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SODIUM ACTIVATION OF CHLORIDE TRANSPORT IN THE FROG CORNEA

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SUMMARY

Na^+ is required on the aqueous or endothelial side of the isolated frog cornea (*Rana catesbeiana*) for Cl^- transport to occur. The activating effect of Na^+ on the Cl^- pump saturates at a Na^+ concentration of about 40 mM and the Na^+ acts as a non-competitive activator of Cl^- transport. The Na^+ stimulation occurs by an increase in the active component of the Cl^- flux.

In previous publications[1-3] it was shown that the isolated cornea of the American bull-frog *Rana catesbeiana*, when placed as a membrane in Ussing-type chambers, transports Cl^- from the aqueous or endothelial side towards the epithelial or lacrimal side. This Cl^- transport, located in the epithelium, controls the hydration and transparency of this cornea[3]. It was found then that Na^+ was required in the bathing fluids for the normal current or potential difference maintained by the cornea to manifest itself fully. The presence of Na^+ was specifically required on the aqueous side of the corneas[1]. Changes of Na^+ concentration on the epithelial side failed to modify the electrical properties of this preparation[1]. In order to obtain more information about this activation, the fluxes of Cl^-* were measured at a Na^+ concentration of 10 mM in

TABLE I

ACTION OF INCREASED SODIUM CONCENTRATION ON CHLORIDE TRANSPORT BY ISOLATED FROG CORNEAS

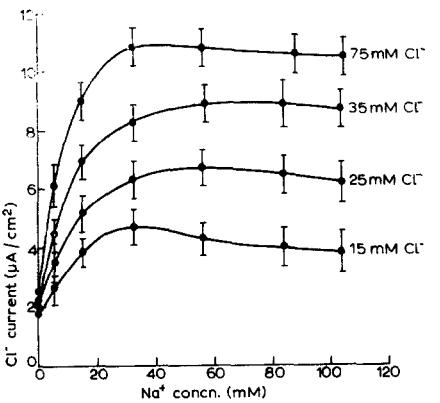
Unidirectional fluxes of $^{36}Cl^-$ measured at the two Na^+ concentrations on the same cornea. Values are mean ± S.E. of results for five corneas for each unidirectional flux. Methods as described in ref. 1.

	10 mM Na^+ in bathing solutions ($\mu equiv \cdot h^{-1} \cdot cm^{-2}$)	*57 mM Na^+ in bathing solutions* ($\mu equiv \cdot h^{-1} \cdot cm^{-2}$)	*Increase* (%)
Aqueous to lacrimal flux	0.436 ± 0.083	0.535 ± 0.077	22.7
Lacrimal to aqueous flux	0.213 ± 0.013	0.233 ± 0.040	9.4
Net Cl^- flux	0.223	0.302	35.4
Short-circuit current	0.206 ± 0.021	0.283 ± 0.021	37.4

* The methods utilized are described in refs 1–3. In all cases choline was used to replace Na^+, and SO_4^{2-} to replace Cl^-, in equimolar amounts. Osmolarity was compensated with sucrose, adjusting all solutions to the same value of 240 mosM.

the bathing solutions, and subsequently at a Na^+ concentration of 57 mM in the same corneas. As will be seen later, the latter concentration is above the saturation value for the activating effect of Na^+ on Cl^- transport. Table I shows the results obtained in ten corneas, five in which $^{36}Cl^-$ influx and five in which $^{36}Cl^-$ efflux was measured at the two Na^+ concentrations, keeping the total Cl^- concentrations at the normal value of 75 mM. It can be observed that the increase in the net flux, with increasing Na^+ concentration, is due to an increase of the aqueous to lacrimal flux of Cl^-, while the passive flux from the epithelial to endothelial side remains almost unchanged. The increase in the net Cl^- flux is reflected in a similar increase in the short-circuit current. This increase in current most probably eliminates the possibility of activation by co-transport in this system. Once it was established that the effect of Na^+ on the short-circuit current of the frog corneas was the consequence of an activation of the Cl^- transport, the kinetics of this activation were studied. In order to determine if the Na^+ effect was produced at the site of Cl^- transport, or acted upon a more distant metabolic region, the experiments shown in Fig. 1 were performed. Several Na^+ concentrations (0, 5, 15, 32, 56, 84 and 104 mM) were tested on each corneal preparation and the short-circuit current recorded continuously. The concentration of Cl^- was also changed (using 15, 25, 35, and 75 mM) but kept constant for each series of Na^+ changes. The same solution was applied to both sides of the cornea in order to avoid diffusion potentials. The resulting effects on the short-circuit current seen in Fig. 1 show that the stimulatory effect of Na^+ saturates when the Na^+ concentration in the medium reaches between 30 and 40 mM, regardless of the concentration of Cl^-. The saturation level of the short-circuit current depends exclusively on the Cl^- concentration in the medium, the higher the Cl^- the higher the Cl^- current, at any given Na^+ concentration.

The data of these experiments were treated as shown in Fig. 2. In a previous



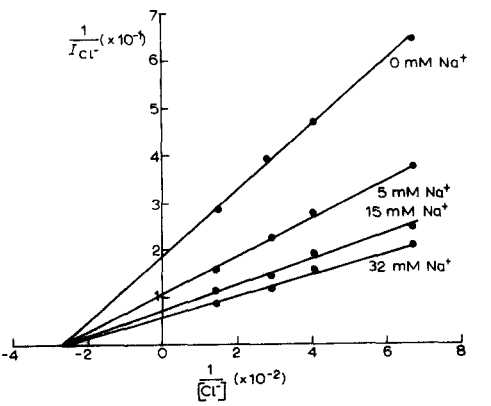


Fig. 1. Saturation curves showing Cl^- current *versus* Na^+ concentration in the medium bathing isolated frog corneas. Note that the level of saturation depends on the Cl^- concentration of the medium while Na^+ has an activating effect with a maximum at about 40 mM. Each curve is the mean of five corneas. The vertical bars represent the S.E.

Fig. 2. Reciprocal plotting of Cl^- current (I_{Cl}) *versus* Cl^- concentration in the medium at several Na^+ concentrations. The curves have the same intercept on the abscissa and this is interpreted as an indication of activation by Na^+ at a site or metabolic region different from the Cl^- site on the carrier. Means for five corneas each line.

paper[1] it was demonstrated that the Cl^--transporting system of the frog cornea followed Michaelis–Menten-type kinetics. Here the data were treated as for the case of an affinity-type activation of the substrate[3,4]. When the initial velocities of transport from Fig. 1 were plotted against Cl^- concentration in a reciprocal manner, a family of straight lines were obtained, each line representing a different Na^+ concentration. Fig. 2 shows that the lines have a common intercept at the level of the abscissa and different intercepts at the level of the ordinate. Extrapolating from enzyme kinetics, it is clear that the action of Na^+ on the Cl^- transport is to change the initial velocity of transport but not the half-saturation constant (K_m) of Cl^-, the substrate, with its enzyme, the carrier responsible for the transport. In other words, Na^+ does not behave as a competitor with respect to Cl^-, and does not affect the carrier enzyme at the site where Cl^- is presumably attached.

The conclusions from previously published work and the results presented here are that Na^+ is required on the aqueous or endothelial side of the corneas, that its activating effect saturates at about 40 mM, and that it does not compete directly at the site of Cl^- transport. It seems appropriate to mention here that in all of the experiments reported in this paper, the short-circuit current is equivalent to the transport of Cl^- (refs 1–3,5).

Evidence has been provided for the existence of a slight Na^+ transport in the cornea of *Rana catesbeiana*[6] in the opposite direction to the Cl^- transport at lower pH. This mechanism does not appear to be related to the Na^+ activation of Cl^- transport because the Na^+ is required on the endothelial or aqueous side of the corneas. Changes of Na^+ concentration on the epithelial side only are unable to produce changes in Cl^- transport[1]. Under similar conditions, Na^+ concentration affects also Cl^- permeability of the frog cornea while ouabain inhibits the Cl^- pump (O.A. Candia, personal communication).

It is likely that the Na^+ concentration in the cells of the epithelium is responsible for the activation effect[7]. If this were the case, then the level of Na^+ in the epithelial cells of the cornea, controlled presumably by a Na^+ pump as in any other tissues, would be important in the regulation of Cl^- transport and in the light-transmission characteristics of the frog cornea.

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