Effects of Ouabain and Furosemide on Transepithelial Electrical Parameters of the Isolated Shark Ciliary Epithelium

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Sections of the ciliary epithelium of adult sharks (Squalus acanthias) were mounted in Ussing-type chambers (area 0.2 cm²) for measurements of transepithelial potential difference (PD), short circuit current (SCC) and calculation of transepithelial resistance (R). In 15 preparations PD was aqueous side negative (-0.51 ± 0.12 mV; SCC 18.3 ± 2.5 µA cm⁻²; R 30.7 ± 3.1 Ohm cm²). However, in 15 other preparations incubated in identical Ringer’s solution PD was aqueous side positive (0.53 ± 0.09 mV; SCC -19.6 ± 2.3 µA cm⁻²; R 27.9 ± 2.8 Ohm cm²). 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 negative or positive PD. When the transepithelial PD was positive on the aqueous side ouabain decreased PD and SCC within 15 to 45 min. When the spontaneous PD was negative both PD and SCC decreased when ouabain was applied to the blood side. When the drug was given to the aqueous side a biphasic response (first stimulation, then inhibition) of PD and SCC was observed. Furosemide when given to the blood side (with aqueous side PD positive) or to the aqueous side (with aqueous side PD negative) decreased PD and SCC. However, a transient stimulation of both electrical parameters was observed when furosemide was applied to either the blood side (with aqueous side PD positive) or to the aqueous side (with aqueous side PD positive). The polarity and magnitude of PD and SCC probably depend on the relative activity of sodium and chloride pumps across the cell membranes of the non-pigmented and/or pigmented cell layer. However, additional transport mechanisms cannot be excluded. Invest Ophthalmol Vis Sci 28:1353–1356, 1987

It has been well established that active secretion of fluid plays an important role in the production of aqueous humor. The rate of fluid secretion and the outflow resistance are the principal factors in determining intraocular pressure. Although a number of electrophysiological studies on isolated iris-ciliary body preparations from rabbit, cat, dog, ox, and amphibians have been performed, the basic transport mechanisms remain obscure. Thus, evidence for transepithelial sodium and chloride transport have been presented. More recently, the existence of sodium/potassium pumps in the basolateral membranes of both the non-pigmented and the pigmented cell layer have been postulated. On the other hand, in a similar preparation of the rabbit neither a net chloride transport nor a chloride-dependence of the short circuit current could be demonstrated. Furthermore, the polarity of the transepithelial potential is an open question. In the rabbit, the origin of the PD appears in part to be related to the presence of HCO₃⁻. This paper presents results on the shark ciliary body, which is large enough to allow isolated sheets of the epithelium to be obtained without iris for transepithelial studies. Similarities between the ciliary body of elasmobranch and mammals suggest that the shark preparation may be suited for ciliary epithelial transport studies in general.

Materials and Methods

Eyes from freshly killed adult sharks (Squalus acanthias, spiny dogfish) were enucleated and stored in cold shark Ringer’s solution. The posterior part of the eye was cut off with razor blades and the lens was removed by cutting the two opposite areas of zonula fibers. The ciliary body of the shark is an easily identifiable band of approximately 4 mm wide. Quartered sections of the ciliary body were mounted horizon-
Table 1. Transepithelial potential, short circuit current and resistance of the isolated shark ciliary body

<table>
<thead>
<tr>
<th>PD aqueous side negative</th>
<th>PD aqueous side positive</th>
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<tr>
<td>PD (mV)</td>
<td>0.53 ± 0.09</td>
</tr>
<tr>
<td>SCC (µA cm⁻²)</td>
<td>18.3 ± 2.5</td>
</tr>
<tr>
<td>R (Ohm cm²)</td>
<td>30.7 ± 3.1</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
</tr>
</tbody>
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* Mean ± SEM.

Methods

Figure 1 summarizes mean responses of PD and SCC upon application of 10⁻⁵ M ouabain. At spontaneous positive PD, addition of ouabain to the aqueous side decreased both PD and SCC within 15 min (P < 0.001). On the original recordings (not shown here) an effect of ouabain could be observed already after 3–5 min. A maximal inhibition of the electrical parameters was obtained after 30–45 min. As can be deduced from the parallel changes of PD
and SCC, transepithelial R did not change significantly. At spontaneous negative PD the effect of ouabain was biphasic; SCC and PD transiently increased over a period of some 15 min and then slowly decreased. Such a biphasic behavior upon ouabain was not observed when the drug was applied to the blood side, and at spontaneous positive and negative PD both electrical parameters decreased.

The differential effects of $10^{-4}$ M furosemide are summarized in Figure 2. The most dramatic effect of the drug was observed when given to the aqueous side at spontaneous negative PD. An almost maximal inhibition of PD and SCC could be demonstrated some 15 min after application. On the original recordings an inhibition was already visible after 1–2 min. A similar effect regarding SCC and PD could be obtained when furosemide was added to the blood side at transepithelial positive PD. However, the decrease of the electrical parameters was only some 50%. Quite different results were recorded when furosemide was given to the aqueous side at positive spontaneous PD or to the blood side at negative PD. Under both conditions SCC and PD increased over a period of some 15 min and then levelled off at values similar to those before the administration of the drug.

Discussion

The present study further supports the assumption that transepithelial electrolyte transport across the epithelium of the shark ciliary body occurs via ouabain-sensitive sodium and furosemide-sensitive chloride transport mechanisms.23,24 In several papers the presence of a Na/K-ATPase in the ciliary epithelium has been demonstrated,5,25-27 including in the shark ciliary body.18 The ATPase activity has been localized mainly to the basolateral membrane of the non-pigmented cell layer.25-27 In addition, there is evidence for some ATPase activity in the basolateral membrane of the pigmented cell layer.25-27 From the histochemical localization of the Na/K-ATPase and from the different effects of ouabain on PD and SCC when applied to the aqueous or blood side, respectively, the existence of a double pump mechanism for sodium has been postulated for the rabbit ciliary epithelium.9 In principle, our data in sharks (Fig. 1) are similar to the observations made in the rabbit ciliary epithelium.7,8,9 and can be partially explained by a model of two Na/K transport processes.

Data on the effect of furosemide on ciliary epithelium are more controversial. In the rabbit preparation application of furosemide had only a minor effect on SCC.10 In the dog ciliary body SCC was reduced only when the drug was given to the aqueous side.13 In the epithelium of the toad the inhibition of SCC was much smaller and slower when furosemide was given to the blood side as compared to application from the aqueous side.13 Furthermore, evidence for net chloride transport directed from blood to aqueous side has been demonstrated in the isolated ciliary body of rabbit7,8,9, dog,13 cat,12 and amphibians.14,15 In recent experiments on the isolated rabbit preparation, no chloride transport could be found.9,10 Our results on shark epithelium support the observation that at transepithelial negative PD, furosemide is more active when given to the aqueous side. The biphasic effect of furosemide under certain conditions and the similarity with the biphasic effect of ouabain (furosemide on blood side at spontaneous negative PD and ouabain on aqueous side at negative PD) are indications that two chloride transport processes may exist, one in the pigmented and one in the non-pigmented cell layer. This hypothesis is further supported by the observation that intracellular chloride activity in both non-pigmented and pigmented cells of the shark ciliary epithelium is above equilibrium.

![Fig. 2. Effect of $10^{-4}$ M furosemide on short circuit current and transepithelial potential differences.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933140/)
and is reduced towards equilibrium by application of furosemide. The localization of the proposed chloride transport mechanisms to the apical or basolateral side of both cell layers is intuitive. At present we do not know whether furosemide effects a coupled Na\(^+/\)Cl\(^-\) transporter, an electrogenic transporter, and/or a passive chloride conductive pathway. Furthermore, the model does not include a mechanism for transepithelial bicarbonate transport and neglects the observation that carbonic anhydrase activity has been localized in the ciliary body of the shark. Due to the structural complexity of the ciliary epithelium, interpretation of transepithelial electrical data is limited. Separation of both epithelial layers or cell culture of non-pigmented and pigmented cells could be used as additional tools for the investigation of the transport systems involved in aqueous humor production.

**Key words:** isolated ciliary epithelium, shark, transepithelial electrical parameters, ouabain, furosemide

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**References**