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Immunocytochemical localization of taurine in the fish retina under light and dark adaptations*

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Summary. Previously we have observed the lack of immunoreactivity of taurine in the rod outer segments from light-adapted fish, such as the ayu *Plecoglossus altivelis* and lefteye flounder *Paralichthys olivaceus*. This finding prompted us to investigate if there is a difference in the immunocytochemical localization of taurine in the rod outer segments between the dark- and light-adapted states. In the retinas of the glass eel *Anguilla japonica* and the young goldfish *Carassius auratus*, extremely intense immunostaining was found in the cone outer segments, rod inner segments, photoreceptor supranuclear region and outer plexiform layer. The rod outer segments were not immunostained in the light-adapted state, while they were intensely immunostained in the dark-adapted state. Consequently, it was suggested that the lack of immunoreactivity in the rod outer segment may depend on light stimulation. In addition, the conspicuous immunocytochemical localization of taurine was discussed with the possible functional roles for taurine in the fish retina.

Keywords: Amino acids – Taurine – Immunocytochemistry – Fish retina – Photoreceptor cells – Light and dark adaptations

Introduction

Extremly high levels of taurine have been demonstrated in many species of marine invertebrates and fishes (Simpson et al., 1959; Allen and Garrett, 1971; Konosu and Shinagawa, 1988; Sakaguchi and Murata, 1988; Sakaguchi, 1991). Taurine in these marine animals may act as a major osmolyte (Lange, 1963; Allen and Garrett, 1971; King et al., 1982; Fugelli and Thoroed, 1986; Sakaguchi and Murata, 1988). During adaptation to sea water, taurine and/or the taurine transporter conspicuously increased in euryhaline fish (Assem and Hanke, 1983; Takeuchi et al., 1999).

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As it is well known, high levels of taurine are found in the photoreceptor cells of the retina (Orr et al., 1976; Voaden et al., 1977). Taurine is regarded as a predominant osmolite in the photoreceptor outer segment (Pasantes-Morales et al., 1998). It is known that light stimulates the efflux of taurine from the rod outer segments of the frog (Salceda et al., 1977). "Protection of the outer segment" may be a functional role of taurine in the photoreceptor cell (Pasantes-Morales and Cruz, 1983, 1985).

Localization of taurine-like immunoreactivity was previously investigated in the fish retina and pineal organ (Omura et al., 1997a). In those studies, we observed that rod outer segments exhibit poor immunoreactivity (see Figs. 1a,b). Changes in immunocytochemical localization of taurine was also investigated in the developing fish retina (Omura and Yoshimura, 1999). We now find that the rod outer segments are lacking in taurine immunoreactivity (see Figs. 1c,d). In order to demonstrate taurine-loaded rod outer segments, the present investigation focusses on the immunocytochemical localization of taurine in the dark-adapted fish retina, since the previously reported experiments (Omura et al., 1997a; Omura and Yoshimura, 1999) were performed under laboratory illumination during the day.

Materials and methods

Glass eels (or elver, 55–60 mm in length) and young goldfish (50–52 mm in length) were maintained in aerated freshwater under a 14L-10D (05:00–19:00 light and 19:00–05:00 dark) photoperiod at 18–23°C for 1 week (light intensity 635 lux). All the experimental procedures under the dark adaptation were performed using Noctovision NVR2015C (NEC).

Under light and dark adaptations, eels and goldfish were anesthetized in 1/1000 MS222 and decapitated. The whole heads of the eels (38 specimens from the light- and 14 specimens from the dark-adapted eels) were immersed in a fixative of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 *M* phosphate buffer or 2.8% glutaraldehyde and 2.0% paraformaldehyde in 0.1 *M* cacodylate buffer containing 0.06 *M* $MgCl_2$. The enucleated eyeballs of the goldfish (4 specimens each from the light- and dark-adaptated states) were immersed in a fixative of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 *M* phosphate buffer. After postfixation in 1% OsO_4 in 0.1 *M* phosphate buffer for 90 min, the specimens were dehydrated and embedded in epoxy resin.

The immunostaining for light microscopy was performed as previously described (Omura et al., 1997). The antiserum diluted to 1:500 in PBS containing 2% BSA was used on the epon sections (0.5–2 μm) after etching with a KOH-methanol-propylene oxide mixture. For visualization the avidin-biotin-HRP complex (VECTOR) was used with intensification by nickel ions for the DAB reaction. The secondary antibody (biotinylated goat anti rabbit IgG, VECTOR) diluted to 1/200 in PBS plus 2% BSA was followed by avidin conjugated HRP (1/400 in PBS plus 2% BSA). Between all steps the tissues were throughly washed with PBS buffer plus Triton X-100.

Control sections were processed either by substituting PBS containing 2% BSA for the primary antibody, or by using antisera which had been preabsorbed with taurine conjugated via glutaraldehyde to BSA.

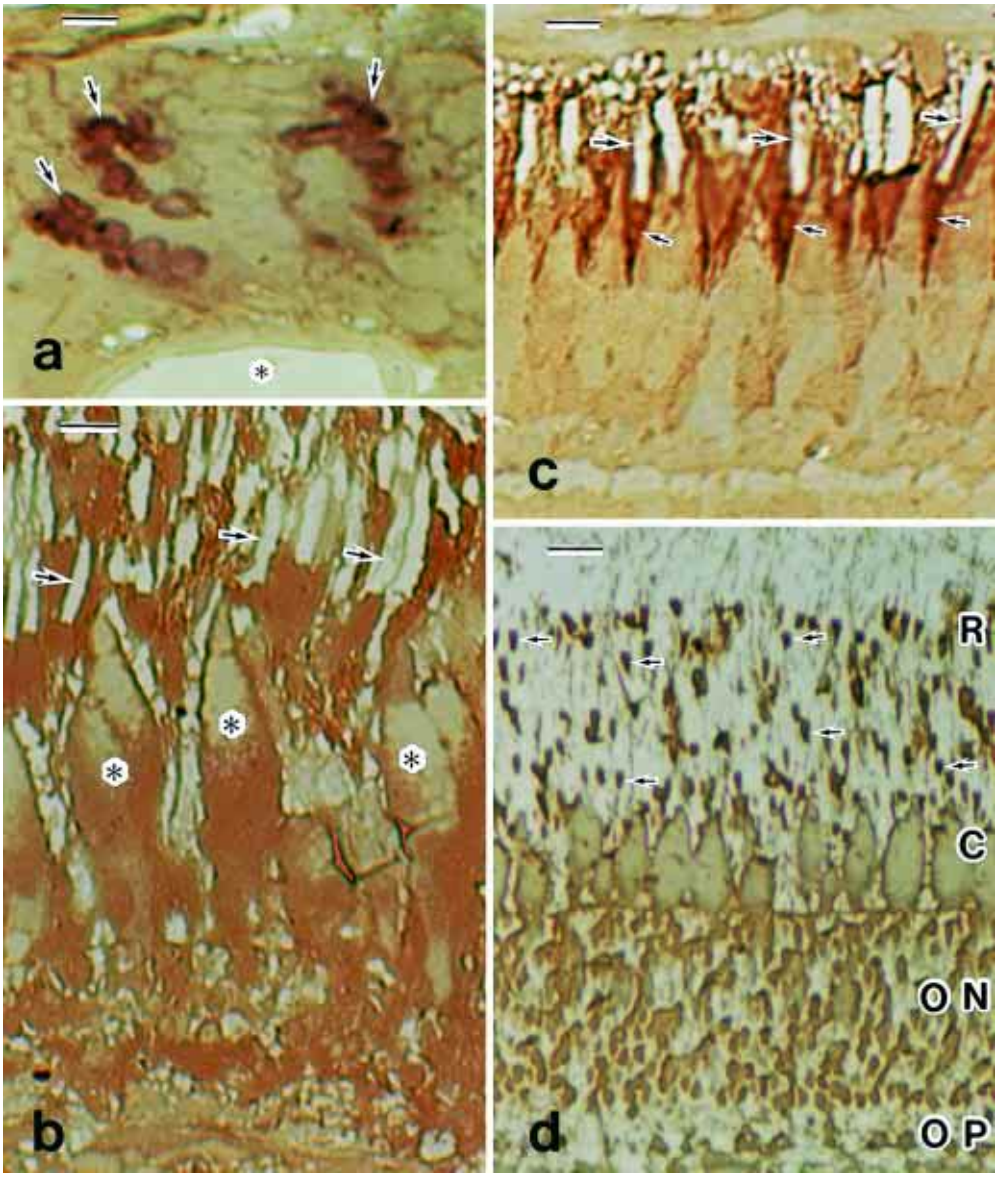


Fig. 1. Light micrographs showing immunocytochemical localization of taurine in the pineal organ (**a**) and retina (**b**) of an anadromous fish, ayu *Plecoglossus altivelis*, and in the retina of 40 days-old (**c**) and 1 year-old (**d**) lefteye flounder *Paralichthys olivaceus*. Compared to those of pineal photoreceptors (arrows in **a**) and retinal cones, the outer segments of rods (arrows pointing to the right in **b**, **c**) exhibit no immunoreactivity at all. Both the newly-matured (**c**) and multilayered outer segments (**d**) of rods are lacking in taurine immunoreactivity, while the inner segments (smaller arrows pointing to the left) exhibit strong immunoreactivity. Asterisks indicate pineal capillary (**a**) and cone cells (**b**); *C* layer of cone outer and inner segments; *ON* outer nuclear layer; *OP* outer plexiform layer; *R* layer of rod outer and inner segments. Scale bars: 10μm (**a**, **b**, **c**), 20μm (**d**)

Results

Retinal structure and retinomotor responses

Both the retina of glass eel and young goldfish possess a well developed stratum of photoreceptor cells, consisting of two major layers of rods (rod outer and inner segments) and cones (cone outer and inner segments), and outer nuclear and plexiform layers. The goldfish retina shows a typical type, consisting of a single layer of rods and cones (Figs. 2a,b). In contrast, the glass eel retina exhibits a double layer of rods and a single layer of cones (Figs. 3a,b): the ratio of rods to cones is approximately 6:1. A typical retinomotor response (photomechanical movement) was observed in the goldfish retina (Figs. 2a,b): under light-adaptation during the day, rods elongate, cones contract, and RPE pigment granules disperse into the apical processes; under dark-adaptation at night, these movements are reversed: rods contract, cones elongate, and RPE pigment granules aggregate into the scleral side. In the eel retina, however, the rods did not show this movement either under the light or dark adaptations (Figs. 3a,b).

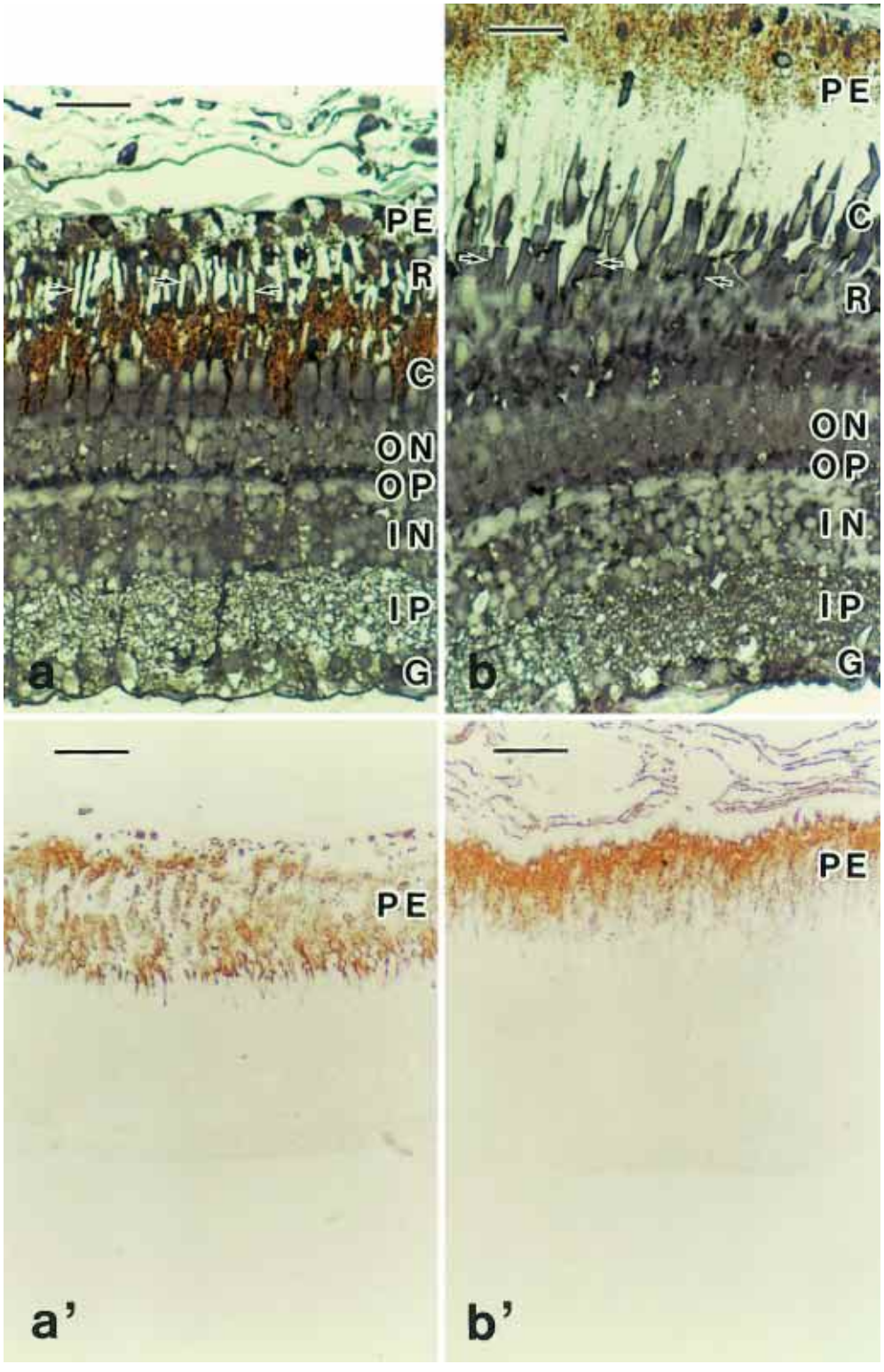
Immunocytochemical localization of taurine in the goldfish retina under light and dark adaptations

Both the light- and dark-adapted goldfish retinas were distinctly stained with the taurine antibody (Figs. 2a,b), although control sections treated with a medium lacking the primary antibody or an antiserum preabsorbed with taurine were not stained at all (Figs. 2a′,b′). Under light adaptation, extremely intense staining appeared in the cone outer segments, the rod inner segments and the outer plexiform layer (Fig. 2a). Intense immunostaining appeared in the cone inner segments and the outer nuclear layer. The rod outer segments were not stained at all. Under dark adaptation, the rod outer segments were conspicuously stained (Fig. 2b).

Immunocytochemical localization of taurine in the eel retina under light and dark adaptations

Immunostaining of taurine in the eel retinas was clearly demonstrated both in the light and dark adaptations (Figs. 3a,b). Extremely intense immunostaining

Fig. 2. Immunocytochemical localization of taurine in the goldfish retina under light (**a**, **a′**) and dark (**b**, **b′**) adaptations. In the light (**a**) rod outer segments (arrows) exhibit no immunoreactivity at all, while cone outer segments, rod inner segments and outer plexiform layer show extremly strong immunoreaction. In the dark (**b**), rod outer segments (arrows) exhibit conspicuous immunoreactivity for taurine. Control sections (**a′**, **b′**) treated with the antigen-preabsorbed antibody do not show immunostaining at all, except for the endogenous staining of pigment granules in the pigment epithelium (*PE*). *C* layer of cone outer and inner segments; *G* ganglion cell layer; *IN* inner nuclear layer; *IP* inner plexiform layer; *ON* outer nuclear layer; *OP* outer plexiform layer; *R* layer of rod outer and inner segments. Scale bars: 50μm (**a**, **b**), 100μm (**a′**, **b′**)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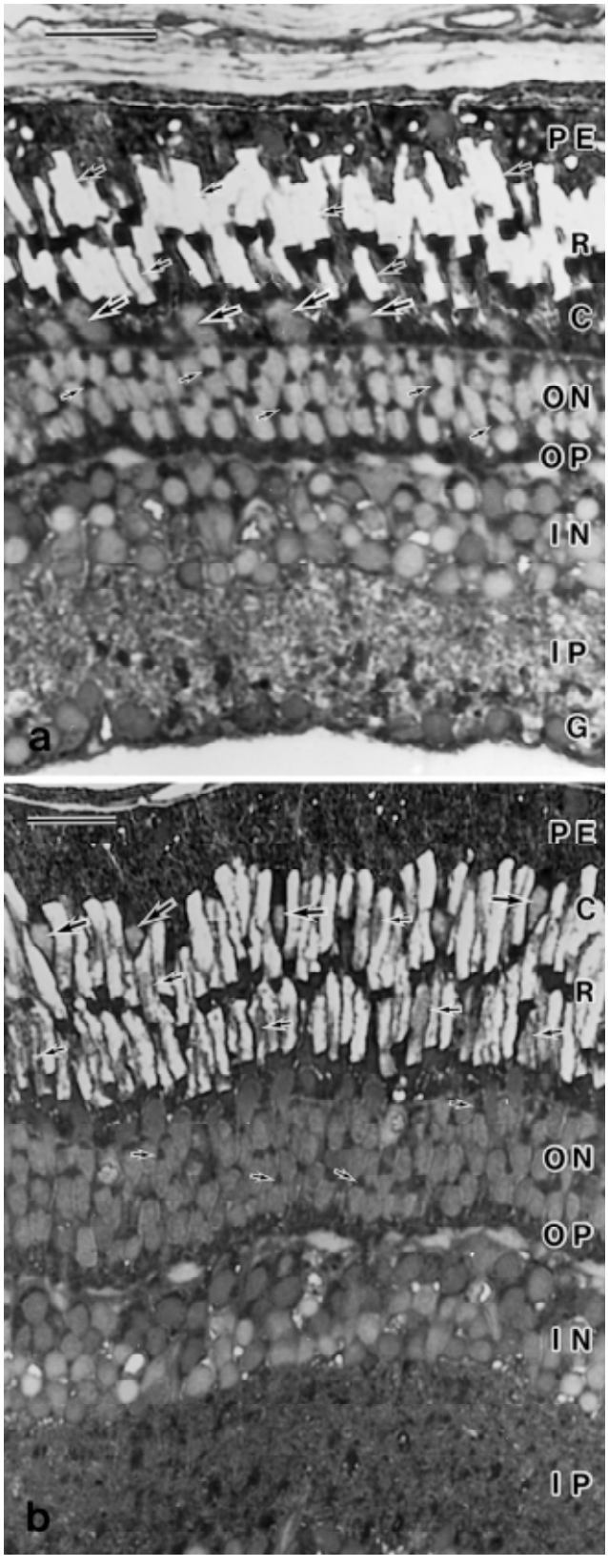
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appeared in the cone outer segments, rod inner segments, outer plexiform layer, and supranuclear region of the photoreceptor cells. (Fig. 3a). Intense immunostaining appeared in the cone inner segments. The rod outer segments were not stained at all in the light adapted state (Fig. 3a). The plasma membrane and/or cytoplsm of the rod outer segment were intensely stained only in the dark adaptation (Fig. 3b). The photoreceptor nuclei of the outer nuclear layer were more intensely stained in the dark, while they were only faintly stained in the light.

Discussion

Taurine is the predominant free amino acid in many tissues of aquatic animals (Simpson et al., 1959; Allen and Garrett, 1971; Konosu and Shinagawa 1988; Sakaguchi and Murata, 1988; Sakaguchi 1991). Taurine in marine invertebrates and fishes is known to act as a major osmolyte (Lange, 1963; Allen and Garrett, 1971; King et al., 1982; Fugelli and Thoroed, 1986; Sakaguchi and Murata, 1988). During adaptation to sea water, taurine concentration and/or mRNA of the taurine transporter increased strongly in many tissues of the euryhaline tilapia (Assem and Hanke, 1983; Takeuchi et al., 1999).

As is well known, both the diadromous eel and the freshwater goldfish are euryhaline teleost fish. In order to clarify the release and/or accumulation of taurine in the rod outer segments in the light condition, the retinas of the glass eel *Anguilla japonica* and young goldfish *Carassius auratus* were investigated using immunocytochemical methods (see Lake and Verdone-Smith, 1989; Omura et al., 1997a). In the eel, the retinal rod cells develop much faster and more plentifully, due to adaptation to their dark habitats (Omura et al., 1997b). The goldfish exhibits a typical photomechanical movement (retinomotor response): the myoids of cone and rod cells elongate and/or contract in response to changes in light conditions (see Ali and Klyne, 1985). The glass eel exhibited a conspicuous elongation and contraction of cone myoids, but little or no movements of rods between light and dark adaptations.

Fig. 3. Comparison of immunocytochemical localization of taurine in the retina of the glass eel *Anguilla japonica* between light (**a**) and dark (**b**) adaptations. Strongly immunostained cone outer segments exhibit a positional change (large arrows): cone myoids contract in the light and elongate in the dark. Rod outer segments (smaller arrows pointing to the left) exhibit conspicuous immunoreactivity under dark adaptation, although they do not show immunoreactivity at all under light adaptation. The photoreceptor nuclei of the outer nuclear layer also demonstrate stronger immunoreactivity under dark adaptation. In addition, the supranuclear region of photoreceptor cells (small arrows pointing to the right) and the outer plexiform layer show extremly strong immunoreaction under both conditions. *C* layer of cone outer and inner segments; *G* ganglion cell layer; *IN* inner nuclear layer; *IP* inner plexiform layer; *ON* outer nuclear layer; *OP* outer Plexiform layer; *PE* pigment epithelium; *R* layer of rod outer and inner segments. Scale bars: 20μm (**a**, **b**)

Table 1. Summary of the taurine-like immunoreactivity in light- and dark-adapted retinas of the glass eel. (See the detailed explanation in the text)

Part	Light	Dark
Rod outer segment	−	++
Cone outer segment	+++	+++
Rod inner segment	+++	+++
Cone inner segment	++	++
Photoreceptor supranuclear region	+++	+++
Outer nuclear layer	+	++
Outer plexiform layer	+++	+++

+++: extremely strong; ++: strong; +: weak; −: undetectable

So far, the distribution of taurine in the eel retina has never been reported, while there have been several reports describing the immunocytochemical localization of taurine in the retina: rat, cat, guinea pig (Lake and Verdone-Smith, 1989), rabbit (Pow et al., 1994), pigeon (Ueck et al., 1995), lungfish (Pow, 1994), goldfish (Marc et al., 1995), ayu (Omura et al., 1997a) and left eye flounder (Omura and Yoshimura, 1999). High levels of taurine immunostaining were found in the outer and inner segments and synaptic terminals of photoreceptor cells (Lake and Verdone-Smith, 1989; Ueck et al., 1995), while no or lower levels of immunostaining were reported in the outer segments of rods and certain cone cells (Omura et al., 1997a; Omura and Yoshimura, 1999).

The present results concerning taurine-like immunoreaction in the light- and dark-adapted eel retina are summarized in Table 1. In the retina of the glass eel and young goldfish, the cone outer segments and rod inner segments exhibited strong immunostaining both under light and dark adaptations. In contrast, the rod outer segments of both fish were not immunostained at all in the light-adapted state, while the plasma membrane and/or cytoplasm of rod outer segments were intensely immunostained in the dark-adaptated state. The photoreceptor nuclei of the dark-adapted retina were also intensely stained in contrast to the photoreceptor nuclei of the light-adapted retina. Taurine might be released from the photoreceptor nuclei by light just as from the rod outer segments. Consequently, it was suggested that the lack of immunoreactivity in the rod outer segment may depend on light adaptation and/or stimulation.

It is known that the release of taurine from the frog rod outer segments is induced by light stimulation (Salceda et al., 1977). Light-evoked release of taurine is also observed in the isolated retinas of chicken, cat and rat (Pasantes-Morales et al., 1973; Schmidt, 1978). In addition, a shielding effect of taurine on the rod outer segments exposed to toxic levels of light and chemicals has been well demonstrated (Pasantes-Morales and Cruz, 1983, 1985). On the other hand, the expression of a taurine transporter has been

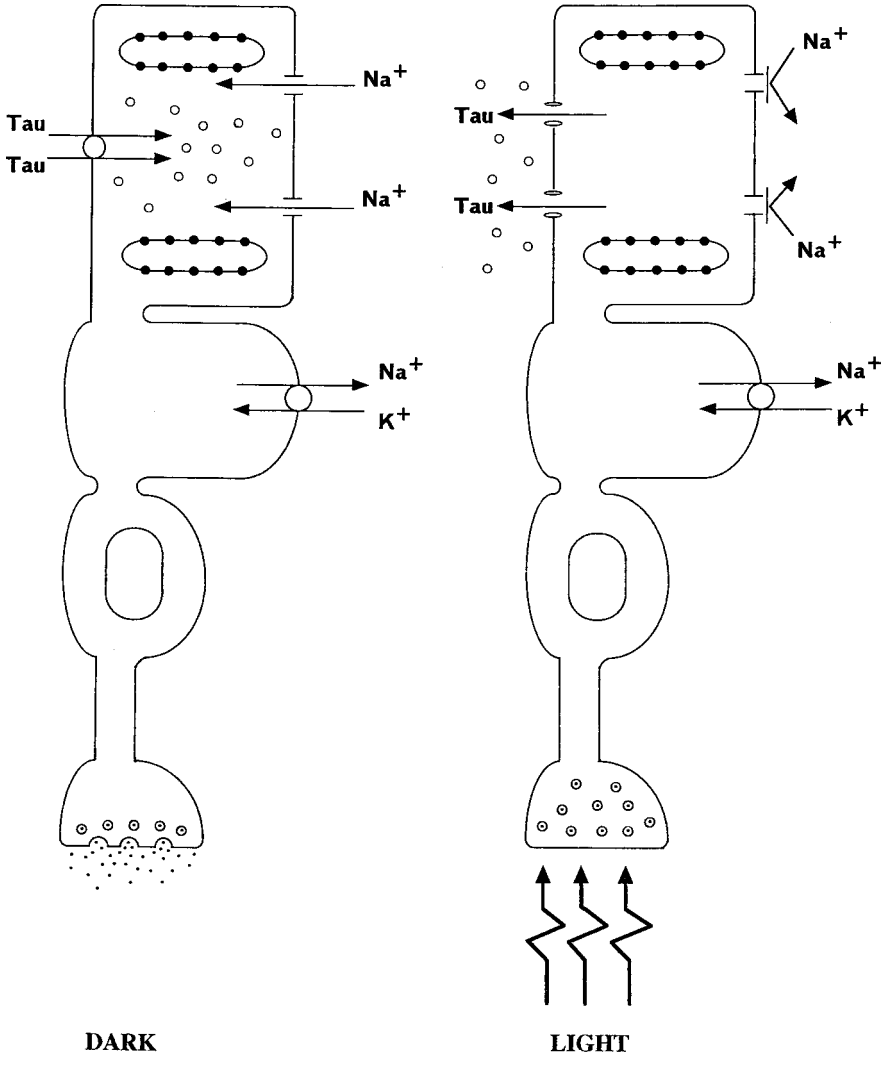

Fig. 4. A diagram showing movements of taurine in the rod outer segment of fish retina under light and dark adaptations. In the dark, taurine may slowly be accumulated by taurine transporters. In the light, after a rapid efflux, the taurine pool of the outer segment may be exhausted

demonstrated in many tissues including the retina of euryhaline telapia *Oreochromis mossanbicus*: after moving the telapia into 70% sea water, the expression of the taurine transporter occurs slowly and reaches a peak after 10 hours (Takeuchi et al., 1999).

As a predominant osmolite, taurine might be released from the rod outer segment by light, when the sodium channel and current are closed and stopped, respectively (see Ganong, 1997). If the dark cycle is then initiated, taurine particles may be transported slowly into the intracellular taurine pool by taurine transporters (see Fig. 4). Therefore, the taurine pool of the rod outer segment might be more concentrated in the dark cycle with time, while the pool is exhausted during the light cycle.

It has been reported that taurine-like immunoreactivity is intense in the photoreceptor synaptic terminals in the outer plexiform layer of the rat, cat and guinea pig (Lake and Verdone-Smith, 1989) as well as in the lungfish retina (Pow, 1994). A possible function of taurine in the synaptic terminal is the regulation of signal transduction (Lombardini, 1991). By means of stimulating Ca^{2+} uptake and inhibiting protein phosphorylation (Lombardini, 1985, 1991), taurine may modulate and/or regulate synaptic transmission in the outer plexiform layer in the eel and goldfish retinas.

In the pigeon retina, the synaptic terminal of the cone pedicle exhibited a higher density of immunogold particles than that of the postsynaptic site (Ueck et al., 1995). This observation at higher magnification demonstrated the immunogold particles not within, but between the synaptic vesicles of the cone pedicle. As the immunogold particles were not associated directly with the synaptic vesicles, it was thus concluded that taurine is not present as a neurotransmitter. Considering its high content, taurine may be released by non-vesicular means from the synaptic terminal. Electron microscopic investigation is necessary for the immunolocalization of taurine in the outer plexiform layer of the eel and goldfish retinas.

The supranuclear region of photoreceptor cells also demonstrated extremely intense immunostaining in the eel retina. It is known that this region is rich in Golgi apparatus and rough-surfaced endoplasmic reticulum, synthesizing opsin that is a main element of the visual pigments (Bok, 1985). Accordingly, it may be suggested that in the eel retina, taurine is also biosynthesized in this region. Retinal taurine levels are maintained by transport across the blood-retinal barrier and/or by endogeneous biosynthesis (Lake and Verdone-Smith, 1989). Cysteinesulfinic acid decarboxylase (CSAD) is the key enzyme in taurine biosynthesis (Tang et al., 1997). CSAD immunoreactivity has been demonstrated in the rat retina: heavy staining in the inner plexiform layer; moderate staining in the inner and outer nuclear layers and ganglion cell layer (Lin et al., 1983). Since the photoreceptor cells were stained mildly with anti-CSAD, it was suggested that only small amounts of taurine may be synthesized in the photoreceptor cell bodies, despite the large content of taurine. Accordingly, more detailed analysis by CSAD immunostaining is necessary to confirm whether there is biosynthesis of taurine in the supranuclear region of the photoreceptor cells in the eel retina.

Additionally, the conspicuous labeling with the taurine antibody may be useful for the marking of photoreceptor cells because the outer and inner segments of cone and rod cells were clearly distinguishable in this study. The physiological role of taurine in the retina of fish is still undetermined. According to the present results of immunocytochemical localization of taurine, it may be suggested that taurine is involved at least in the protection of the rod outer segments, and in the modulation of signal transduction.

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