# EVIDENCE FOR TRANSEPITHELIAL OUABAIN AND FUROSEMIDE SENSITIVE MECHANISMS ACROSS THE CILIARY EPITHELIUM OF THE SHARK (SQUALUS ACANTHIAS)

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Sections of the ciliary epithelium of adult sharks were prepared for measurements of intracellular membrane potential (membrane PD) and intracellular chloride activity ($a_i$ -Cl) as described previously (Wiederholt and Zadunaisky, Bulletin, MDIBL 24:89, 1984). In addition, isolated sections of the same tissue were mounted in Ussing-type chambers (area 0.2 $cm^2$) for measurement of transepithelial membrane potential (Transepithelial PD), short circuit current (SCC) and transepithelial resistance (R).

The membrane PD across the basolateral membrane of non-pigmented cells (facing the aqueous humor) was - 53.2 $\pm$ 1.3 mV (n = 35) and decreased to -18.0 $\pm$ 1.2 mV (n = 17) when 40 mM potassium was superfused. The calculated slope of 42 mV/decade potassium change indicates a high potassium permeability. $10^{-5}$ M ouabain depolarized the membrane PD to -36.3 $\pm$ 3.4 mV (n = 10) and $10^{-4}$ M furosemide hyperpolarized the membrane PD to -65.2 $\pm$ 1.5 mV (n = 14).

The chloride intracellular activity was 64.2 $\pm$ 3.3 mM (n = 15) well above the predicted value (26mM). After application of $10^{-4}$ M furosemide, $a_i$ - Cl was 25.5 $\pm$ 1.3 mM (n = 13) approaching the equilibrium value of 19 mM for this series. The data indicates that a furosemide-sensitive anion mechanism exists in this epithelium.

In 15 preparations transepithelial PD was aqueous side negative (-0.51 $\pm$ 0.12 mV; SCC 18.3 $\pm$ 2.5 uA $cm^{-2}$; R 30.7 $\pm$ 3.1 Ohm $cm^2$). However, in 15 other preparations the spontaneous transepithelial PD was aqueous side positive (0.53 $\pm$ 0.09 mV; SCC - 19.6 $\pm$ 2.3 UA $cm^{-2}$; R 27.9 $\pm$ 2.8 Ohm $cm^2$). The polarity of the transepithelial PD probably depends on the relative activity of cation and anion pumps across the cell membrane of the non-pigmented and or pigmented cell layer. To test this hypothesis further $10^{-5}$ M ouabain or $10^{-4}$ M furosemide were applied either to the aqueous or blood side of the isolated ciliary epithelium at transepithelial positive or negative PD. Figure 1 summarizes data when ouabain was given to the aqueous side. At transepithelial positive (aqueous side ) PD ouabain decreased PD and SCC within 15 to 45 min. A biphasic response of PD and SCC was observed when the spontaneous transepithelial PD was negative. A similar effect of ouabain has been reported on the isolated rabbit iris-ciliary body preparation (Krupin et al., Exp Eye Res 38:115-123, 1984). Ouabain given to the blood side inhibited transepithelial PD and SCC both at conditions of spontaneous positive and negative transepithelial PD.

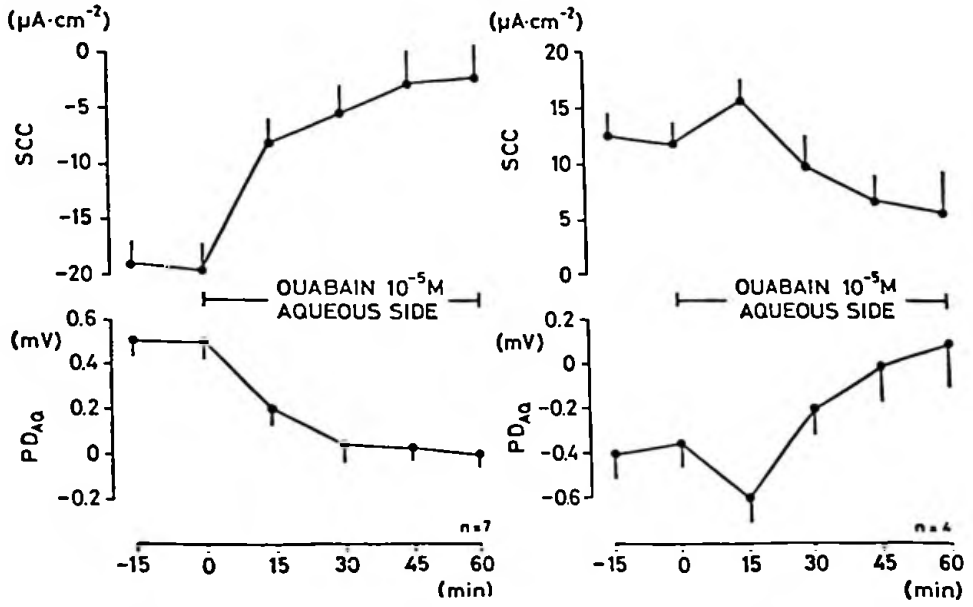


Figure 1. Effect of ouabain on transepithelial potential and short circuit current of the isolated ciliary epithelium of the shark.

Evidence for active chloride transport has been demonstrated in the isolated iris-ciliary body of toad, cat and rabbit. However, in recent experiments on the isolated rabbit preparation neither a significant net chloride transport nor a chloride or furosemide-dependence of SCC could be found (for literature see, Krupin et al., Exp Eye Res 38: 115-123, 1984). In our experiments on shark ciliary epithelium furosemide inhibited both PD and SCC when given to the aqueous side at negative transepithelial PD or given to the blood side at positive spontaneous PD. A biphasic response of PD and SCC to application of the drug was observed at positive PD/furosemide aqueous side and negative PD/furosemide blood side.

The results from transepithelial electrical measurements confirm our conclusion derived from intracellular measurements as to the presence of both a ouabain and a furosemide sensitive mechanism in the shark ciliary epithelium. Concerning the localization of the pumps our data are compatible with the hypothesis postulated for the amphibian (Watanabe and Saito, Exp Eye Res 27:215-226, 1978) and rabbit (Krupin et al., Exp Eye Res 38:115-123, 1984) preparation. In addition, for the ciliary epithelium of the shark we postulate furosemide-sensitive chloride mechanisms. Thus magnitude and net direction of overall transepithelial electrolyte transport might depend on the balance between a cation and an anion mechanisms.

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