THE EFFECT OF FORSKOLIN ON THE ISOLATED CILIARY EPITHELIUM OF THE SHARK, SQUALUS ACANTHIAS.

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In the isolated ciliary epithelium of the shark it has been well established that both sodium and chloride transport mechanisms are involved in generating transepithelial electrical parameters (Wiederholt, M., Zadunaisky, J.A., Invest. Ophthalmol.Vis.Sci. 28: 1353-1356, 1987). The polarity of transepithelial potential (PD) and short circuit current (SCC) depend on the relative activity of sodium and chloride (and additional not yet defined) pumps across the cell membranes of the non-pigmented and pigmented cell layer. Forskolin, which directly activates adenylate cyclase and thus increases intracellular cyclic AMP, has recently been shown to lower intraocular pressure by reducing secretion of aqueous humor (Caprioli, J. et al., Invest. Ophthalmol. Vis.Sci.25: 268-277, 1984).

Isolated sections of the ciliary epithelium of adult male sharks were mounted in Ussing-type chambers (area 0.1 or 0.2cm^2) as described before (Wiederholt, M. and Zadunaisky, J.A., Invest. Ophthalmol.Vis.Sci.28: 1353-1356, 1987). Shark Ringers was gassed with 1% CO_2 in air for a final pH of 7.6. Both parts of the Ussing-chambers were perfused at a rate of 1 - 3ml/min. Forskolin and bumetanide were dissolved in ethanol (final concentration 0.04%).

The addition of forskolin (10^{-6} and 10^{-5}M) to the aqueous side of the ciliary epithelium resulted in an change of SCC of some 20 to 40% (Fig.1). Forskolin in concentrations of 10^{-5}M was ineffective when added to the solution bathing the blood side. Forskolin changed SCC when either SCC (and transepithelial PD) at the beginning of the experiment was positive or negative. Under both conditions forskolin changed SCC (and PD) to less positive/more negative values, compatible with a stimulation of transepithelial anion transport or inhibition of cation transport. Since the ciliary body of the shark possesses an active, furosemide-sensitive chloride transport mechanism (Wiederholt, M. and Zadunaisky, J.A., Pflügers Arch.407 (Suppl.2): S112-S115, 1986) it is tempting to speculate that forskolin stimulates chloride transport as has been demonstrated in other chloride secreting epithelia such as the rabbit iris-ciliary body (Chu, T.E. et al., Current Eye Res. 5: 511-516, 1986), and the rectal gland of the shark (Greger, R. and Schlatter, E., Pflügers Arch. 402: 63-75, 1984).This assumption is further supported by the observation (Fig.1B) that the response in SCC to forskolin was completely reversed by bumetanide, a substance which similarly to furosemide is a potent inhibitor of chloride secretion.

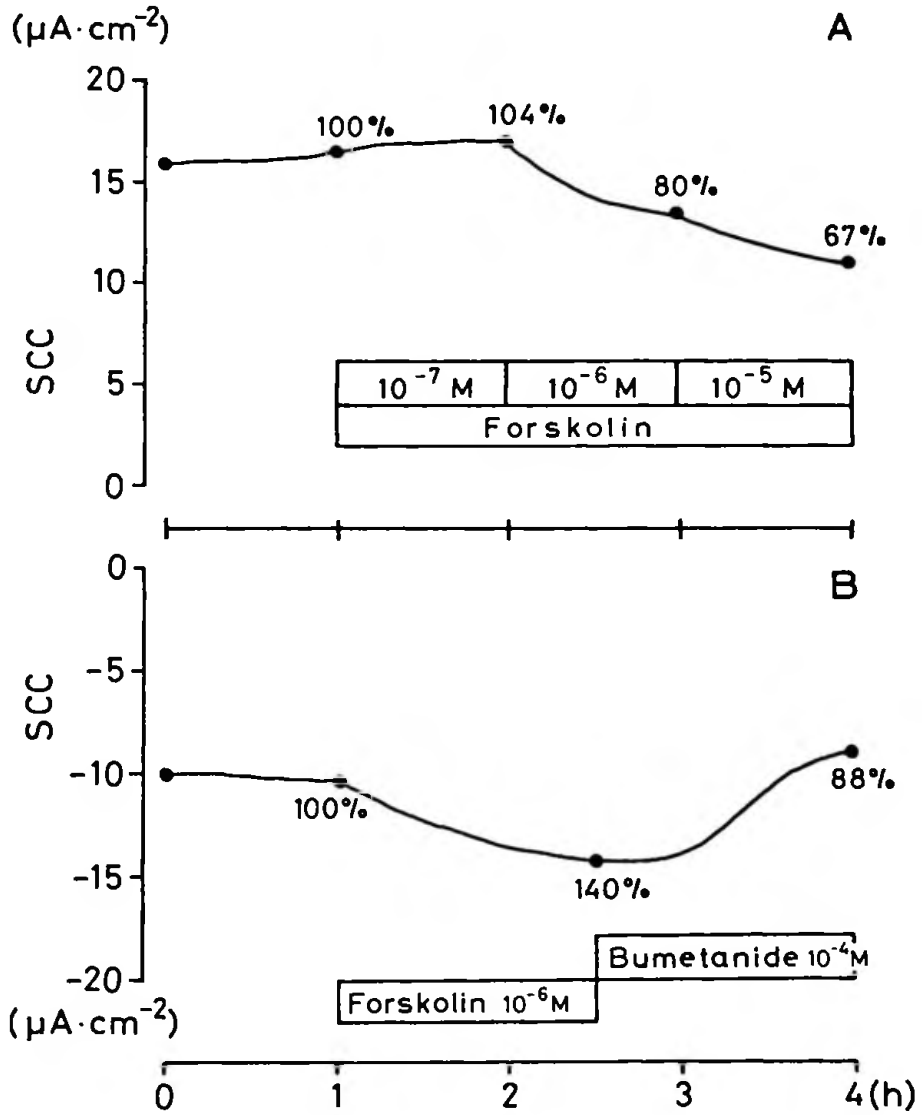

Figure 1: Original recordings of the SCC response of the isolated ciliary epithelium to application of forskolin (aqueous side) or bumetanide (aqueous and blood side). Positive SCC denotes spontaneous SCC (and transepithelial PD) aqueous side positive versus blood side. Negative SCC indicates aqueous side negative versus blood side. Similar effects of forskolin and bumetanide were obtained in 3 experiments. It has been shown before (Invest.Ophthalmol. Vis.Sci.28:1353-1356, 1987) that without application of drugs SCC (and PD) can be kept constant for up to 4 hours.

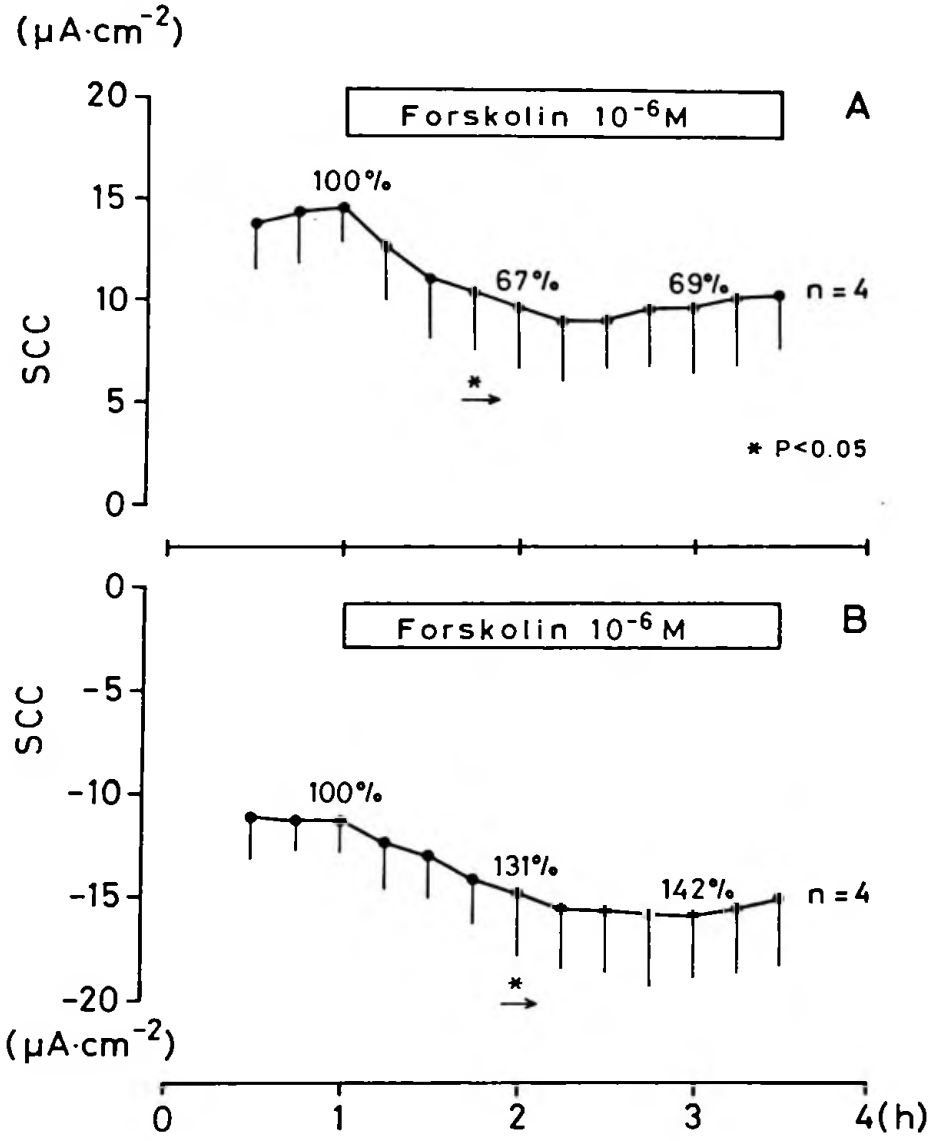

Figure 2: Effect of forskolin (aqueous side) on SCC at spontaneous positive or negative electrical parameters. Mean values +SEM.
*Significantly different from values at 100%, P<0.05.

Fig.2 summarize mean responses of SCC upon application of 10^{-6} M forskolin to the aqueous side. At spontaneous positive (Fig.2A) or negative SCC (Fig.2B) the mean change of SCC amounted to 30 to 40%. There was a similar change in PD (from + 0.65mV to + 0.40 mV, and from -0.65 mV to -0.84 mV) while R (range 50-61Ωm^2) was constant.

The data support the the hypothesis that in the ciliary epithelium of the shark the adenylate cyclase system is present and may be involved in transepithelial ion transport via increase of intracellular cAMP.

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