HISTOCHEMICAL STUDY OF NA^+-K^+-ATPASE AND CARBONIC ANHYDRASE IN THE CILIARY EPITHELIUM OF THE SPINY DOGFISH, SQUALUS ACANTHIAS.

Cassandra Flügel*, Michael Eichhorn*, Elke Lütjen-Drecoll*, Michael Wiederholt**, José A. Zadunaisky+

*Anatomisches Institut, Universität Erlangen-Nürnberg, 8520 Erlangen, FRG; **Institut Klinische Physiologie, Klinikum Steglitz, Freie Universität Berlin, 1000 Berlin 45, FRG; +Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016, USA.

Na^+-K^+-ATPase and carbonic anhydrase are two enzymes which are known to be involved in the active transport processes of aqueous humor production in mammalian eyes. Biochemical and physiological studies have indicated that both enzymes may be present also in the ciliary epithelium of the spiny dogfish eye (Jampol and Forrest, Exp. Eye Res. 13:315-319, 1972; Maren, Comp. Biochem. Physiol. 5:201-215, 1962; Maren et al., Invest. Ophthalmol. 14:662-673, 1975; Wiederholt and Zadunaisky, Pflügers Arch. 407 (Suppl.2):S112-S115, 1986; Wiederholt and Zadunaisky, Invest. Ophthalmol. Vis. Sci. 28:1353-1356, 1987). From these findings it was concluded that the mechanism of aqueous humor formation could be similar in elasmobranchs and mammals. In mammals (rabbits and monkeys) we have demonstrated histochemically that both enzymes are distributed unevenly in the different regions of the ciliary epithelium. (Lütjen-Drecoll and Lönnerholm, Invest. Ophthalmol. Vis. Sci. 21:782-792, 1981; Flügel and Lütjen-Drecoll, Histochemistry, in press). We have now studied the distribution of the enzymes in the eye of elasmobranchs.

Eyes of 3 spiny dogfishes were enucleated, cut equatorially, and from the anterior part of the bulbus small pieces were prepared containing the ciliary body from the iris towards the retina. In the shark eye, the inferior quadrant contains the ciliary muscle called ciliary papilla while the superior part contains the suspensory ligament for the lens. Therefore, sections were taken predominantly from the nasal and temporal quadrant.

For the demonstration of Na^+-K^+-ATPase at the light microscopic level, the method of Chayen et al. (Histochemistry 71:553-554, 1981) was used. Fresh specimens were deep-frozen in liquid nitrogen, 10 µm sections prepared at -20° C and thawed on glass slides. The sections were then preincubated for 5 min in a moist chamber at 37° C with a drop of medium consisting of 0.2 M Tris-HCl buffer (pH 7.5) in which potassium acetate at 0.1 M and Polypep 5115 (Sigma) at 40 % w/v had been dissolved. The preincubation medium was than replaced by the complete incubation medium: 0.2 M Tris-HCl buffer (pH 7.5) containing 2 mM sodium acetate, 410 mM sodium chloride, 20 mM magnesium chloride, 37.5 mM potassium chloride, 16.5 mM ATP, 40 % Polypep and lead ammonium citrate/acetate complex (Sigma L 4009), 32 mg/ml. The final pH of 7.5 was adjusted with 1 N HCl. We modified the method slightly by adding 2.5 mM levamisole (Sigma) to the incubation medium following Mayahara et al. (Histochemistry 67:125-138, 1980) for incubation of the alkaline phosphatase. Incubation

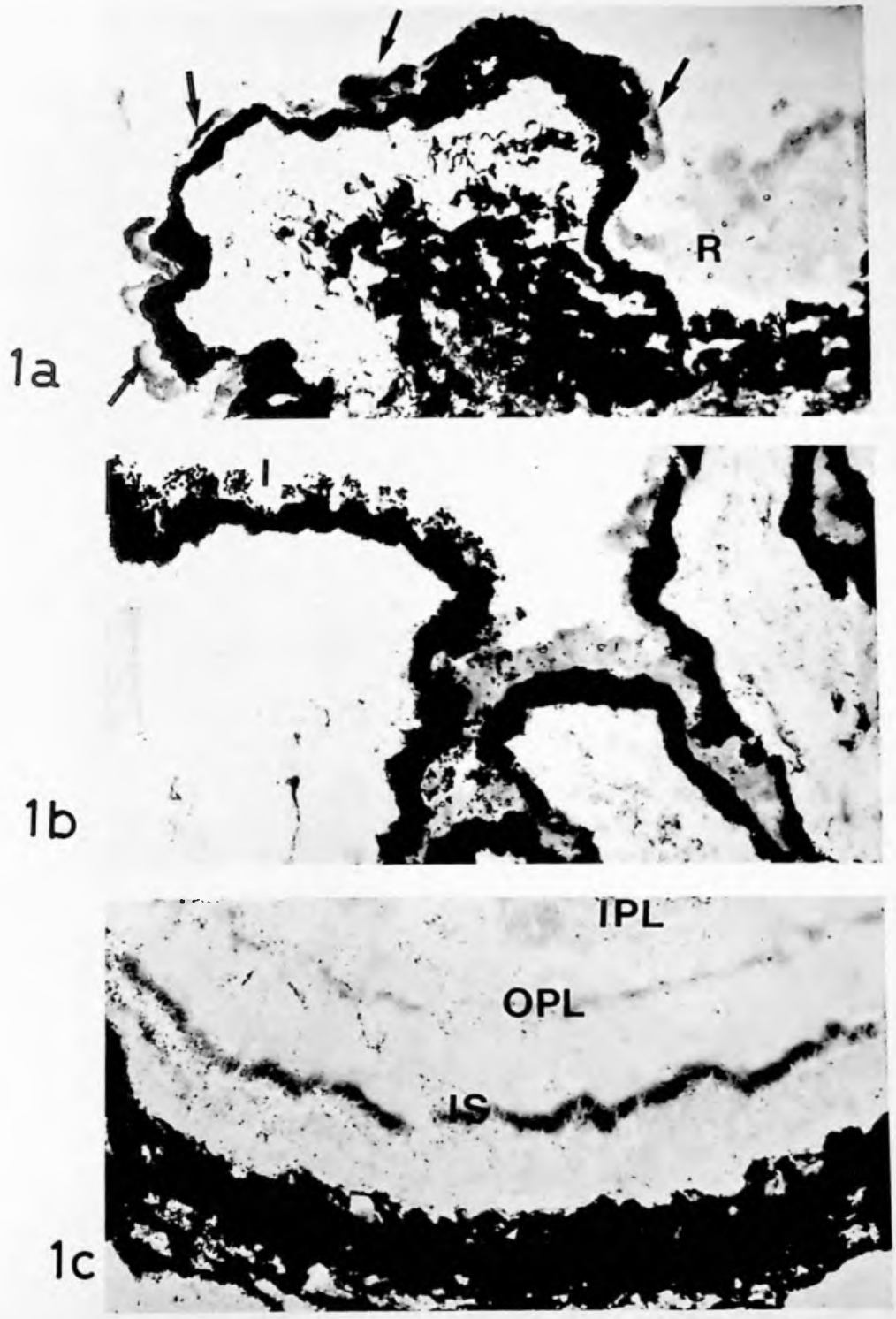

Fig. 1 : Frozen sections of ciliary body (a), iris (b) and retina (c) of a spiny dogfish eye incubated for Na^+-K^+-ATPase for 40 min. a : Reaction products are localized posteriorly in the non-pigmented epithelial cells (arrows) of the ciliary body; R = retina (x 600). b : Anteriorly the non-pigmented epithelial cells of the ciliary body are stained up to the transition into the iris (I) (x 750). c : In the retina staining is found in the inner segments of the photoreceptors (IS) and in the outer (OPL) and inner plexiform layers (IPL) (x 800).

periods were 10-60 min. Then the sections were rinsed several times in 0.2 M Tris HCl buffer (pH 7.4) at 37° C, stained for 1 min in 1 % ammonium sulphide, washed in distilled water and mounted in Kaiser's glycerol gelatin (Merck). For controls, 10 mM ouabain was added to the preincubation and incubation media.

For the histochemical demonstration of carbonic anhydrase, small pieces of ciliary epithelium were immediately put into cold 2.5 % glutaraldehyde solution, buffered by 0.1 M cacodylate buffer (pH 7.4) for 3 hours, then washed in the same buffer and embedded according to Ridderstråle (Acta Physiol. Scand. 98:465-469, 1966) in hydroxypropylmethacrylate (JB4, Polysciences, Pennsylvania) as prescribed. 2 µm sections were cut and stained, following the Hansson procedure (Histochemie 11:112-128, 1967) with 1.75 mM $CoSO_4$ in the solution. Incubation times were 1-10 min.

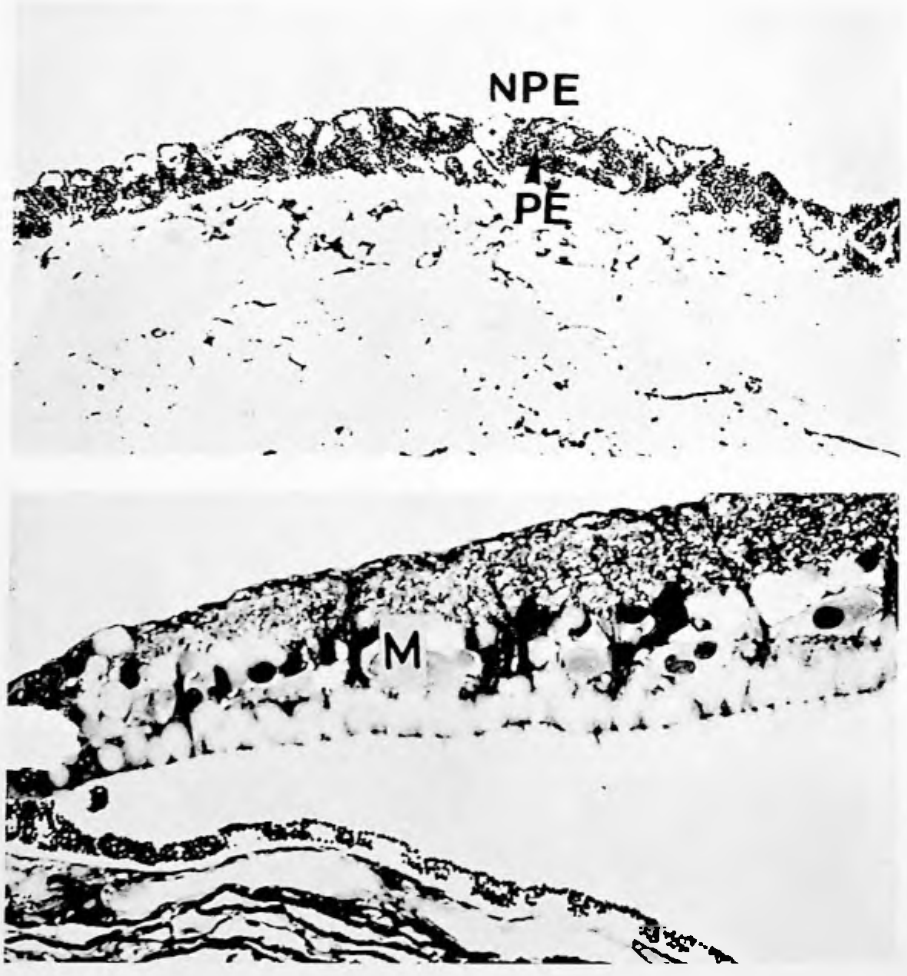

Fig. 2 : Sections of ciliary body (a) and retina (b) stained for carbonic anhydrase, incubated for 10 min. a : In the ciliary body no staining is to be seen in the pigmented (PE) and non-pigmented (NPE) epithelium (x 1100). b : In the retina the main staining is localized in the Müller (M) cells (x 1040).

The reaction products for Na^+-K^+-ATPase staining could be seen after 15 min of incubation. They were located basolaterally in the non-pigmented epithelium. All cells posteriorly from the ora serrata to the first pigmented cells anteriorly at the transition zone between ciliary epithelium and iris were stained equally (Fig. 1a,b). Only the first two cells at the ora serrata often showed a weaker staining. In the adjacent retina, an intensive staining could be seen in the inner segments, somewhat weaker also in the outer and inner plexiform layer (Fig. 1c). The epithelium of the iris did not stain. The pigmented epithelium of the ciliary body could not be investigated because of the intense pigmentation of this cell layer.

No staining for carbonic anhydrase could be seen in the ciliary body (Fig. 2a). In the adjacent retina, mainly the Müller cells stained (Fig. 2b).

Our results indicate that the Na^+-K^+-ATPase activity can be localized in the basolateral membrane foldings of the non-pigmented ciliary epithelium. This localization corresponds to the localization of the enzyme in the pars plicata of rabbits and primates. In contrast to these species, no regional differences could be seen in the spiny dogfish. The absence of carbonic anhydrase staining only indicates that there is less concentration of this enzyme in the spiny dogfish than in rabbits and primates. The staining procedure was performed only on fixed methacrylate-embedded sections and we know that there is loss of enzyme activity with this procedure. Therefore, absence of staining does not indicate total absence of enzyme activity.

This study was supported by DFG grant Wi 328/14-1 and NIH grants EY-1340 and GM-25002.