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Effect of hypertonicity on the increase in basolateral conductance of *Necturus* small intestine in response to Na^+-sugar cotransport

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Exposure of *Necturus* small intestine to a galactose-containing perfusate that is 20% hypertonic compared to the galactose-free (control) perfusate results in a rapid depolarization of the electrical potential difference across the apical membrane, ψ^{mc}, and a decrease in the ratio of the resistance of the apical membrane to that of the basolateral membrane, (r^m/r^s); however, the slow repolarization of ψ^{mc} and increase in (r^m/r^s), observed under isotonic conditions, is blocked. These findings are consistent with the notion that the increase in the conductance of the basolateral membrane in response to Na^+-coupled sugar (or amino acid) transport across the apical membrane may be a 'volume regulatory response' to cell swelling.

The addition of D-galactose or L-alanine to the solution bathing the mucosal surface of *Necturus* small intestine, in vitro, results in an increase in transcellular Na^+ transport and, hence, Na^+-pump activity at the basolateral membrane, that is paralleled by an increase in the conductance of the basolateral membrane to K^+; the latter is blocked by metabolic inhibitors or by the presence of Ba^{2+} in the serosal solution [1–3]. Exposure of this epithelium to a moderately (12%) hypotonic solution also brings about an increase in the conductance of the basolateral membrane that can be blocked by Ba^{2+} or by exposure of the tissue to metabolic inhibitors [3]. These findings raised the possibility that the increase in the basolateral membrane K^+ conductance following the addition of sugars or amino acids to the mucosal bathing solution may be a 'volume regulatory response' to cell swelling resulting from the intracellular accumulation of these solutes in osmotically active forms [3]. The present study was designed to determine whether preventing cell swelling by exposing the tissue to a galactose-containing solution that is hyperosmotic compared to the galactose-free, control perfusate blocks the slow increase in basolateral membrane conductance.

Necturus maculosa (Lemberg, Oshkosh, WI) were stored at 4°C in tap water until use. The animals were anesthetized by immersion in tap water containing 0.1% tricaine methanesulfonate (Sigma) until all reflexes were absent. The proximal one-third of the small intestine was excised, stripped of the underlying musculature by blunt dissection and mounted, mucosal surface-up, between two halves of a perfusion chamber having an exposed surface area of 0.19 cm^2. Conventional microelectrodes for the measurement of the electrical potential difference across the apical membrane with reference to the mucosal solution, ψ^{mc}, were fabricated from glass capillary tubing (Omega Dot, W.P.I., Brunswick, ME; 1.2 mm o.d.) and,

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when filled with 0.5 M KCl, had tip resistances (measured in 0.5 M KCl) of 90–100 MΩ. The methods for determining and recording ψ^{mc} and the ratio of the slope resistance of the apical membrane to that of the basolateral membrane (r^m/r^s) have been described in detail elsewhere [1].

After mounting in the chamber, both surfaces of the tissue were perfused with a control solution containing (mM): NaCl, 70; $CaCl_2$, 1; $MgCl_2$, 0.5; KCl, 2.5; $KHCO_3$, 1; 4-morpholinepropanesulfonic acid (Mops) (Sigma), 2; and, mannitol, 90; the pH of this solution was adjusted to 7.2–7.4 using Tris-base and the osmolarity of this solution was 243 ± 1 mosM. After successfully impaling a cell across the apical membrane, the perfusion solution was switched to one that was either (i) isosmotic with the control solution in which 10 mM mannitol was simply replaced by 10 mM galactose or (ii) hyperosmotic compared to the control solution having the same electrolyte composition and containing 10 mM galactose and 130 mM mannitol; the osmolarity of the hyperosmotic solution was 291 ± 3 mosM. Thus, the effects of 10 mM galactose on ψ^{mc} and (r^m/r^s) were determined when the galactose-containing perfusate was isosmotic with the control solution and when the galactose-containing perfusate was 20% hyperosmotic compared to the control solution.

The results of a typical experiment illustrating the effects of the addition of 10 mM galactose to the mucosal bathing solution under isosmotic conditions are shown in Fig. 1a. The initial responses are a marked depolarization of ψ^{mc} and a decrease in the value of (r^m/r^s); these responses can be attributed to the activation of carrier mechanisms that mediate the coupled entry of Na^+ and galactose across the apical membrane into the cell that are both rheogenic (electrogenic) and conductive [1]. These initial, rapid responses are followed by a slower repolarization of ψ^{mc} and an increase in (r^m/r^s) which are, at least in part, the results of an increase in the conductance of the basolateral membrane to K^+ [2,3]. The introduction of phlorizin to the mucosal solution after a steady state was reached results in a small hyperpolariza-

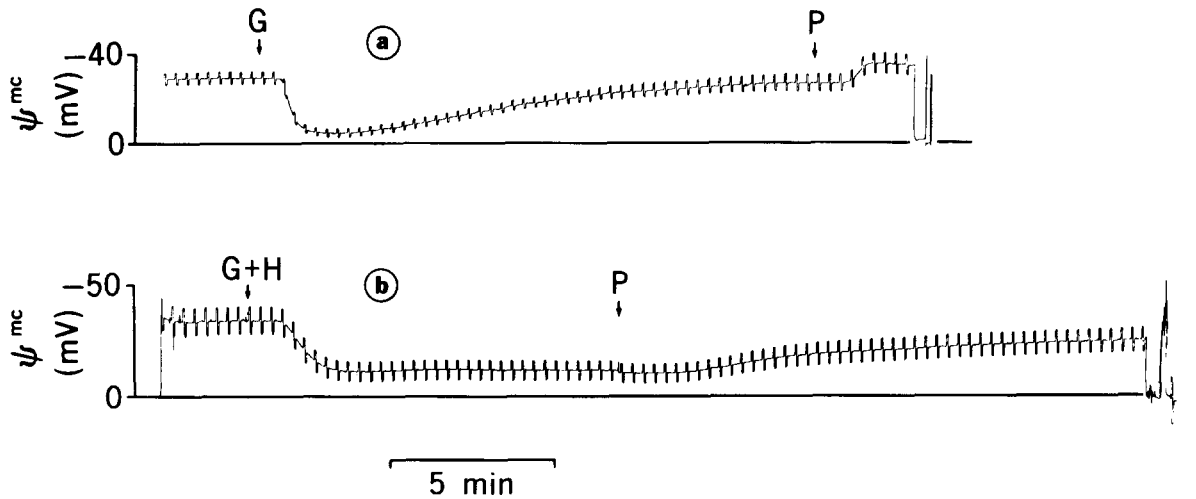

Fig. 1. (a) The typical responses of ψ^{mc} and (r^m/r^s) to the addition of 10 mM galactose to the mucosal perfusate. Following the addition of galactose (G) there is a rapid depolarization of ψ^{mc} and a decrease in the value of (r^m/r^s). The latter is determined from the magnitudes of the periodic bipolar deflections of ψ^{mc} and ψ^{ms} (not shown) in response to bipolar transepithelial current pulses of 150 $\mu A/cm^2$ as described previously [1]. The addition of $3 \cdot 10^{-6}$ M phloridzin to the mucosal solution (P) brings about a hyperpolarization of ψ^{mc} and an increase in (r^m/r^s) to values that exceed those prior to the addition of galactose. (b) Typical responses of ψ^{mc} and (r^m/r^s) when the galactose-containing solution is, at the same time, 20% hypertonic compared to the galactose-free perfusate (G + H). Note that while ψ^{mc} depolarizes and (r^m/r^s) decreases there is no spontaneous repolarization of ψ^{mc} or increase in (r^m/r^s). Under these conditions the addition of phloridzin to the mucosal solution simply restored ψ^{mc} and (r^m/r^s) to, or near, the control values.

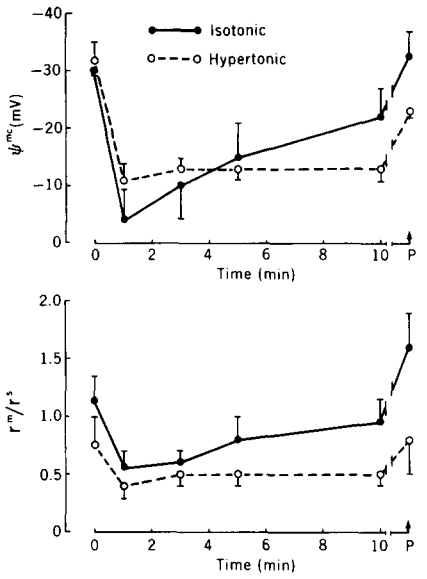

Fig. 2. Summary of the time-courses of the effects of 10 mM galactose and phloridzin (P) on ψ^{mc} and (r^m/r^s). At zero-time the perfusate was switched from the galactose-free solution to one that contained 10 mM galactose and that was either isotonic to the control solution (●) or 20% hypertonic (○).

tion of ψ^{mc} and a significant increase in (r^m/r^s) above the control values. The average results of four such experiments are summarized in Fig. 2 (filled circles); these are typical of a large number of similar experiments reported previously [1,2].

The results of a typical experiment in which the galactose-containing perfusate was, at the same time, 20% hyperosmotic to the control (galactose-free) perfusate are illustrated in Fig. 1b and the average results of five such experiments are given in Fig. 2 (open circles). There is an initial depolarization of ψ^{mc} and a decrease in the value of (r^m/r^s) similar to the effects observed under isosmotic conditions; but, the slower repolarization of ψ^{mc} and increase in (r^m/r^s) are aborted. Further, under these conditions, the addition of phloridzin to the mucosal solution simply restored (r^m/r^s) to the control value and ψ^{mc} did not hyperpolarize but instead remained below the control value.

These findings, together with those reported previously [3] are consistent with the notion that cell swelling in response to the intracellular accumulation of sugar or amino acid in osmotically active forms may be a 'signal' that somehow results in an increase in the K^+ conductance of the basolateral membrane, inasmuch as prevention of cell swelling by rendering the galactose-containing perfusate hypertonic essentially abolishes the slow increase in (r^m/r^s) and the accompanying hyperpolarization of ψ^{mc} observed under isosmotic conditions.

These findings parallel those reported by Kristensen [4] and Kristensen and Folke [5] for Na^+-coupled alanine uptake by isolated hepatocytes. These investigators have shown that this uptake process is accompanied by increases in cell volume and the permeability of the plasma membrane to K^+. If, however, the increase in cell volume is prevented by rendering the suspension solution hypertonic with sucrose, the increase in K^+ permeability was significantly reduced.

Finally, parallelisms among the rate of Na^+ entry across the apical membrane, Na^+-pump activity at the basolateral membrane and the K^+ conductance of the basolateral membrane have been reported for a number of other Na^+-absorbing epithelial including: *Necturus* [6], toad [7] and frog [8] urinary bladders; frog skin [9]; *Necturus* [10] gallbladder; and, *Necturus* renal proximal tubule [11]. It is tempting to speculate on the basis of the present findings that this 'cross-talk' [12–14] between the apical and basolateral membranes associated with changes in the rate of transcellular Na^+ transport under isotonic conditions and 'volume regulatory' responses to anisotonic conditions share common homocellular regulatory mechanisms designed to preserve cellular homeostasis [15] with respect to volume and ionic activities.

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