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Cell swelling increases a barium-inhibitable potassium conductance in the basolateral membrane of *Necturus* small intestine

(cotransport/sugar absorption/sodium absorption)

KIM R. LAU, RANDALL L. HUDSON, AND STANLEY G. SCHULTZ*

Department of Physiology and Cell Biology, University of Texas Medical School, P.O. Box 20708, Houston, TX 77225

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ABSTRACT **Previous studies have shown that, immediately after the addition of galactose or alanine to the solution bathing the mucosal surface of *Necturus* small intestine, there is a rapid depolarization of the electrical potential difference across the mucosal membrane (ψ^{mc}). This is followed by a repolarization of ψ^{mc} that is paralleled by an increase in the ratio of the effective resistance of the mucosal membrane to that of the basolateral membrane (r^m/r^s); the latter was shown to be, at least in part, due to a marked increase in the conductance of the basolateral membrane. We now report the following. (*i*) Exposure of this epithelium to a 12% hypotonic solution results in a hyperpolarization of ψ^{mc} and an increase in r^m/r^s. These effects are blocked by metabolic inhibitors and by the presence of 5 mM Ba^{2+} in the bathing solution; indeed, in the presence of Ba^{2+}, ψ^{mc} depolarizes and r^m/r^s decreases to low values. (*ii*) Addition of 15 mM galactose to the mucosal solution when the serosal solution alone contains 5 mM Ba^{2+} results in a depolarization of ψ^{mc} but no subsequent repolarization of ψ^{mc} or increase in r^m/r^s; however, ψ^{mc} repolarizes and r^m/r^s increases when Ba^{2+} is subsequently removed from the serosal bathing solution. We conclude that (*i*) the basolateral membrane normally possesses a Ba^{2+}-inhibitable K conductance, which appears to be reduced in the presence of metabolic inhibitors; (*ii*) after exposure of the tissue to a hypotonic solution or the addition of galactose to the mucosal solution, this conductance increases; and (*iii*) these responses can be blocked by metabolic inhibitors. These findings suggest that the delayed response of this tissue to the addition of sugars or amino acids to the mucosal solution may be the result of cell swelling resulting from the intracellular accumulation of these solutes in osmotically active forms.**

Previous studies from this laboratory have disclosed that the addition of galactose or alanine to the solution bathing the mucosal surface of *Necturus* small intestine, *in vitro*, brings about a rapid depolarization of the electrical potential difference across the mucosal (or apical) membrane and an increase in the conductance of that barrier (1). This initial response, which can be attributed to the activation of rheogenic Na^+-coupled cotransport processes for sugar and/or amino acid entry across the mucosal membrane, is followed by a slower repolarization of the electrical potential difference across that barrier that is blocked by the presence of metabolic inhibitors in the bathing solutions and appears to be the result of an increase in the K^+ conductance of the basolateral membrane (1, 2); however, the "intracellular signal" that elicits this delayed increase in basolateral membrane conductance was not definitively resolved in these studies.

It has long been known that sugars and amino acids are accumulated within small intestinal cells in osmotically active forms and, thus, are accompanied by an increase in cell water content and volume (3–5). Recently, studies on several epithelia have indicated that the initial increase in cell volume resulting from exposure to hypotonic bathing solutions is followed by a partial or full restoration of the original cell volume (6–8). It appears that this "volume regulatory response" is due, at least in part, to an increase in the permeability of the basolateral membrane to K^+, which results in a loss of K^+ from the cells (presumably accompanied by Cl^-).

The purposes of the present series of studies were to examine the electrophysiological response of *Necturus* small intestine to exposure to a hypotonic bathing solution and to determine whether the delayed response of this tissue to sugars and amino acids could be related to cell swelling.

METHODS

Segments of small intestine were stripped of the underlying musculature and mounted between two halves of a perfusion chamber as described (1). For the experiments designed to examine the effects of exposure to a hypotonic solution, the control solution contained NaCl (70 mM), $KHCO_3$ (1 mM), K_2HPO_4 (1 mM), KH_2PO_4 (0.5 mM), $CaCl_2$ (1 mM), $MgCl_2$ (0.5 mM), and mannitol (90 mM). The hypotonic solution had the same composition except that the mannitol concentration was reduced to 45 mM. The osmolality of the control solution was 237 ± 1 mosM (n = 9) and that of the hypotonic solution was 208 ± 1 mosM (n = 9). Thus, the osmolarity of the control solution was *decreased* by 12%, which is approximately the percentage increase in cell water content observed (3–5) when small intestine is exposed to sugars or amino acids.

For the experiments designed to examine the effect of serosal Ba^{2+} on the electrophysiologic response to the addition of galactose to the mucosal solution, the composition of the control solution was identical to that given above except that NaCl was at 110 mM and mannitol was at 15 mM. Solutions containing galactose (15 mM) or $BaCl_2$ (5 mM) were maintained isosmolar by appropriately adjusting the mannitol concentration. All solutions had a pH of 7.2–7.4 when gassed with air. In some instances verapamil (10 μM) was added to the perfusion solutions to inhibit smooth muscle movement; we have demonstrated previously that this agent has no effect on the electrophysiologic properties of the intestinal cells (1). All experiments were carried out at 22°C.

After mounting, the tissues were perfused with the control solution and short-circuited, with correction for fluid resistance, with an automatic voltage clamp. Villus cells were impaled across the mucosal membrane with microelectrodes filled with 0.5 M KCl having tip resistances between 60 and 90 Mohm. Criteria for an acceptable impalement were those adopted previously (1), with the additional restriction that the electrical potential difference across the apical mem-

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*To whom reprint requests should be addressed.

brane must not vary by more than 1 mV for a period of at least 2 min before changing solutions. The methods for recording the short-circuit current (I_{sc}) and the electrical potential difference across the mucosal membrane (ψ^{mc}) and for determining the transepithelial electrical resistance (r_t) and the ratio of the effective resistances of the mucosal and serosal membranes (r^m/r^s) have been described (1). All results are given as the mean ± the SEM. Statistical significance was evaluated using the Student *t* test (paired or unpaired).

RESULTS

A typical recording of ψ^{mc} when both surfaces of the tissue initially were perfused with the control solution and then were perfused with the hypotonic solution is shown in Fig. 1. (Both surfaces of the tissue were bathed with the hypotonic solution to prevent complications that could arise from "streaming potentials.") After exposure to the hypotonic solution there was a gradual hyperpolarization of ψ^{mc} and a parallel increase in r^m/r^s. The average time courses of these responses for 11 tissues are illustrated in Fig. 2. The average values of I_{sc}, r_t, ψ^{mc}, and r^m/r^s for these tissues in the presence of the control solution and after a new steady-state was achieved in the presence of the hypotonic solution are given in Table 1, experiment A. Thus, reduction of the osmolality of the bathing solutions by 12% results in a highly significant hyperpolarization of ψ^{mc} and a nearly 2-fold increase in r^m/r^s. These effects were completely blocked when 1 mM NaCN and 1 mM iodoacetamide were present in the control and hypotonic bathing solutions. Exposure of the tissue to these metabolic inhibitors for 1 hr abolished the I_{sc} and resulted in higher values of r_t and lower values of ψ^{mc} and r^m/r^s than usually encountered (Table 1, experiment B). Subsequent exposure of the tissues to the hypotonic solution resulted, if anything, in small decreases in ψ^{mc} and r^m/r^s; in no instance was there a hyperpolarization of ψ^{mc} or increase in r^m/r^s.

Fig. 3 illustrates a typical result of experiments in which the hypotonic solution contained 5 mM Ba^{2+}. Under these conditions, ψ^{mc} depolarized and r^m/r^s decreased. The average time courses of these responses for eight tissues are also illustrated in Fig. 2, and the initial and final steady-state values of I_{sc}, r_t, ψ^{mc}, and r^m/r^s are given in Table 1, experiment C. Thus, Ba^{2+} not only prevents but also reverses the effects of exposure of the tissue to a hypotonic solution on ψ^{mc} and r^m/r^s. Further, as shown in Fig. 3 and Table 1, experiment C, the effects of Ba^{2+} were largely reversible; thus, when the

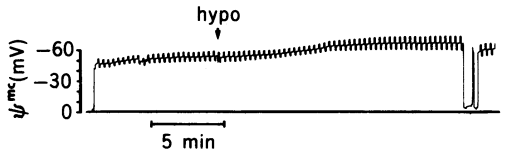

FIG. 1. Effects of switching from the control solution to a (12%) hypotonic solution (arrow) on ψ^{mc} and r^m/r^s. The periodic deflections are the changes in ψ^{mc} ($\Delta\psi^{mc}$) resulting from periodic displacements of the transepithelial electrical potential difference ($\Delta\psi^{ms}$) by ± 10 mV as described previously (1). Changes in r^m/r^s are reflected by changes in the magnitudes of these periodic deflections. The values of r^m/r^s are calculated from $[\Delta\psi^{mc}/(\Delta\psi^{ms} - \Delta\psi^{mc})]$.†

†As discussed by Boulpaep and Sackin (9), the assumption that $r^m/r^s = [\Delta\psi^{mc}/(\Delta\psi^{ms} - \Delta\psi^{mc})]$ is not strictly valid because of "distributed effects" in the lateral intercellular spaces. However, narrowing of the lateral intercellular spaces alone because of cell swelling in the presence of hypotonic solutions would, if anything, produce effects opposite to those observed; that is, if the resistance of the lateral intercellular spaces increases, the effect would be to decrease the calculated value of r^m/r^s.

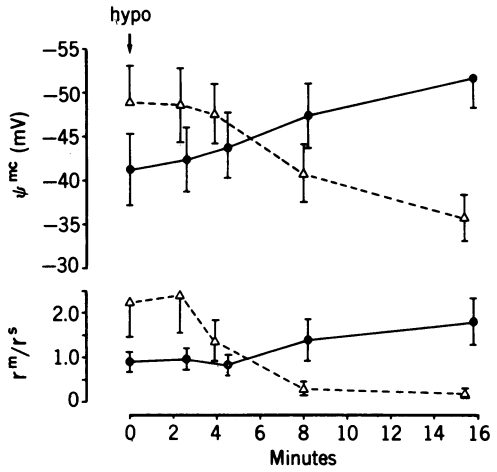

FIG. 2. Average effects of exposing the tissues to (12%) hypotonic bathing solutions in the absence (●) and the presence (△) of 5 mM Ba^{2+} on ψ^{mc} and r^m/r^s. The average values at "zero time" were those observed in the presence of the control solution for at least 2 min prior to changing the bathing solutions.

Ba-containing hypotonic perfusion solution is switched back to the control solution, there is a highly significant repolarization of ψ^{mc} and an increase in r^m/r^s.

Finally, the effects of the presence of Ba^{2+} in the serosal solution alone on the response of ψ^{mc} and r^m/r^s to the addition of galactose to the mucosal bathing solution are shown in Fig. 4; the results shown are typical of four such experiments. Initially r^m/r^s was very low and did not differ significantly from the value observed when Ba^{2+} was present in both solutions. The addition of galactose (15 mM) to the mucosal solution was followed by the expected depolarization of ψ^{mc}, but there was little or no secondary repolarization of ψ^{mc} or increase in r^m/r^s; this response resembles that observed in tissues exposed to metabolic inhibitors (1). However, when the solution bathing the serosal surface of the tissue was switched to one that did not contain Ba^{2+}, r^m/r^s increased and ψ^{mc} repolarized. As noted previously (1), the repolarization of ψ^{mc} was accompanied by a marked increase in I_{sc}, which is almost certainly due to an increase in the driving force for Na^+ entry (coupled to the entry of galactose) across the apical membrane.

DISCUSSION

Previous studies from this laboratory have disclosed that after the addition of sugars or amino acids to the solution bathing the mucosal surface of *Necturus* small intestine, there is a gradual increase in the conductance of the basolateral membrane that is prevented by metabolic inhibitors (1); inferential evidence has been presented that the increase in basolateral membrane conductance is due, at least in part, to an increase in the K^+ conductance of that barrier (2).

The results of the present series of studies indicate the following.

(*i*) Bathing *Necturus* small intestine with a moderately (12%) hypotonic solution, which presumably brings about cell swelling, results in a gradual increase in the ratio r^m/r^s that is prevented and, indeed, reversed when Ba^{2+} is present in the perfusion solutions. The responses to exposure to the hypotonic bathing solution also are blocked completely by metabolic inhibitors.

(*ii*) The increase in the conductance of the basolateral membrane after the addition of galactose to the solution bathing the mucosal surface of *Necturus* small intestine is reversibly blocked by the presence of Ba^{2+} in the serosal bathing solution. Indeed, in the presence of serosal Ba^{2+}, the

Table 1. Effect of reduced osmolarity on electrical properties of *Necturus* small intestine

Exp.	Conditions	*n*	I_{sc}, μA/cm²	r_t, Ω·cm²	ψ^{mc}, mV	r^m/r^s
A	Control	11	9.1 ± 2.1	171 ± 12	−41 ± 4	0.97 ± 0.21
	Hypotonic		8.5 ± 2.3	160 ± 13*	−52 ± 4**	1.81 ± 0.47*
B	Control + metabolic inhibitors	4	0	236 ± 31	−11 ± 1	0.42 ± 0.14
	Hypotonic + metabolic inhibitors		0	272 ± 40	−10 ± 1	0.38 ± 0.10
C	Control	8	5.7 ± 0.7	169 ± 13	−49 ± 4	2.26 ± 0.77
	Hypotonic + Ba^{2+}		3.8 ± 0.7	153 ± 17	−36 ± 3**	0.20 ± 0.10*
	Control		2.8 ± 1.5	153 ± 13	−46 ± 5	0.96 ± 0.32

n designates the number of tissues; *P* values for significant difference from control are: *, <0.025; **, <0.001.

tissues behaved qualitatively as if they were exposed to metabolic inhibitors.

There is compelling evidence that Ba^{2+} inhibits the K^+ conductance of the basolateral membranes of a number of epithelia (10–14), presumably by "plugging" K^+-selective channels (15, 16). Thus, it is not unreasonable to conclude that the Ba^{2+}-inhibitable increase in r^m/r^s observed when the tissue is exposed to a hypotonic bathing solution is the result of an increase in the K^+ conductance of the basolateral membrane and, thus, a decrease in r^s, which is blocked by treatment of the tissue with metabolic inhibitors.

This conclusion is in accord with, and extends, earlier findings dealing with the response of several epithelia to hypotonic bathing solutions. Dellasega and Grantham (6) found that exposure of isolated segments of renal tubules to a 50% hypotonic solution resulted in initial rapid cell swelling followed by a slower, partial restoration of the original cell volume. This decrease in cell volume could be blocked by cooling the bathing solution to 5–7°C, the presence of 1 mM KCN in the bathing solution, or elevating the bath K^+ concentration. These investigators concluded that the "volume regulatory response" may be a consequence of an energy-dependent increase in K^+ permeability permitting a loss of KCl and water from the cell. Finn and Reuss (17) have reported that reducing the osmolarity of the solution bathing the serosal surface of toad urinary bladder results in an increase in r^m/r^s because of a decrease in both r^m and r^s, with the latter predominating. More recently Davis and Finn (7) have provided evidence that the "volume regulatory response" of frog urinary bladder is the result of an increase in the K^+ conductance of the basolateral membrane. These investigators also demonstrated that inhibiting Na^+ entry across the apical membrane with amiloride blocks the "volume regulatory response" by decreasing the K^+ conductance of the basolateral membrane. These results are consistent with the notion that the K^+ conductance of the basolateral membrane is somehow responsive to the rate of Na^+ entry into the cell across the apical membrane and/or the activity of the Na^+/K^+ pump at the basolateral membrane (1, 2, 18, 19). In this regard, it is of interest that, in the present studies, when transcellular Na^+ transport was abolished by metabolic inhibitors, the observed values of r^m/r^s were uniformly lower than those encountered in "nonpoisoned" tissues (Table 1, experiment B). Finally, Foskett and Spring (8) have provided preliminary data that the volume regulatory decrease in *Necturus* gallbladder after osmotic swelling is due to enhanced efflux of KCl across the basolateral membrane and have suggested a possible role of vesicle fusion in the activation of this process; however, it is unclear from those studies whether KCl exit is via conductive pathways or not.

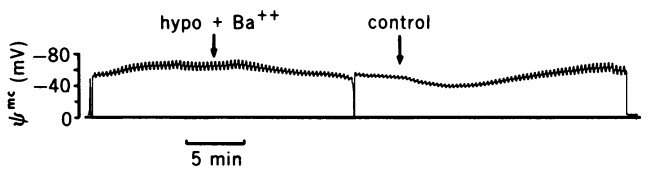

FIG. 3. Effects of switching from the control bathing solution to a (12%) hypotonic bathing solution containing 5 mM Ba^{2+} on ψ^{mc} and r^m/r^s and then switching back to the control solution. Changes in r^m/r^s are reflected by changes in the magnitudes of the periodic deflections.

Inasmuch as serosal Ba^{2+} also inhibits the increase in r^m/r^s observed after the addition of sugars, and presumably amino acids, to the mucosal solution, it is reasonable to conclude that this response is due to an increase in the K^+ conductance of the basolateral membrane, as inferred previously (1, 2).

Taken together, these findings suggest that the increase in basolateral K^+ conductance after the addition of sugars or amino acids to the mucosal solution is, at least in part, a response to cell swelling resulting from the intracellular accumulation of these solutes in osmotically active forms (3–5). This increase in basolateral K^+ conductance results in a hyperpolarization of the basolateral membrane and, by virtue of the low-resistance paracellular pathways, the mucosal membrane as well. As discussed previously (1, 2, 18–20), hyperpolarization of the mucosal membrane increases the driving force for these rheogenic Na^+-coupled entry processes. In addition, hyperpolarization of the basolateral membrane will increase the driving force for conductive anion exit from the cell across that barrier to accompany K^+ exit as part of the "volume regulatory response." Finally, as noted previously, an increase in the K^+ conductance of the basolateral membrane in response to an increase in the rate of Na^+ entry across the apical membrane would serve to prevent a large increase in cell K^+ activity in response to an increase in the activity of the Na^+/K^+ pump (1, 2, 18–20). In short, these findings suggest a link between "volume regulatory responses" and transcellular solute transport mediated by changes in the transport properties of the basolateral membranes in response to changes in the rate of solute entry across the mucosal membrane.

In this regard it is of interest to note that "volume regulatory responses" after exposure to a hypotonic bathing solution have been reported for a number of cells and tissues that are never exposed to major changes in the osmolality of their extracellular milieu under physiological conditions; in a

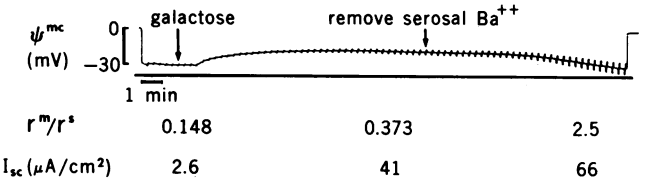

FIG. 4. The effects of the addition of 15 mM galactose to the mucosal bathing solution when the serosal bathing solution contained 5 mM Ba^{2+} and after switching the serosal bathing solution to one that was Ba^{2+}-free. Also shown are the values of r^m/r^s and I_{sc} before the addition of galactose, at the "peak" of the response, and when a new steady-state was achieved in the absence of Ba^{2+}.

number of reported instances, these responses appear to be associated with an increase in membrane K^+ permeability (21). The present findings suggest that the physiological importance of this regulatory mechanism(s) may be to preserve cell volume in the face of changes in intracellular solute content rather than changes in extracellular osmolality.

Finally, the finding that r^m/r^s is very small when 5 mM Ba^{2+} is present in both solutions or the serosal solution alone, averaging only 0.2 (Table 1, experiment C), suggests that the conductance of the basolateral membrane is largely attributable to passive K^+ channels. A question of central importance is whether the increase in the K^+ conductance of that barrier in response to an increase in cell volume is the result of an increase in the conductive capacity of single channels or whether cell swelling somehow elicits an increase in the number of open channels either by activation of previously silent channels already present in the membrane or by recruitment of new channels into the membrane from intracellular sources. Needless to say, the reason(s) why these changes are blocked by metabolic inhibitors and the intracellular messengers that are immediately responsible for these changes remains to be elucidated. Clearly, the blocking effects of metabolic inhibitors do not necessarily imply that the increase in K^+ conductance is directly dependent upon ATP; it is quite possible that changes in intracellular composition secondary to metabolic inhibition (e.g., increased Ca^{2+} activity, increased H^+ activity, etc.) are immediately responsible for the block.

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