the conformation of membrane constituents to an extent that is directly related to transmitter release.

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Light and temperature affect retinyl ester hydrolase activity and visual pigment composition

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Summary. Dim light, in combination with high water temperature, resulted in a significant increase in the retinyl ester hydrolase activity in the goldfish retina. This rise in enzyme activity may relate to a selective increase in the availability of retinal chromophores thereby favoring the formation of rhodopsin under these light and temperature conditions.
Key words. Goldfish; rhodopsin and porphyropsin; retinyl ester hydrolase; light and temperature treatments.

Visual pigments are light sensitive molecules located in the photoreceptor cells (rods and cones) of the eye, serving the function of photo-transduction[1]. These photopigments are derived from the conjugation of opsin (a protein) to either retinal or 3,4-didehydroretinal[2]. Because retinal and 3,4-didehydroretinal based pigments exhibit different absorption properties[3], an alteration of visual pigment composition (i.e. from retinal based pigments to 3,4-didehydroretinal based pigments or vice versa) will result in a change in the visual sensitivity, thereby improving the animal's adaptation to its (new) environment[4]. This change in the visual pigment composition occurs seasonally in certain species and in others, during spawning migrations[2, 5–7]. Although laboratory light and temperature treatments have been successful in the (artificial) induction of visual pigment changes in some animals[8–10], the mechanism underlying this visual pigment change is not understood. In the present communication, experimental evidence is presented to show that light and temperature exert a profound influence on the ocular retinyl ester hydrolase activity (which governs the release of retinol from retinyl esters in the eye) as well as on visual pigment composition. Assuming that the activity of retinyl ester hydrolase is specific for retinyl palmitate, an increase in this enzyme activity should selectively increase the amount of retinal (in comparison to 3,4-didehydroretinal) available for rhodopsin (over porphyropsin) formation. These results, therefore suggest that the hydrolase enzyme may hold a control mechanism through which light and temperature exert their influence on the visual pigment composition.

Goldfish were used in these experiments because they possess both rhodopsin and porphyropsin in their retinas[13]. The composition of these visual pigments is known to change in response to an artificial alteration of their light and temperature environments[9, 15]. Visual pigment analyses were performed by standard procedures[9, 10]. Retinyl ester hydrolase (REH) activity was assayed by a method using HPLC (high performance liquid chromatography) and described in detail previously[11, 12]. Retinyl palmitate was used as the substrate of the REH. The enzyme activity was linear to 3 mg protein. The distribution of REH activity in goldfish is shown in table 1. The highest REH activity was found in retinal homogenates, followed by homogenates prepared from the retinal pigment epithelium (RPE/choroid). These values are similar to those from the bovine retina (72.5 pmol retinol/mg/h, n = 19)[11] and RPE/choroid (100.8 pmol retinol/mg/h, n = 10, unpublished observation). Both brain and liver tissues had significantly lower REH activity than those in ocular tissues (table 1). The unusually low REH activity found in the liver (i.e. compared to 130 pmol retinol/mg/h in the bovine liver[11], and 571 pmol retinol/mg/h in the rat[12]) may be attributable to the low level of vitamin A stored in the goldfish hepatic tissue[15].

The effect of light and temperature on the REH activity in ocular tissues is shown in table 2. The REH activities in the retina and RPE/choroid of fish in group 1 (held under room

Table 1. Distribution of REH activity in the goldfish

Tissue type	Specific enzyme activity (pmol retinol/mg/h)
Retina	114.3 ± 18.9 (n = 12)
RPE/choroid	105.1 ± 10.4 (n = 8)
Brain	41.7 ± 5.2 (n = 4)
Liver	13.4 ± 3.3 (n = 4)

Fish were dark adapted 2–5 h before decapitation and eye removal. Two retinas of a fish were dissected from their underlying retinal pigment epithelium (RPE) and homogenized in 0.5 ml Tris-maleate buffer (0.05 M, pH = 8). The RPE/choroid were also peeled from the eye cup and homogenized in 0.5 ml of Tris-maleate. The liver and the brain were dissected from the fish and homogenized in 1 ml each of Tris-maleate. The specific activities of REH (mean ± SD, n = number of samples analyzed) are given. The animals possessed in excess of 90% porphyropsin in their retinas. Boiling (by adding the tissue homogenate in test tube placed in 100 °C for 30 min) completely abolished the REH activity in the retina and the RPE/choroid.

Table 2. Light and temperature influence on the visual pigment composition and REH activity in the goldfish

Group	Light and temperature conditions	Porphyropsin %	Specific activity (pmol retinol/mg/h) Retina	RPE/choroid
1	Room light 20 °C	100	105.6 ± 7.2 (n = 6)	118.4 ± 15.5 (n = 8)
2	Room light 30 °C	90	143.3 ± 9.6 (n = 8)	115.4 ± 20.3 (n = 8)
3	Dim light 16L/8D, 30 °C	30	223.5 ± 11.5 (n = 18)	146.8 ± 33.2 (n = 18)

Upon arrival from the supplier, goldfish were acclimated for 30 days in the specified conditions (for details of these light and temperature conditions)[9] before visual pigment (percent porphyropsin: mean value from two fish) and REH (mean ± SD, n = number of samples) analyses.

light and at room temperature for 30 days) were comparable to those (table 1) obtained from fish upon arrival. This suggests that REH activity in these animals remained unchanged during the 30-day duration of the experiment. Likewise, the composition of photopigments in the retina remained high in porphyropsin proportions in fish held under room conditions.

Although light and temperature did not exert a significant influence on the REH activity in the RPE of the eye (table 2), these environmental factors affected both visual pigment composition as well as REH activity in the retina (table 2). An increase in temperature (compare results from groups 2 to 1) associated with a 40% increase in REH activity in the retina but little change in photopigment composition. A combined effect of light and temperature (compare results from groups 3 to 1) resulted in a large reduction of porphyropsin proportion (from 100% to 30%, which is in agreement with our earlier findings)[9] and a significant increase (Student's t-test, $p \geq 0.001$) in the REH activity.

The effect of temperature acclimation on the enzyme activity has been well established in the literature[16]. For example, the K_m values of acetylcholinesterase in rainbow trout acclimated to 2 °C and to 18 °C were completely different when it was assayed at 5, 10, 15 and 20 °C. Examples of these 'thermal modulations' are also shown in other enzymes such as phosphoenolpyruvate, lactate dehydrogenase (and others) and in other species such as rabbit, tuna (and others)[16]. Data from the present study show that light and temperature give rise to a significant change in the ocular REH activity in goldfish. Although the present study has not elucidated whether light/temperature had influenced either the K_m or the V_{max} of the REH, this light/temperature induced increase in the ocular REH activity will clearly increase the rate of release of retinol in the eye, leading to an increase in the availability of retinal chromophore for the formation of rhodopsin. Furthermore, it is also important to study whether this REH activity is specific for retinyl ester in comparison to 3,4-didehydroretinyl ester in order to establish the significance of this control mechanism.

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The influence of different nutrients on plasma cholecystokinin levels in the rat[1]

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Summary. Isocaloric and isovolemic amounts of protein (casein), fat (intralipid) and carbohydrate (saccharose) and an isovolemic control solution of water were administered intragastrically to conscious rats. The plasma CCK levels, determined by a sensitive and specific radioimmunoassay, showed an increment of 6.3 ± 0.6, 2.7 ± 0.5, 1.7 ± 0.4 and − 0.9 ± 0.4 pM, respectively (basal value 2.5 ± 0.3 pM). The threshold increment of plasma CCK to stimulate pancreatic enzyme secretion by exogenous CCK was found to be 1.5 pM. It is therefore concluded that casein is a potent stimulus for CCK secretion and pancreatic secretion, but that fat and even carbohydrate, although less potent, also produce a CCK increment above the threshold for pancreatic secretion.

Key words. Rats; nutrients; cholecystokinin; pancreatic secretion.