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Research report

Localisation of annexins in the retina of the rainbow trout—light and electron microscopical investigations

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Abstract

We present a first description of annexin immunoreactivity within the teleost retina. Antibodies against annexins V and VI were used in light and electron microscopic sections of light- and dark-adapted retinae. Strong immunoreactivity could be found in retinal layers with high synaptic input, such as the outer and inner plexiform layers and dendritic regions within the inner plexiform layer, in cells that are involved in negative feedback control such as horizontal and amacrine cells, in the membrane metabolism of photoreceptor outer segments, and in close relation to cytoskeletal components. Our findings suggest that both annexins V and VI are involved in the regulation of transmitter release, particularly of transmitters that are not directly involved in phototransduction. The annexins appear also to be involved with structures that support morphological changes in light and dark adaptation.

Theme: Sensory systems
Topic: Retina and photoreceptors

Keywords: Annexin; Cytoskeleton; Feedback synapses; Light and dark adaptation; Membranes; Vesicles

1. Introduction

Annexins form a family of at least 13 Ca^{2+} and phospholipid binding proteins that are highly homologous in their structure but lack an EF hand-type calcium binding site [11,13]. A core domain of approximately 70 amino acids occurs as a tetrad repeat in all annexins except annexin VI, which is unique in its structure as it has eight repeats. Unlike the highly conserved C-terminus, the amino terminal domains of annexins are highly variable in both length and amino acid composition. From the fact that the amino terminal is unique comes the widely held belief that it is this region that mediates the specific functions of individual annexins. Annexins are highly abundant intracellular proteins and are expressed up to 1% of the total cell protein in all vertebrate cell extracts examined to date. Their conservation and abundance suggest important functions, but despite their potential importance, their biological functions are not well defined for any of these proteins [12,33].

A most significant current problem in annexin research is to obtain a clear indication of their physiological roles. Proposed functions include the regulation of membrane trafficking and exocytosis, the regulation and inhibition of protein kinase C activity, the mediation of cytoskeletal–membrane interactions, the mediation of mitogenic signal transduction, and the formation of ion-specific channels (reviewed in Refs. 4,6,9,12).

Abbreviations: BC, bipolar cell; CP, calycal process; D, dendrite; ELM, external limiting membrane; GCL, ganglion cell layer; HC, horizontal cell; INL, inner nuclear layer; MY, myoid; ONL, outer nuclear layer; OS, outer segment; PR, photoreceptor layer; SR, synaptic ribbon

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Annexins V and VI, investigated in the present study, are highly abundant in nervous tissues [36]. Annexin V is not only found in glial cells as previously reported [14,36], but also in pituicytes of the neurohypophysis [34], anterior pituicytes [19], and synaptic vesicles of neural tissues [15]. Annexin V is concentrated in the axon terminal and found to be associated with synaptic vesicles, but not with cell organelles or the plasma membrane, whereas glial cells seem to express a battery of Ca^{2+}-activated proteins such as S100B, calmodulin, and annexins II and V [34]. The addition of calcium results in a clustering of vesicles (aggregation), which points to a function in neurotransmitter release with its ability to bind membranes of synaptic vesicles and/or the plasma membrane. Annexin IV has been found to be associated to the plasma membrane of rat Purkinje cells [36], and interaction of annexin VI with synapsin I has been reported [17]. Presumably both annexins can act as modulators of vesicle release. However, both annexin A5 and A6 knockout mice so far lack a clear phenotype, confounding hypothesis about their functions [3,16].

Within the rat eye, annexin V has been reported for the basal cornea, the posterior iridal epithelium, the eye muscle and lens fibres, in the retina only in Müller cells, and within the optic nerve in close association with glial cells and the axon periphery [14]. In molluscs (*Aplysia* and *Helix*), a 40-kDa protein was found in the eyes and in neurons that show strong similarities with annexins [20,28], and is regulated by light and serotonin in opposite ways. A possible circadian rhythm regulation through intracellular calcium modulation and/or that of other second messengers (even by the formation of ion channels, a "hydrophilic pore") has been proposed [18,28].

The retina of teleosts contains a set of active neurotransmitters (e.g., dopamine, glutamate, melatonin, and serotonin). An endogenous clock controls synthesis, storage, and release of several of these transmitters [37,38]. In this study, we show that both annexins V and VI are expressed in the trout retina especially in layers with high synaptic output (V and VI), in cells that are involved in negative feedback control (V and VI), and in membrane metabolism of photoreceptor outer segments (V), but also in close relation to cytoskeletal components (VI).

2. Materials and methods

Adult rainbow trouts (*Oncorhynchus mykiss*) were purchased from a local hatchery, kept in freshwater tanks under constant conditions (15 °C, 12-h light/12-h darkness) for several weeks prior to experiments, and sacrificed by decapitation. Eyecups were prepared, removing sclera and lens and fixed in 4% phosphate-buffered paraformaldehyde for several hours at room temperature. After thorough washing in phosphate buffer (0.1 M, pH 7.3), eyecups were processed for further treatment. To obtain tissue in a dark-adapted condition, fish were sacrificed at night in complete darkness and all preparations including fixation performed with the aid of infrared optics and an array of five infrared diodes.

For light microscopy, eyecups were incubated in 30% phosphate-buffered sucrose overnight, cut in half with a razor blade, and embedded in Tissue Tek (Miles, USA) for cryosectioning on a Reichert Cryocut E microtome. Cross and tangential sections of 14 μm were mounted on poly-L-lysine-coated slides, dried for an hour, and incubated for immunocytochemistry. Preincubation with 0.5% bovine serum albumin (BSA), 1% Triton X-100, and 10% normal goat serum (NGS) in phosphate-buffered saline lasted for an hour at room temperature. All antibodies (annexins V and VI: polyclonal, antirabbit; CY3: antirabbit) were diluted in phosphate-buffered saline containing 0.5% BSA, 1% Triton X-100, and 3% NGS. Incubation with first antibodies (1:1000) was carried out in a humid chamber at 4 °C overnight. For fluorescence detection, a CY3 secondary antibody (1:100; Jackson, USA) was applied for 90 min in the dark at room temperature. For controls, the first antibody was omitted from the incubation medium. Sections were examined with a Reichert Polyvar microscope.

For electron microscopy, retinae were separated from scleral and chorioidal tissues, cut in small pieces, dehydrated, and embedded in Epon using propylene oxide as an intermedium. Epon blocks were sectioned on a Reichert Ultracut microtome and sections of silver to golden interference colour mounted on formvar-coated nickel grids. Grids were washed in bidistilled water before the removal of resin with 10% H_2O_2 (1 h). After thorough washing with 0.1 M Tris buffer (pH 7.2) and a short incubation in the same Tris buffer containing 0.1% BSA, grids were preincubated for 30 min in 5% NGS, following incubation with annexin antibodies for 2 h (1:50; diluted in Tris buffer containing 0.1% BSA). For controls, the first antibody was omitted from the incubation medium. With the next washing step, the pH value was gradually shifted to 8.4 and the concentration of BSA increased to 1%. The secondary antibody (1:30, goat antimouse/Au, 5 μm; Amersham, USA) was diluted in this buffer, sections were incubated for 1 h, followed by several washing steps in Tris buffer, which brought the pH value gradually back to 7.2. Sections were fixed in 2% Tris-buffered glutaraldehyde for 10 min, washed in Tris buffer and bidistilled water, counterstained in a LKB Ultrastainer with uranyl acetate (45 min at 40 °C) and lead citrate (3 minutes at 20 °C), and dried before examination in a Zeiss EM 910 transmission electron microscope.

All chemicals were purchased from Sigma-Aldrich. The specificity of primary antibodies has been tested earlier [29].