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Changes in the apparent chloride permeability of *Necturus* enterocytes during the sodium-coupled transport of alanine

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The membrane potential and intracellular Cl^- activity of *Necturus* enterocytes were measured with double-barrelled ion-selective microelectrodes and apparent permeability coefficients ($\bar{P}_{Cl}$) for the apical membrane calculated from Cl^--replacement experiments. In the presence of L-alanine in the mucosal solution an increase in $\bar{P}_{Cl}$ took place. It is proposed that this might reflect the activation of a Cl^- conductance during active substrate transport.

It is generally accepted that the transport of sugars and amino acids across the mucosal border of small intestinal cells is coupled to that of Na^+ and that, consequently, the intracellular accumulation of these substrates is dependent on the electrochemical potential difference for this cation (see Ref. 1 for review). This coupled entry is accompanied by an augmented rate of Na^+ pumping at the basolateral membrane as revealed by an increase in an associated ouabain-sensitive K^+-influx [2].

Cells engaged in transport of sugars and amino-acids face an osmotic problem created by the increase in intracellular substrate concentration and an associated rise in K^+ concentration due to the secondary effect on the Na^+ pump. This latter effect is never seen to take place, however, due to the switching on of K^+ channels leading to an increased passive loss of K^+ from transporting cells [3,4]. This change in permeability to K^+ seems to occur through the opening of Ca^{2+}-activated K^+ channels, which are sensitive to Ba^{2+} and to the bee-venom neurotoxin apamin [3,5,6]. The final effect in both rabbit and *Necturus* enterocytes is a decrease in the intracellular K^+ content [2,4]. It has been proposed that this phenomenon is part of a regulatory mechanism preventing gross changes in cell volume and membrane potential that would otherwise occur during nutrient transport [2,6].

It is well documented that changes in Cl^- permeability accompany the regulation of K^+ permeability during volume regulation in other cell types [7]. We have explored if this is also the case in enterocytes by measuring membrane potential (E^s) and intracellular Cl^- activity (a^c_{Cl}) with double-barrelled, Cl^--sensitive microelectrodes in *Necturus* small intestine. Our results suggest that during Na^+-coupled transport an apparent increase in enterocyte Cl^- permeability also takes place.

Initial measurements of a^c_{Cl} in *Necturus* enterocytes indicated that this anion can be accumulated intracellularly above the value expected for electrochemical equilibrium [8]. The results

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illustrated in Fig. 1 confirm this view: the trace of a^c_{Cl} in the upper part of the figure reveals an initial activity of 27 mM, the Nernst distribution predicting 10 mM, and shows that E_{Cl} is positive with respect to E^s by 23 mV. This uphill accumulation is consistent with previous measurements in other intestinal preparations [9–13]. Some information about the magnitude of Cl^- fluxes can be obtained by observing the effect of altering the extracellular ionic composition. Fig. 1 shows the effect of Cl^- removal from the mucosal solution (0 Cl^-) on E^s and a^c_{Cl}. Immediately after the removal of Cl^- a^c_{Cl} starts to decrease with only a small depolarisation (see E^s trace) *. The same experiment performed in the presence of 20 mM L-alanine produces a marked depolarisation of E^s and an apparently faster drop in a^c_{Cl}. The effect of L-alanine on E^s was similar to that described in previous work in the presence of normal Cl^- concentrations [4,8]. The understanding of the a^c_{Cl} transient requires more elaboration. In both 0 Cl^- transients the observed decrease in a^c_{Cl} followed time-courses that could be fitted to single decreasing exponentials (Fig. 2). Initial rates of decrease in a^c_{Cl} after 0 Cl^- were 2.0 and 3.9 mM $\cdot$ min^{-1} in the absence and presence of L-alanine, respectively. Assuming that the observed changes in a^c_{Cl} are due solely to transmembrane fluxes of chloride, the net efflux from the cells in 0 Cl^- can be estimated from the rate of decrease in a^c_{Cl} and the volume to surface ratio. For an activity coefficient of 0.77 [14] and a volume to surface ratio of $25 \cdot 10^{-4}$ cm (our preliminary estimates from light microscopy preparations), initial effluxes of 108 and 210 pmol $\cdot$ $cm^{-2} \cdot s^{-1}$ can be calculated in the absence and presence of L-alanine, respectively. The value in the absence of L-alanine represents the net outward movement of chloride across the apical membrane, as the driving forces across the basolateral membranes are virtually unaltered at $t = 0$. The same cannot be said of the efflux in the presence of L-alanine, however, due to the marked

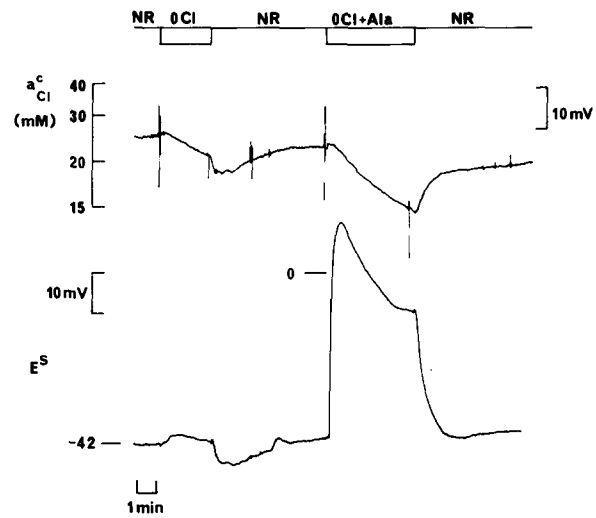

Fig. 1. Effect of chloride substitution on membrane potential (E^s) and intracellular chloride activity (a^c_{Cl}) of a *Necturus* enterocyte measured with a double-barrelled microelectrode. The signal from the conventional barrel (lower trace), subtracted electronically from that of the Cl^--sensitive barrel, is proportional to a^c_{Cl} (upper trace). For the periods indicated at the top of the figure the mucosal solution was changed from normal Ringer (NR) to one in which all chloride had been replaced by gluconate (0-Cl^-) or a similar chloride-free solution containing 20 mM L-alanine (0-Cl^- + Ala). A segment of the upper third of *Necturus* small intestine was used. Once stripped of its underlying muscle layers by blunt dissection, the intestine was mounted in a modified Ussing chamber and superfused with a solution (NR) of the following composition, in mM: NaCl 70, KCl 2.5, Hepes 5, $CaCl_2$ 1, $MgCl_2$ 1, $KHCO_3$ 0.5 and D-mannitol 95. In 0-Cl^- 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 in order to achieve the same free Ca^{2+} concentrations as in chloride-containing solutions. Osmolality was maintained constant throughout by omitting the appropriate amount of D-mannitol. The response of the chloride-sensitive microelectrode was calibrated as described previously [26]. The Cl^- electrode was filled with Cl^--sensitive ion exchanger (Corning 477913 [27]) and gave about 58 mV for 10-fold change in chloride activity. The response time was faster than the time taken to change the calibration solution (around 1 s). Measured potentials are referred to a 3 M KCl agar bridge as external electrode located in the solution bathing the serosal side of the tissue. The transepithelial potential difference (E^t) was also measured. The potential difference across the mucosal membrane (E^m) was obtained from $E^t = E^m + E^s$, and was used in the calculation of forces across this membrane.

* The trace of a^c_{Cl} illustrated in Fig. 1 is not corrected for drift which amounted to 4 mV during the 20 min stretch of record illustrated. Figures used for flux calculation were all drift-corrected. For the experiment in Fig. 1 corrected a^c_{Cl} values were: 27 mM before 0 Cl period, and 26 mM before 0 Cl + Ala period. Recovery at the end of the period illustrated was to 90% of the starting value. In six separate experiments recoveries after 0 Cl varied between 87 and 111% (26.1 ± 2.3 to 26.4 ± 2.9 mM before and after 0 Cl, respectively, mean ± S.E.).

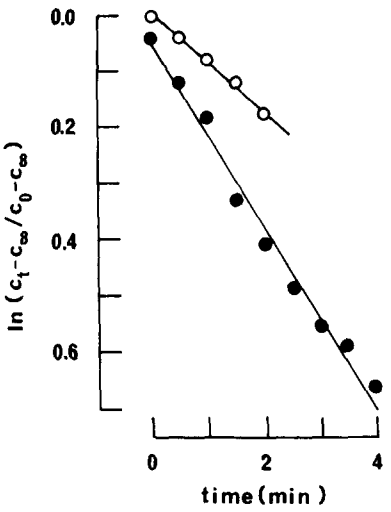

Fig. 2. Effects of mucosal Cl^- removal on intracellular chloride concentration in *Necturus* small intestine. The points were derived from continuous recordings in a^c_{Cl} corrected for an activity coefficient of 0.77 (see Fig. 1). The concentration at a given time is plotted semilogarithmically: c_t, c_0 and c_∞, concentrations at times t, 0 and ∞, respectively. A value of c_∞ was calculated by iteration from the best fit of the data to $c_t = (c_0 - c_\infty)\, e^{-kt} + c_\infty$. The rate constants for efflux derived from the slopes of the straight lines are -0.08 min^{-1} for 0-Cl^- experiment (○) and -0.17 min^{-1} for 0-Cl^- in the presence of 20 mM L-alanine (●). The initial change in chloride concentration was calculated by multiplying this rate constant by the initial chloride concentration [28].

depolarisation which increases the gradient for Cl^- entry through the basolateral membrane. We have no information on the electrodiffusional permeability of the basolateral membrane to Cl^-, but Ba^{2+}-blockade experiments in *Necturus* enterocytes show a high selectivity for K^+ [6] and would then suggest a low Cl^- permeability for this membrane. Similarly studies in another urodele, *Amphiuma*, also suggest a negligible basolateral membrane conductance for Cl^- [15]. On the basis of these considerations we must conclude that the calculated figures represent net effluxes across the brush-border membrane.

As a first approximation, it can be considered that the observed Cl^- efflux is an electrodiffusional movement down the electrochemical gradient. The apparent Cl^- permeability of the apical membrane ($\bar{P}_{Cl}$) in this case can be calculated using the constant-field theory [16]. For an efflux of 108 $pmol \cdot cm^{-2} \cdot s^{-1}$, and the initial E_s values after the transient, the calculated $\bar{P}_{Cl}$ was $1.5 \cdot 10^{-6}$ $cm \cdot s^{-1}$. The equivalent $\bar{P}_{Cl}$ for the 0 Cl^- transient in the presence of L-alanine * was $7.8 \cdot 10^{-6}$ $mm \cdot s^{-1}$. Therefore in the presence of L-alanine there appears to be a 5-fold increase in the brush-border membrane permeability to Cl^-. A change in permeability of this order should be detectable immediately after adding L-alanine to the mucosal solution in the presence of normal Cl^- concentrations. This was tested for during the cell impalement shown in Fig. 1 and the results for this have already been published in an earlier abstract [8]. The rapid depolarisation caused by L-alanine was accompanied in this case by a transient increase in a^c_{Cl}. This corresponds to a $\bar{P}_{Cl}$ of a $5.1 \cdot 10^{-6}$ $cm \cdot s^{-1}$ if the initial increase in a^c_{Cl} is assumed to reflect Cl^- influx across the apical membrane only, i.e. a 3.4-fold increase over the basal permeability (see above). The agreement with the $\bar{P}_{Cl}$ derived from the 0 Cl^- + L-alanine transient is fairly good considering that the influx of chloride might be underestimated in the 77 Cl^- + L-alanine experiment due to the rapidity of the rise in a^c_{Cl} ‡. We encountered a great cell to cell variability both in the response of E^s to L-alanine and in the rate of a^c_{Cl} decrease after Cl^--replacement in the course of these experiments ($\bar{P}_{Cl}$ ranged from $1.5 \cdot 10^{-6}$ to $43 \cdot 10^{-6}$ $cm \cdot s^{-1}$, six experiments in four animals), and this has also been reported previously [8]. For this reason it became mandatory to compare apparent $\bar{P}_{Cl}$ values derived from experiments during single impalements in individual cells and this proved to be technically very difficult. The results of some experiments in which this was successfully achieved, however, are shown in Fig. 3. The plot shows the relationship between $\bar{P}_{Cl}$ in

* This $\bar{P}_{Cl}$ value is derived for the efflux value and the peak depolarised value of E^s, i.e. +12 mV. If the calculation is done at the last point of the transient ($E^s = -7$ mV) $\bar{P}_{Cl}$ becomes $8.2 \cdot 10^{-6}$ $cm \cdot s^{-1}$. Thus the apparent permeability coefficient is not greatly affected by E^s within this range.

‡ The $\bar{P}_{Cl}$-values calculated here could be overestimated in the 0-Cl^- transient experiments if part of the observed efflux occurred via a carrier-mediated pathway unresponsive to the membrane potential. The increase in $\bar{P}_{Cl}$ in the presence of L-alanine, however, appears to reflect an increase in the electrodiffusional Cl^--permeability as it was observed under conditions in which concentration gradients for Cl^- were of opposite signs; i.e. L-alanine transients in the presence or absence of chloride.

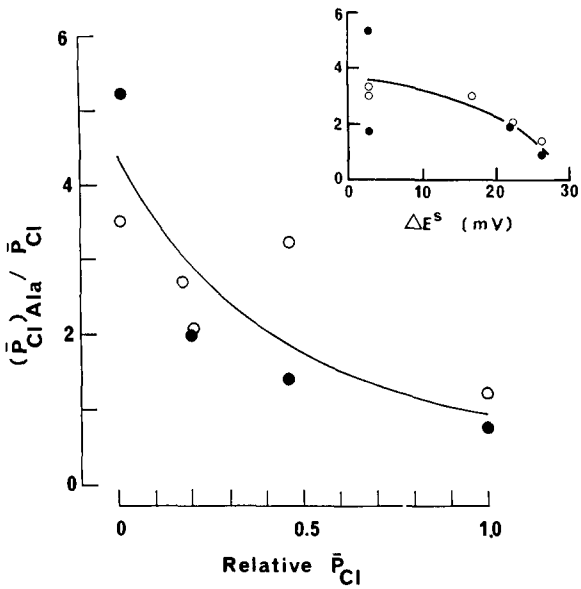

Fig. 3. Extent of the change in apparent chloride permeability as a function of the basal permeability. $\bar{P}_{Cl}$ values (see text) measured from transients of a^c_{Cl} in response to replacement of all mucosal Cl^- by gluconate are expressed as a fraction of the highest $\bar{P}_{Cl}$ measured this way (Relative $\bar{P}_{Cl}$). Permeability values derived from transients in the presence of L-alanine $(\bar{P}_{Cl})_{Ala}$ in the presence (○) or absence (●) of chloride, are given as ratios to basal $\bar{P}_{Cl}$ measured in the absence of alanine during the same cell impalement. The inset shows the same ratios plotted against the change in membrane potential observed during total Cl^- replacement by gluconate. Lines were drawn by eye.

the presence of L-alanine, derived from either 0-Cl^- transients in the presence of L-alanine or from the L-alanine-induced a^c_{Cl} transient measured in the presence of Cl^-, and that measured simply by Cl^- replacement. It can be seen that in the presence of L-alanine the apparent chloride-permeability is greater than the basal $\bar{P}_{Cl}$. This effect becoming smaller for higher basal Cl^--permeabilities.

The results presented above are consistent with the idea that during transport of L-alanine across *Necturus* enterocytes an increase in their apparent apical membrane permeability of Cl^- occurs. We have recently also observed that under certain circumstances a conductance to Cl^- dominates the ionic selectivity of the apical membrane in *Necturus* enterocytes (Giráldez and Sepúlveda, unpublished data). The inset in Fig. 3 shows that the ratio $(\bar{P}_{Cl})_{Ala}/\bar{P}_{Cl}$ follows an inverse relation when plotted against the magnitude of the depolarisation induced by 0-Cl^- solution. The latter being a function of the Cl^- conductance of the apical membrane. It is tempting therefore to speculate that the effect of Na^+-coupled transport is to enhance this Cl^- conductance. Maximal effects of L-alanine were observed in cells showing low basal Cl^- permeability, while more permeable cells were less responsive (Fig. 3). This suggests that in some instances, and for reasons which are not clear to us at the moment, $\bar{P}_{Cl}$ is already fully activated in a proportion of cells. One function for the phenomenon described here could be in promoting cell Cl^- loss as part of a volume regulatory mechanism preventing large increases in enterocyte volume during absorption [2,6,8,17].

It is interesting to note that the substrate-dependent increase in apparent Cl^--permeability we report here could provide an explanation at the cellular level for the repeatedly reported observation that sugars or amino acids either abolish transepithelial Cl^- absorption or induce a net Cl^- secretion [18–23]. In addition, the activation of a conductive Cl^- permeability pathway also located in the apical membrane is thought to mediate the copious intestinal secretion of H_2O, Na^+ and Cl^- which occurs under the action of some microbial toxins [24,25]. More work will be needed to see if these relations are coincidental or not, and to elucidate the signalling system linking amino acid absorption and this putative activation of chloride permeability.

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