TRANSPORT PROPERTIES OF THE ISOLATED PIGMENTED EPITHELIUM OF THE SHARK (SQUALUS ACANTHIAS) CILIARY BODY

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In previous studies both an ouabain-sensitive sodium transport and a furosemide/bumetanide sensitive chloride transport (Wiederholt et al., Invest. Ophthalmol. 28:1353-1356, 1987; Bull. MDIBL 27:6-8,1987/88) have been demonstrated in the isolated ciliary epithelium of the shark (Squalus acanthias). However, the localization of the transporters to the non-pigmented (NPE) or pigmented (PE) cellular layers has not been possible.

Sections of the ciliary epithelium of adult male sharks were isolated as previously described (Invest. Ophthalmol.28: 1353-1356,1987) and then mechanically stripped to obtain isolated sheets of NPE and PE. In preparations taken for light and electron microscopy it could be seen that the stripped PE layer revealed intact epithelial cells. In particular, the basal third of the PE cells were in very close contact with each other - their attachment zones may have the appearance of tight junctions.

Isolated sections of the ciliary epithelium (containing NPE and PE) and sections of the stripped NPE and stripped PE were mounted in Ussing-type chambers (area 0.1cm^2). Shark Ringer's solution was gassed with 1% CO_2 in air for a final pH of 7.6.

In the intact ciliary epithelium (NPE and PE) addition of forskolin 10^{-5}M (TABLE 1) to the aqueous side resulted in an increase of short circuit current (I_{sc}) and transepithelial voltage (V) as has been shown before (Bull.MDIBL 27:6-8,1987/88). The data are consistent with the assumption that forskolin stimulates chloride transport as has been demonstrated in several chloride secreting epithelia.

In isolated NPE mounted in Ussing-type chambers, we were unable to obtain preparations with transepithelial resistance (R) higher than 10 Ohm cm^2 or which were stable for more than 20 min. This is consistent with the morphological observation that although tight junctions were demonstrable in NPE, the apical membranes were damaged by the stripping method. However, even in the most leaky NPE preparation transepithelial V was always apical side positive as compared to basolateral side.

TABLE 1 Effects of forskolin (10^{-5}M) and bumetanide (10^{-4}M) on transepithelial electrical parameters of isolated shark ciliary epithelium (NPE and PE) and isolated stripped pigmented epithelium (PE).

	I_{sc} ($\mu A \cdot cm^{-2}$)	V (m V)	R (Ω cm^2)	n
PE + NPE				
Control	16.3±1.0	-0.84±0.09	51.4±3.2	7
Forskolin$^+$	31.1±2.1*	-1.53±0.17*	48.9±3.3	
PE				
Control	11.5±2.1	-0.16±0.03	14.2±1.7	
Forskolin$^+$	26.3±1.7*	-0.36±0.03*	14.3±1.7	6
Forskolin + Bumetanide^{++}	14.2±1.5*	-0.19±0.01*	14.5±1.8	

Values are means ± SEM. I_{sc}, short circuit current; V, transepithelial potential difference; R, transepithelial resistance.
$^+$ 45 min after control; $^{++}$ 45 min after forskolin.
* $P < 0.001$ (paired t - test)

As shown in TABLE 1, transepithelial V and R in the isolated stripped preparation of PE were approximately 20-30% of values for intact ciliary epithelium (NPE + PE). The polarity of the PE preparation was always apical side negative. In the stripped PE forskolin increased I_{sc} and V by approximately 100 %, an effect similar to that of forskolin on the intact tissue. Furthermore, forskolin-induced stimulation of I_{sc} and V was blocked by bumetanide.

The data support the hypothesis that in the ciliary epithelium of the shark, the furosemide/bumetanide-sensitive chloride transport is localized to the pigmented epithelium. This transport is modulated by the adenylate cyclase/cAMP system. In preparations of the mammalian ciliary body the existence of such a chloride transport mechanism is highly controversial.

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