SITE AND MECHANISM OF ENDOLYMPH PRODUCTION IN SQUALUS ACANTHIAS

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The inner ear of the elasmobranch, Squalus acanthus, is ideally suited for the study of endolymph secretion. The canals were readily removed from the surrounding cartilage. Segments of canals were cannulated and used for determination of electrical propoperties or fluid transport. The segments of semicircular canals used for these experiments ranged in inside diameter from 300 to 650 u, and in length from 8.3 to 22.5 mm. To investigate the electrical characteristics of the semicircular canals, canals were mounted in a chamber in such a manner that the lumen of the canal could be perfused and the bath exchanged. A coaxial Ag/AgCl wire served as the luminal electrode and the bath was grounded with a second Ag/AgCl wire. The transepithelial potential difference (PD) was 0.8 mV, lumen positive with respect to bath, when canals were filled and bathed with shark Ringer. The electrical resistance was 37.0 ohm-cm^2 under the same conditions calculated from the voltage deflections induced by current clamping the tissue with 0.5 uA pulses which were symmetrical about zero. Dilution potential experiments, in which the NaCl concentration of the bath or perfusate was reduced, showed that the epithelium was selective for Na over Cl (P_{Na}/P_{Cl} = 0.98) when compared to free solution (P_{Na}/P_{Cl} = 0.66) It is likely that this selectivity is due to the paracellular pathway since: a) in a low resistance tissue the characteristics of the paracellular pathway dominate the characteristics of the epithelium; and b) the selectivity was the same whether the gradient was oriented from bath to lumen or lumen to bath. Biionic potential experiments, in which a 30 fold Na gradient and a 30 fold K gradient oriented in opposition were created, showed that the paracellular shunt was selective for Na over K (P_{Na}/P_{K} = 0.93) when compared to free solution (P_{Na}/P_{K} = 0.68). Transepithelial PD was unaffected by 0.1 mM amiloride (a Na channel blocker) or 1 mM DIDS (a Cl-HCO_3 exchange inhibitor), but hyperpolarized to -1.1 mV when 105 mM K Ringer (simulated endolymph) replaced normal Ringer in the lumen.

Fluid secretion was demonstrated by introducing a test droplet, isolated between columns of stained mineral oil, into the canal lumen. At the end of an experiment the droplet was collected for analysis. The rate of fluid secretion was calculated from the increase in droplet length and cross sectional area of the canal. When lumen and bath contained normal Ringer, the rate of fluid secretion was 0.33 ul/cm^2.min (or 5.56 x 10^{-6} cm/sec). The rate of fluid secretion was not altered when the lumen contained 105 mM K Ringer, normal Ringer plus barium (a K channel blocker), or when 10 uM forskolin (which stimulates adenylate cyclase) was added to the bath. Addition of the Na,K-ATPase inhibitor, ouabain (10^{-4} M), or a carbonic anhydrase inhibitor, methazolamide (10^{-4} M), to the bath reduced the rate of fluid secretion. Addition of a Na/K/Cl cotransport inhibitor, bumetanide (10^{-5}), to either the lumen or bath abolished fluid secretion. Analysis of collected droplets showed that K and Cl had entered the lumen while Na was absorbed under control conditions.

The morphology of the canals was investigated by both light and electron microscopy. A thickened ridge of epithelium exists along the length of the canal; this ridge may be analogous to the stria vascularis the site of endolymph secretion in the mammalian membranous labyrinth.

The semicircular canals of the shark apparently use the cotransport of K, Cl and possibly Na to drive fluid secretion. This process is dependent upon a

functioning Na/K ATPase. Fluid secretion also apparently involves either proton or bicarbonate transport since methazolamide inhibited fluid secretion. Carbonic anhydrase activity is associated with transport of these ions in other epithelia. Although the paracellular shunt is selective for Na over K, active Na absorption must occur as the electrochemical gradient for Na favors its entry into the lumen. The lack of an effect of forskolin indicates that fluid secretion is not regulated by cyclic AMP. The lack of an effect of amiloride, DIDS and barium show respectively that diffusional Na entry, $Cl\text{-}HCO_3$ exchange and barium sensitive K channels are not likely to be involved in transepithelial ion movement but further investigation is needed. This low resistance, fluid secreting tissue should serve as a simple model for the study of endolymph production. (Supported by a grant from the Lucille P. Markey Trust.)