tsin and Santos 1985; Lai et al. 1985). In the present study, saponified esters and alcohols were eluted with 10% dioxane in *n*-hexane at a flow rate of 1 mL/min, whereas aldehydes were eluted with 12% diethyl ether in *n*-hexane at the same flow rate. The HPLC column was 5 (μm Ultrasphere. Retinoids and 3,4-didehydroretinoids were detected at 340 nm. Quantitation was attained by comparing integrated peak areas of retinoids and 3,4-didehydroretinoids with those obtained by injections of known amounts of authentic standards. Mainly all *trans* isomers were observed in the present study and *cis* isomers (except visual pigment chromophores; see Table 1) constituted only a small portion (less than 10%) of all retinoids and 3,4-didehydroretinoids measured in the present study.

Results and discussion

Table 1 shows that most (86%) retinoids and 3,4-didehydroretinoids in the goldfish existed in ester forms found mainly in the RPE of the eye. Aldehydes constituted about 10% of all retinoids and 3,4-didehydroretinoids and they were found only in the retina (Table 1). In contrast, most alcohols were recovered from the liver and the (remainder of the) body

instead of from the eye, although these alcohols only accounted for 4% of all retinoids and 3,4-didehydroretinoids in the goldfish.

It is of particular interest to point out that in the goldfish, the quantity of 3,4-didehydroretinoids (in either ester, aldehyde, or alcohol forms) exceeded that of retinoids. This high ratio of 3,4-didehydroretinoids/retinoids was observed in all body compartments with the exception of that in the liver (71% retinyl esters), the GI tract (100% retinyl esters), and the RPE (67% retinol). The retinol/3,4-didehydroretinol ratio in the RPE (i.e., 67% retinol) seems to reflect the relative proportion of retinol/3,4-didehydroretinol in the blood of the goldfish (61% retinol; Castillo and Tsin 1984) suggesting that blood retinol may be in close equilibrium with the retinol in the RPE. However, the high proportion of 3,4-didehydroretinyl esters in the RPE (80% 3,4-didehydroretinyl esters, Table 1) suggests that within the RPE itself some level of compartmentalization must exist in order to accommodate this difference in the composition of alcohols and esters (Tsin et al. 1983).

In summary, our data indicate that in the goldfish ocular tissues are important locations of retinoid and 3,4-didehydro-retinoid metabolism, which favors the formation and (or) storage of 3,4-didehydroretinoids.

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Table 1

Quantity of all *trans* retinoids and 3,4-didehydroretinoids in all body compartments of goldfish. Mean values (in nmol/fish, $n = 11$) for retinoids (A_1) and 3,4-didehydroretinoids (A_2) are indicated

	Esters		Aldehydes		Alcohols		Total
	A ₁	A ₂	A ₁	A ₂	A ₁	A ₂	
Liver	0.05	0.02	0.00	0.00	0.02	0.21	0.30
GI tract	0.01	0.00	0.00	0.00	0.01	0.03	0.05
RPE	2.50	9.30	0.00	0.00	0.08	0.04	11.92
Retina*	—	—	0.26	1.64	—	—	1.90
Eye cup	0.70	3.30	0.00	0.00	0.00	0.02	4.02
Body	0.12	0.29	0.00	0.00	0.04	0.26	0.71
Total	3.38	12.91	0.26	1.64	0.15	0.56	18.90
% total	17.88	68.31	1.38	8.68	0.79	2.96	100.00
% of each form	86.19		10.06		3.75		100.00

* Only visual pigments were analysed in the retina. Values indicated in the table were derived from the amount of rhodopsin and porphyropsin, assuming that 1 mol of opsin conjugates with 1 mol of 11-*cis* retinal (or 3,4-didehydroretinal) to form rhodopsin (or porphyropsin) (Hubbard et al. 1971; Suzuki and Makino-Tasaka 1983). Free (not conjugated to opsin) retinal and 3,4-didehydroretinal exist in extremely low quantity in the retina (Suzuki and Makino-Tasaka 1983). Alcohols and esters were not measured in the retina.