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POTENTIAL DIFFERENCE AND FLUID TRANSPORT ACROSS RABBIT CORNEAL ENDOTHELIUM

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SUMMARY

An electrical potential difference of 0.5 mV (blood side positive) has been found across rabbit corneal endothelium in addition to the reported transport of fluid. Such inhibitors as $5 \cdot 10^{-5}$ M ouabain, 2 mM cyanide and 2 mM iodoacetate, which are known to suppress fluid transport, were found to abolish the potential difference. Both fluid transport and potential difference are depressed reversibly either in the absence of HCO_3^-, Na^+, or K^+ or in the presence of cytochalasin B. The suggestion is made that in this system the potential difference is associated to the fluid transport, and some possible mechanisms for this association are discussed.

Many such epithelial tissues as frog skin, gastric mucosa and urinary bladder which transport water and electrolytes are known to display electrical potential differences of tens of millivolts across their cellular layer[1-3]. This potential difference is related to and serves as an index of that transport[2,4]. It is also known that other such epithelial preparations as gall bladder, kidney proximal tubule and rabbit corneal endothelium which also transport fluid have so small potential differences (0–10 mV) that the measurement of these has given rise to discrepancies (*cf.* ref. 5). This last group of tissues are generally classed apart from the rest of the epithelia and their transport systems are referred to as being "electrically neutral"[6,7]. In the present communication, however, the rabbit corneal endothelium has been found to generate unequivocally a potential difference. Even though comparatively small (0.5 mV), this potential difference clearly varies in the same way as the rate of fluid transport across this tissue when both are subject to comparable experimental procedures. These findings point to the need for a re-examination of the evidence and a re-definition of the criteria by which transport systems are labelled electrically neutral. They also raise the possibility that some of the mechanisms underlying transport and the generation of an electrical potential are common to all epithelia.

The technical demands imposed by the determination of the small d.c. potential difference required separate measurements of potential difference and rate of fluid transport. For the measurement of the potential difference, corneas were scraped free of epithelium, and the endothelium, supported by the stroma, was mounted in a chamber and bathed on both sides with a solution modified from ref. 8, which contained (in mM) NaCl 110, $KHCO_3$ 3.8, $NaHCO_3$ 39, $MgSO_4$ 0.8, KH_2PO_4 1.0, $CaCl_2$

1.7, glucose 6.9, adenosine 5.0 and glutathione 0.24 mM, and which was adjusted to pH 7.4 with CO_2. Electrical contact with the solutions was made through movable agar–saline bridges prepared with the same solution used in the chamber. The drift of these bridges was less than 50 μV per hour and was compensated by means of a series battery frequently during the experiments. The potential difference was measured with an electrometer (Keithley 610C); the error of individual readings was $\pm$ 10 μV. The temperature was 37 °C and there was no hydrostatic pressure difference across the tissue.

Fluid transport across the endothelium was measured following procedures developed earlier[8–10] and modified for the present purposes. The corneas were again scraped free of epithelium, mounted in a chamber and bathed on both sides with the solution described above. The corneal stroma swelled by imbibition of fluid through its outer (epithelial) surface, but at a given time the swelling was arrested by replacing the outside solution with silicone oil. The subsequent variations in stromal thickness thus corresponded to fluid movements across the endothelium[11]. The thickness of stroma (around 350–400 μm when fresh) *plus* endothelial cells (3 to 5 μm) was measured by the excursion of a microscope focused on the tissue–fluid interfaces[8,9]. Temperature was 37 °C and a pressure head of 20 cm H_2O was applied to the inner (endothelial) side.

The potential difference across the endothelium was 500 $\pm$ 37 μV (inside negative; S.E., $n = 32$) and ranged from 200 to 900 μV. It was usually stable for 3–4 h and then decreased nearly to zero after 5–6 h; it could be transiently decreased by wrinkling the tissue and was immediately eliminated by scraping off the endothelium. The remaining stroma had a negligible potential difference (0–30 μV). The

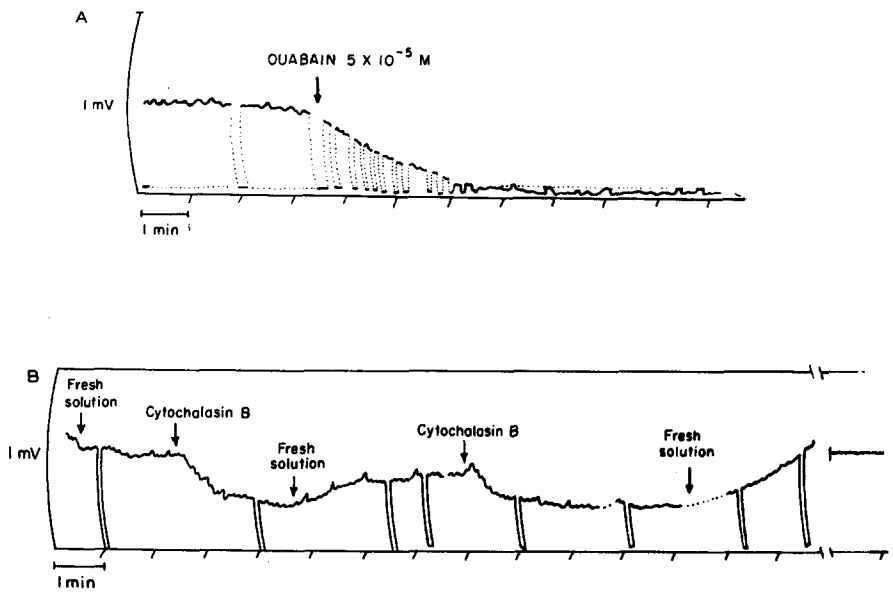

Fig. 1. Effects of ouabain $5 \cdot 10^{-5}$ M (A) and cytochalasin B 20 μg/ml (B) on the electrical potential difference across the endothelium. The curves were traced from the recorder's chart; the plots are intercepted by periodic calibrations of the baseline. The lower dotted line (A) denotes the baseline.

potential difference and the fluid transport across the endothelium were affected similarly by inhibitors and by ambient ionic substitutions. Among the inhibitors, $5 \cdot 10^{-5}$ M ouabain abolished the potential difference very swiftly (Fig. 1, representative of four experiments). Cytochalasin B also had an immediate effect in decreasing the potential difference, which was seen at all concentrations tried (1–20 μg/ml), was reversible and could be obtained several times during the same experiment. An example (representative of five experiments) is shown in Fig. 1. Cyanide and iodoacetate, both $2 \cdot 10^{-3}$ M, acted more slowly, and abolished the potential difference in 1–1.5 h. Several ionic substitutions affected the potential difference in a reversible

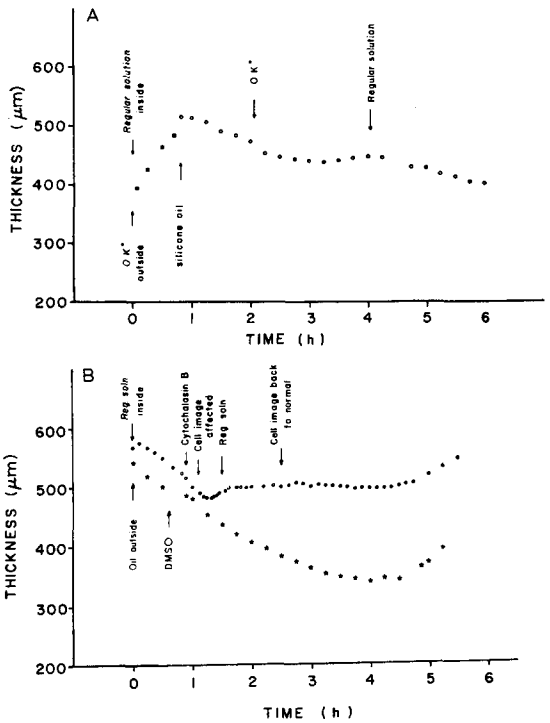

Fig. 2. Effects of the absence of K^+ and of cytochalasin B on the transport of fluid across the endothelium. Individual thickness readings accurate to $\pm$ 2 μm. A: The corneal stroma, denuded of its epithelium, swelled by imbibition of the outside solution; after this solution was replaced by oil, subsequent readings corresponded to fluid movements across the endothelium. The rate of fluid transport across the endothelium was reversibly affected by changing the solution perfusing the inside to one that was K^+ free; the delay was due to chamber dead volume. B: ○, (test) and *, (control) are readings taken from two corneas of one rabbit. The two experiments were run at the same time. The corneas were denuded and oil covered the outside. The intervals during which swelling was induced are omitted for clarity. Cytochalasin B 20 μg/ml (solubilized in dimethylsulfoxide (DMSO) at 25 mg/ml) was perfused at the arrow; after the microscopic images of the endothelial cells showed alterations, the fluid transport was impaired. Upon return to regular solution a long period of washing was required for the image of most endothelial cells to return to normal; this incomplete recovery was reflected by the subsequent transport of fluids which took place at a low rate. At the arrow below the control curve a solution with a concentration of dimethylsulfoxide equal to that used in the test experiment was perfused without ill effects. After 5–6 h of perfusion *in vitro*, both preparations deteriorated.

way; total replacement of HCO_3^- by Cl^-, of Na^+ by Li^+ and of K^+ by Na^+ abolished the potential difference in 1–2 min.

All the experimental procedures just described either were known or have been presently found to affect the fluid transport. Ouabain, cyanide and iodoacetate are known to suppress it[8,12]. Cytochalasin B was seen in five experiments to impair it in a partially reversible way which is exemplified in Fig. 2. The drug caused a marked change in the microscopic image of the endothelial cells which became corrugated. This morphological change was largely reversible upon return to perfusion with fresh solution, but a few cells, which appeared to have been damaged, might account for the incomplete recovery of the transport. Replacement of HCO_3^- by Cl^- and of Na^+ by Li^+ arrests fluid transport (ref. 8, 13; also J. Fischbarg, unpublished); replacement of K^+ by Na^+ was presently seen to produce an analogous effect (Fig. 2, representative of seven experiments).

The present results show the existence of a small electrical potential across the corneal endothelium. They also show that this potential, as well as the transendothelial fluid transport, are decreased or abolished in a similar fashion by several ionic substitutions and by inhibitors; this behavior suggests an association between the potential difference and the fluid transport. There are several possible transport mechanisms which would explain this association. The endothelium might be the site of an ionic pump in parallel with a large "leak" pathway; the solvent would follow the ionic movements. The value of the potential difference is within an order of magnitude consistent with this possibility, since 500 μV across the endothelial resistance of some 50 $\Omega \cdot cm^2$ (ref. 14) represent an ionic current of $\pm$ 0.4 μequiv/h per cm^2, while the fluid flow observed (e.g. $\simeq$ 40 μm/h or 4 μL/h per cm^2 in Fig. 2), if assumed isotonic, corresponds to some 0.6 μequiv/h per cm^2 of net monovalent ion transfer. The fast effect of ouabain on both potential and transport may be taken as an indication that such pump could be electrogenic, while the slower effects of cyanide and iodoacetate are consistent with a metabolically dependent mechanism. In a similar vein, the effects of the ionic substitutions on potential difference and fluid transport might reflect a deprivation of the ionic substrates possibly being transported (Na^+, HCO_3^-) or an interference with the transport mechanism (K^+-free solutions). Yet, all this indirect evidence is not unequivocally matched by other lines of evidence. In this preparation the junctions at the apical end of the intercellular spaces are permeable to horseradish peroxidase[15]; also, when the stroma is made to swell, the fluid dilates the basal end of the intercellular spaces, which subsequently regain their normal width after several hours in which fluid transport takes place (G. K. Smelser and J. Fischbarg, unpublished.) The intercellular spaces may hence be a route for fluid movement. Cytochalasin B inhibits cellular movements in other systems[16,17]. In the present one, its mechanism of action is unclear so far; it could simply have an indirect effect on both fluid transport and potential difference, or it could inhibit peristaltic movement of the intercellular spaces such as those which have been hypothesized to underlie fluid movements in this preparation[18]. In this last scheme, the potential difference would not correspond to an electrogenic ionic pump, but would be rather explained as a streaming potential induced by fluid flow across a matrix with fixed charges. Further evidence is needed to establish which of these mechanisms is responsible for the potential difference and the fluid transport observed.

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