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Hypotonicity increases apical membrane Cl^- conductance in *Necturus* enterocytes

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The effect of hypotonicity on epithelial cells of *Necturus* small intestine has been studied using conventional and Cl^--selective microelectrodes. Exposure to a mucosal solution made hypoosmotic by 70 mosmolal provokes a rapid dilution of intracellular Cl^-, consistent with a perfect osmometer behaviour of the cell. The swollen cells showed an increased apical membrane Cl^- conductance. The increased Cl^- conductance might be involved in regulatory volume decrease.

Epithelial cells in the small intestine of *Necturus maculosus* have recently been shown to have a conductance to Cl^- which can dominate the ionic selectivity of the apical membrane [1]. This conductance is modulated by cyclic AMP and has been proposed to be the permeability pathway activated during sodium-coupled transport of solutes and during secretagogue-induced fluid secretion [1,2]. An increase in electrodiffusional membrane permeability to Cl^- has also been shown to take place in other cell types as a consequence of cell volume expansion, during what has been termed regulatory volume decrease [3]. In the present report we have used intracellular microelectrodes to investigate whether *Necturus* apical membrane Cl^- conductance can be regulated by cell swelling. Our data show that apical Cl^- conductance is effectively activated when enterocytes are swollen in hypotonic medium.

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A continuous recording of the apical membrane potential, E^m, of a *Necturus* enterocyte is shown in Fig. 1. Replacement of all mucosal Cl^- by gluconate produced a fast depolarisation which, as it has been shown elsewhere [1], reflects the presence of a Cl^- conductance in the apical membrane. Decreasing the osmolality of the mucosal solution by 70 mosM evoked a hyperpolarisation. Under hyposmotic conditions, the replacement of Cl^- produced a depolarisation which was larger than in normal, isotonic Ringer (50 compared with 33 mV). This change in the size of depolarisation corresponds to an increase in the relative Cl^- permeability of the mucosal membrane, β, from 2.7 to 6.3 (see legend to Table I for calculation of β). In these experiments the tissue was short-circuited except for short periods in which transepithelial potential, E^t, was clamped at 10 mV. The deflections produced on E^m are proportional to the apical membrane resistance, and the ratio $\Delta E^m/\Delta E^t$ can be taken as a measure of the fractional resistance of the mucosal membrane, fR^m. In hypotonic Ringer the size of the deflections in the E^m trace was reduced, suggesting that in this

situation the conductance of the mucosal membrane had been increased. A summary of the effects of hypotonicity on electrical parameters is shown in Table I. Hypotonicity produced, on average, a 10 mV hyperpolarisation and a fall in fR^m by about 20%. This occurred with a mean time constant of about 14 s. The relative Cl^- permeability of the mucosal membrane, β, calculated from the size of the depolarisation in $0Cl^-$, increased by a factor of 2.2 in hypotonic medium. A reasonable explanation for this change in selectivity associated with a fall in fR^m is that it reflects an increase in the mucosal permeability to Cl^-. This increase might be underestimated if a parallel increase in K^+ permeability occurred [4].

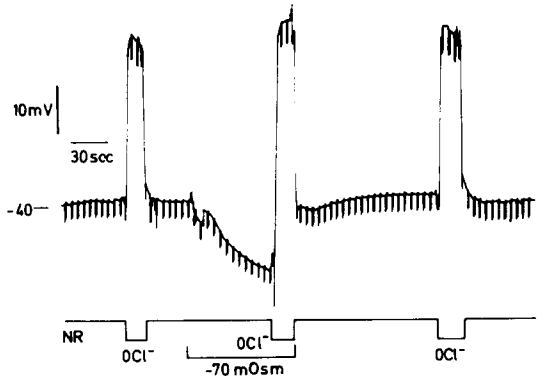

Fig. 1. Effect of hypotonicity on apical membrane potential and Cl^- selectivity of *Necturus* enterocytes. The record starts with a conventional microelectrode inside the cell. For the periods indicated at the bottom the mucosal solution was changed from normal Ringer (NR) to one in which all chloride was replaced by gluconate ($0Cl^-$). Also indicated is a period during which the solution was made hyposmotic by 70 mosM. A segment of the upper third of *Necturus* small intestine was stripped of underlying muscle layers and mounted in a modified Ussing chamber. The electrical arrangement and microelectrode technique were as described previously [1]. The epithelium was short-circuited except for brief periods during which the transepithelial potential was clamped at 10 mV. The composition of normal Ringer was, in mM: NaCl 70, KCl 2.5, $CaCl_2$ 1, $MgCl_2$ 1, $KHCO_3$ 0.5, D-mannitol 95 and Hepes-Tris 5 (pH 7.2). Hypotonic solutions were prepared by removing 70 mM D-mannitol. In $0Cl^-$ solutions all chloride salts were replaced by equimolar amounts of the corresponding D-gluconate salts except for calcium gluconate which was used at a concentration of 10 mM to achieve the same free Ca^{2+} concentration as in chloride-containing solutions [14]. Osmolality was kept at the desired value by removal of the appropriate amount of D-mannitol. Osmolalities measured by freezing point depression were 244 ± 2 and 175 ± 2 mosmol per kg H_2O for the isosmotic and the hyposmotic solutions, respectively. All experiments were conducted at room temperature.

TABLE I

EFFECT OF HYPOTONICITY ON MEMBRANE POTENTIAL AND RESISTANCES

E^m is the membrane potential and fR^m the fractional resistance of the mucosal membrane measured as $\Delta E^m/\Delta E^t$ for constant transepithelial current pulses. $(\Delta E^m)_{0Cl}$ is the depolarisation of the mucosal membrane following the removal of Cl^- from the mucosal medium, corrected for the liquid junction potential at the reference electrode. β is the chloride to potassium permeability ratio, P^m_{Cl}/P^s_K, and it was calculated according to

$$\beta = \left(C^{out}_{Cl}/C^c_K\right) \exp\left(zF(\Delta E^m)_{0Cl}/RT\right) - C^{out}_{Cl}/C^c_K$$

where C stands for concentration of the indicated ion either in the cellular compartment (c) or the bathing solution (out) (see Ref. 1). Values are means $\pm$ S.E. of seven experiments for E^m and fR^m and five experiments for $(\Delta E^m)_{0Cl}$. Hypotonic values were significantly different from isotonic values by paired t-test ($P < 0.01$ except for fR^m, $P < 0.05$). Data were obtained from continuous recordings in experiments where full recovery of the initial state was achieved.

	E^m (mV)	fR^m	$(\Delta E^m)_{0Cl}$ (mV)	β
Isotonic	-31.3 ± 1.9	0.25 ± 0.03	34.8 ± 2.8	3.1 ± 0.5
Hypotonic	-41.6 ± 3.2	0.20 ± 0.03	48.0 ± 4.7	6.8 ± 1.3

The rapid hyperpolarisation of E^m upon rendering the mucosal side hypotonic is itself of some interest. It could be caused in principle by an increase in K^+ permeability. This, however, does not seem to be the case as a similar hyperpolarisation is observed in the presence of serosal Ba^{2+} which is known to block K^+ channels in *Necturus* enterocytes [4,5]. A change of permeability to Na^+ can also be ruled out because the selectivity of the membrane to Na^+ remained low during hypotonicity as revealed by Na^+-removal experiments (not shown). Finally the observed increase in Cl^- permeability would produce the opposite effect on membrane potential because Cl^- is accumulated above its electrochemical equilibrium in *Necturus* enterocytes (see below and Ref. 6). A plausible explanation for this hyperpolarisation can however be devised from the known fact that the mucosal membrane is highly

TABLE II

EFFECT OF HYPOTONICITY ON MEMBRANE POTENTIAL AND CHLORIDE ACTIVITY

E^m is the membrane potential and a^c_{Cl} the intracellular chloride activity measured with double-barrelled Cl-selective microelectrodes. Double-barrelled Cl^--selective microelectrodes were made according to the method described by Zeuthen [15] and filled with Corning 477913 liquid ion exchange (see also Refs. 1 and 2). E_{Cl}, the equilibrium potential for Cl^-, was calculated from $E_{Cl} = (RT/zF)\ \ln(a^{out}_{Cl}/a^c_{Cl})$. The initial rate of change in intracellular chloride activity after replacement of the mucosal medium by hypotonic solution is given (da^c_{Cl}/dt). Values are means ± S.E. of eight experiments in which continuous measurements with full recovery were obtained. All values in hypotonic solution were significantly ($P < 0.01$) different from isotonic values as judged by paired t-test.

	E^m (mV)	a^c_{Cl} (mM)	$E^m - E_{Cl}$ (mV)	da^c_{Cl}/dt (mM·min^{-1})
Isotonic	−33.9 ±1.7	20.0 ±1.1	6.6 ±1.6	-
Hypotonic	−40.1 ±1.5	14.4 ±1.1	4.2 ±1.7	10.5 ±1.2

selective to Cl^-, on the assumption that hypotonicity leads to cell swelling and consequent dilution of intracellular contents. The cell being highly permeable to Cl^-, any decrease in intracellular Cl^- concentration produced by the increase in cell volume in hypotonic Ringer, should then produce a cell hyperpolarisation since E^m follows E_{Cl}. If this were the case, the change in E^m should parallel a simultaneous decrease in the intracellular Cl^- activity, a^c_{Cl}. This was tested by measuring E^m and a^c_{Cl} with double-barrelled, Cl^--selective microelectrodes during hypotonic replacement of the mucosal medium. Table II shows that the membrane potential measured by the reference barrel of the microelectrode detected a hypotonicity-linked hyperpolarisation of similar magnitude as that measured with conventional single-barrelled microelectrodes. The average value of a^c_{Cl} in isotonic medium was similar to that reported before for *Necturus* enterocytes [6] and shows that Cl^- is above electrochemical equilibrium by about 7 mV. Hypotonic challenge produced a decrease in a^c_{Cl}, which in the steady-state was about 30%, and which occurred with a time course similar to the change in E^m (time constant 10–15 s). Restoring the tonicity led to an increase in a^c_{Cl} to control values with a similar time course. Recovery of E^m also occurred in parallel. This concomitant change in a^c_{Cl} and E^m suggests very strongly that the hyperpolarisation is due to the fall in a^c_{Cl} after cell dilution. It is interesting to note that the fall in a^c_{Cl} is very similar to that predicted for a cell that behaves like a perfect osmometer when exposed to a solution whose osmolality has been reduced by 70 mosM, i.e. a 28% dilution. As already observed in other leaky epithelial [7,8], this suggests that the basolateral membrane has low permeability to water or that the osmotic pressure of the basolateral spaces equilibrates very rapidly with that of the mucosal solution. Two immediate effects, therefore, seem to take place when enterocytes are exposed to hypotonic solutions: first, a dilution of the cell contents caused by the osmotic behaviour of the cell and secondly, an increase in apical membrane Cl^- conductance. The precise relation between these two events could not be traced in these experiments and remains to be elucidated.

If the initial change in a^c_{Cl} induced by hypotonicity is assumed to be the consequence of a change in cell volume only, then knowledge of the volume to surface ratio [1] and the initial rate of change in a^c_{Cl} (da^c_{Cl}/dt in Table I) allows the calculation of the net initial water influx. From these values estimates of the hydraulic permeability coefficient ($L_p = J_v/\Delta\pi$, where J_v is the net water flow and $\Delta\pi$ the difference in osmotic pressure) and the osmotic permeability ($P_{os} = L_p\ RT/\bar{V}_w$, where $\bar{V}_w$ is the partial molar volume of water and R and T have their usual meaning) can be obtained. For the present experiment the value of L_p was $1.4 \cdot 10^{-3}$ cm · s^{-1} · osM^{-1} and that of $P_{os} = 7.7 \cdot 10^{-3}$ cm · s^{-1}. These figures are remarkably similar to previous estimates in other leaky epithelia obtained by optical and electrophysiological methods [7,9].

Various cell types swollen by exposure to hypotonic solutions regulate their volume by loss of KCl through independent electrodiffusional pathways for K^+ and Cl^- [3]. Exposure of *Necturus* enterocytes to hypotonic solutions has previously been shown to produce a slow hyperpolarisation of E^m (new steady state reached in about 16 min) which is blocked by serosal Ba^{2+} and that was attributed to an increased basolateral K^+ permeability [4]. The results presented above indicate that

hypotonicity also produces an immediate cell swelling and an increase in the Cl^- conductance of the apical membrane. The latter was already present after 1 min of changing to hypotonic solution. The observation by Lau et al. [4] that in the presence of Ba^{2+} hypotonicity produces a marked decrease in the fractional resistance of the mucosal membrane is entirely consistent with the increased apical Cl^- conductance reported here. Na^+-coupled electrogenic transport of sugars or amino acids in enterocytes is accompanied by an apparent increase in K^+ permeability [10–12]. Studies in *Necturus* small intestine suggest that this permeability is localised to the basolateral membrane and might be part of a mechanism countering the tendency of the cells to swell due to accumulation of intracellular substrates [13]. Measurements of Cl^- fluxes in *Necturus* enterocytes have shown that during Na^+-coupled amino acid transport an increase in the apparent apical Cl^- permeability also takes place [2] and it has been proposed that the recently characterised cAMP-regulated apical Cl^- channels are involved in this response [1]. It is tempting to speculate that the same conductance is the one demonstrated here to be activated by cell swelling. In conclusion the activation of apical membrane Cl^- conductance, together with changes in K^+ permeability reported previously [4], might constitute the elements of a mechanism to prevent cell swelling which would be analogous to the process of regulatory volume decrease.

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