The Postnatal Development of Sodium Transport in the Proximal Small Intestine of the Rabbit

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Summary

To investigate the postnatal development of intestinal Na^+ transport, a major determinant of fluid absorption, we measured spontaneous and glucose-coupled Na^+ transport across short-circuited epithelium and in isolated villus enterocytes from rabbit jejunum at age intervals after birth. Villus cells from suckling animals actively transported Na^+ and responded to glucose, but their capacity to do so was less than that of villus cells from older animals. Net Na^+ fluxes across short-circuited epithelium from suckling animals failed to respond to glucose, remaining negligible and less than adult values. This lack of response to glucose in tissue from younger animals was associated with marked paracellular shunting as evidenced by greater unidirectional fluxes and greater tissue conductance. Villus enterocytes from suckling animals compared to those from adults had reduced $(\text{Na}^+ - \text{K}^+) \text{ATPase}$ activity, but were rich in thymidine kinase. We conclude that proximal intestinal epithelium in suckling animals has a limited capacity for active Na^+ transport, is incompletely differentiated, and is leaky, with a greater permeability for ions compared with adult intestine.

Speculation

Changes which occur in intestinal Na^+ transport during postnatal development may be important in the pathogenesis of infant diarrhea, and relative limitations of intestinal transport capacity may contribute to the infant's susceptibility to dehydration following enteric infection.

Advances in our understanding of normal intestinal ion and water transport have contributed greatly to modern concepts of the pathophysiology and treatment of diarrheal disease (1, 18). The young of all species seem particularly susceptible to diarrhea. Development of the digestive and absorptive functions of the small intestine has been extensively studied (10, 13, 15); but little information is available regarding intestinal electrolyte and water absorption during early postnatal life. Recent studies have suggested that changes occur in ileal transport function during development of the rabbit (4), and in the rat, altered intestinal response to hypertonic solutions has been shown in young compared with adult animals (22). The present study examines jejunal Na^+ transport during postnatal development of the rabbit. Using established techniques, we measured spontaneous and glucose-coupled Na^+ transport *in vitro* across short-circuited jejunal epithelium and in isolated jejunal villus cells at intervals from birth to maturity. We focused our attention on sodium because this actively transported ion is a major determinant of the passive flow of water across the intestine (18).

MATERIALS AND METHODS

We studied nonfasted New Zealand White rabbits at ages 3 to 5 days (100 to 170 g), 10 to 14 days (200 to 260 g), 24 to 28 days

(300 to 350 g), or as adults (1500 to 3500 g). After weaning at 21 days, they received standard rabbit chow *ad libitum*. The animals were killed with an injection of Phenobarb sodium, and a 15-cm segment of jejunum starting 5 to 10 cm distal to the ligament of Treitz was used for ion transport studies in short-circuited chambers or for isolation of villus enterocytes for Na^+ efflux studies and enzyme analyses. Steady-state unidirectional mucosa to serosa (J_{ms}), serosa to mucosa (J_{sm}) and net (J_{net}) Na^+ fluxes were measured in Ussing-type short-circuited chambers under basal conditions and after glucose 30 mM by a modification of a technique previously described (16), exposing 0.4 cm^2 mucosal and serosal surfaces of 4 adjacent segments of jejunal epithelium to 15 ml of warmed oxygenated Krebs-bicarbonate phosphate buffer. Small (0.4 cm^2) chambers were used for all age groups to minimize differences in villus surface area with age. Three consecutive 10-min fluxes and 1 overall 30-min flux were measured for each period after 15-min equilibration. The spontaneous potential difference (PD) was determined at intervals, and a short-circuit current (I_{sc}) appropriate to neutralize the electrical gradient was introduced (19). Na^+ fluxes were expressed as $\mu\text{Eq}/\text{cm}^2/\text{hr}^{-1}$, PD as mV, I_{sc} as microamps, and conductance as mmho/cm^2 . Initially, the *in vitro* study of very fragile epithelium from these young, small animals presented certain technical problems. Because stripping the mucosa from underlying muscularis damaged the tissue of the younger rabbits, we studied full thickness intestine at all ages, first comparing Na^+ flux in stripped and unstripped tissue from adult rabbits. In unstripped tissue, J_{ms}^{Na} , J_{sm}^{Na} , and J_{net}^{Na} ($n = 6$) did not differ from values in stripped mucosa ($n = 8$) (J_{net}^{Na} , stripped versus unstripped, 0.02 ± 0.57 versus 1.05 ± 0.53 $\mu\text{Eq}/\text{cm}^2/\text{hr}$; mean $\pm$ S.E.); in both tissue preparations, J_{net}^{Na} and I_{sc} were equally stimulated by glucose (J_{net}^{Na} stripped, 2.92 ± 0.63 ; unstripped, 3.08 ± 0.88 , $P < 0.001$). Tissue conductance across stripped intestine was significantly greater than that across full thickness mucosa throughout the experiment in the absence or the presence of glucose ($P < 0.001$). The Ussing chamber data for the 3- to 5-day-old rabbits is not presented because the tissue failed to maintain stable electrical characteristics and histologic sections taken after the experiment showed exfoliation of cells and mechanical damage. In all other age groups, histologic sections taken after the experiment showed minimal abnormality, and the electrical characteristics of the tissue remained stable for at least 2 hr under the conditions of the experiment.

Villus cells could be selectively and reliably isolated from jejunum in all age groups by a vibration technique which excludes crypt cells (7); the presence of intact crypts was confirmed by light microscopy after the isolation procedure. The technique for Na^+ efflux studies using these villus cells was identical to that described previously (7-9). Briefly, Na^+ efflux rate constants were measured after loading the enterocytes with $^{22}\text{Na}^+$, washing with MgCl_2 , resuspending them in a tracer-free medium, and measuring the rate of appearance of $^{22}\text{Na}^+$ in the medium, expressed as the amount of Na^+ exchanged per hr. Rate constants were studied with and without maximal inhibition by ouabain 1 mM, defined as passive and total efflux (7), respectively, in both a glucose-free

(mannitol, 10 mM) and glucose (10 mM) -containing medium. Isolated villus cells were also homogenized (9) and assayed for sucrose and lactase by the method of Dalhqvist (5), Na⁺K⁺ ATPase by a modification (9) of the method of Kelly *et al.* (11), thymidine kinase by a modification (9) of the method of Klemperer and Haynes (12) and Breitman (3), and protein by the method of Lowry *et al.* (14).

Results were analyzed by Student's *t* test or paired *t* test where appropriate. Analysis of enzyme data were based on a logarithmic distribution, and the results were expressed as antilogarithms.

RESULTS

SHORT-CIRCUIT CHAMBER STUDIES

Na⁺ Flux (Table 1). Net fluxes under basal (glucose-free) conditions did not differ significantly between the age groups studied. Glucose produced expected significant increments in $J_{\text{net}}^{\text{Na}}$ and $J_{\text{ms}}^{\text{Na}}$ in intestine from 24- to 28-day and adult animals resulting in net absorption of Na⁺ ($P < 0.005$), whereas in contrast, fluxes across intestine from 10- to 14-day animals failed to respond to glucose. Unidirectional fluxes, $J_{\text{ms}}^{\text{Na}}$ and $J_{\text{am}}^{\text{Na}}$ were significantly greater in the absence or presence of glucose in the 10- to 14-day age group compared with that in older animals and decreased progressively with increasing age.

Electrical Data (Fig. 1). Throughout the basal study period, mean PD of tissues from all age groups remained stable, and no significant differences were observed between groups. After 30 mM glucose, a significant increment in PD was observed in 24- to 28-day and adult groups within 5 min ($P < 0.005$), but in 10- to 14-day animals, the response was delayed and became significant only after 15 min. The mean I_{sc} (at each interval) during the glucose-free period was greater in 10- to 14- than in 24- to 28-day ($P < 0.01$) and adult rabbits ($P < 0.001$). Similar differences occurred in the presence of glucose, which increased I_{sc} at all ages ($P < 0.05$). Tissue conductance across intestine from 10- to 14-day animals exceeded that of the older groups ($P < 0.001$) and fell with age; glucose increased conductance in intestine from 10- to 14-day and 24- to 28-day animals ($P < 0.01$). Conductance across stripped adult intestine was greater than across full thickness intestine (see "Materials and Methods") but still less than we observed in full thickness from these younger animals.

Na⁺ TRANSPORT IN ISOLATED VILLUS CELLS (TABLE 2).

Under basal conditions, total Na⁺ efflux rate constants were significantly less in 3- to 5- and 10- to 14-day age groups than in 24- to 28-day and adult rabbits which did not differ. Glucose significantly stimulated the total Na⁺ efflux rate constant in all age groups, although the total glucose stimulated rate constant remained significantly lower in the 3- to 5- and 10- to 14-day

groups when compared to 24- to 28-day and adult groups. Passive efflux rate constants with and without glucose did not differ significantly between the age groups.

ENZYME ACTIVITIES OF ISOLATED VILLUS CELLS (TABLE 3).

When compared with data from adult animals, isolated villus cells from 3- to 5-day animals were deficient in Na⁺K⁺ ATPase and sucrose and rich in lactase and thymidine kinase activities. Na⁺K⁺ ATPase activity, lowest at 3 to 5 days, progressively increased with age, whereas thymidine kinase activity greatest at 3 to 5 days, progressively decreased. Lactase activity, high at 3 to

Fig. 1. Electrical data: PD, Short-circuit current (I_{sc}) and Conductance ($COND$) across proximal rabbit jejunum during postnatal development. Points, mean of 24 to 40 measurements. $\times$, age 10 to 14 days; $\circ$, age 24 to 28 days; Δ , adult animals.

Table 1. Sodium ion flux in short-circuit jejunal epithelium during postnatal development¹

Age group	n ²	Study conditions	$J_{\text{ms}}^{\text{Na}}$	$J_{\text{am}}^{\text{Na}}$	$J_{\text{net}}^{\text{Na}}$
10-14 days	10	Glucose-free	18.19 $\pm$ 1.36	18.48 $\pm$ 1.20	-0.29 $\pm$ 0.59
		Glucose, 30 mM (P) ³	19.64 $\pm$ 1.49 (ns)	20.73 $\pm$ 1.80 (ns)	-1.09 $\pm$ 0.46 (ns)
24-28 days	8	Glucose-free	10.23 $\pm$ 0.30	8.78 $\pm$ 0.22	1.45 $\pm$ 0.25
		P ⁴	<0.001	<0.001	ns
		Glucose, 30 mM (P) ³	12.24 $\pm$ 0.45 (<0.001)	8.71 $\pm$ 0.24 (ns)	3.54 $\pm$ 0.55 (<0.001)
		P ⁴	<0.001	<0.001	<0.001
Adult	8	Glucose-free	7.15 $\pm$ 1.27	6.08 $\pm$ 1.12	1.05 $\pm$ 0.53
		P ⁴	<0.001	<0.001	ns
		Glucose, 30 mM (P) ³	10.94 $\pm$ 0.84 (<0.005)	7.87 $\pm$ 1.17 (ns)	3.08 $\pm$ 0.88 (<0.001)
		P ⁴	<0.005	<0.001	<0.01

¹ Each value represents the mean flux $\pm$ S.E. expressed in $\mu\text{Eq}/\text{cm}^2/\text{hr}$.

² n, number of animals; ns, not significant.

³ Paired *t*-test of increments after 30 mM glucose.

⁴ Compared to flux value in 10- to 12-day-old age group.

Table 2. Na^+ efflux from isolated enterocytes during postnatal development

Age	Study conditions	Total Na^+ efflux rate constant	n^1	Passive Na^+ efflux rate constant (1 mM ouabain)	n^1
3–5 days	Glucose-free ²	9.6 ± 0.4^3	41	7.2 ± 0.5	24
	Glucose, 10 mM (p) ⁴	12.7 ± 0.5 (<0.001)	38	8.7 ± 0.6 (ns)	25
10–14 days	Glucose-free p^5	13.0 ± 0.6 <0.001	23	7.4 ± 0.4 ns	29
	Glucose, 10 mM (p) ⁴ p^5	15.7 ± 0.7 (<0.01) <0.001	29	8.7 ± 0.4 (ns) ns	32
24–28 days	Glucose-free p^5	15.9 ± 0.6 <0.001	33	8.3 ± 0.4 ns	26
	Glucose, 10 mM (p) ⁴ p^5	18.2 ± 0.7 (<0.05) <0.025	35	8.5 ± 0.5 (ns) ns	23
Adult	Glucose-free p^5	15.8 ± 0.6 ns	41	7.7 ± 0.3 ns	34
	Glucose, 10 mM (p) ⁴ p^5	18.8 ± 0.7 (<0.001) ns	38	8.0 ± 0.3 (ns) ns	36

¹ n , number of observations.² Mannitol, 10 mM.³ Mean $\pm$ S.E.⁴ Compared to glucose-free conditions.⁵ Compared to value at preceding age.Table 3. Enzyme activities in isolated villus enterocytes during postnatal development¹

Age group	n^2	Lactase	Sucrase	Thymidine kinase	(Na^+K^+) ATPase
3–5 days	8	77.6 (55.0–109.6)	0.67 (0.05–9.21)	15.5 (11.9–20.2)	6.2 (5.3–7.2)
10–14 days p^3	6	75.9 (64.5–89.1) ns	0.06 (0.05–0.08) <0.005	9.8 (8.0–11.9) <0.005	8.6 (7.4–9.8) ns
24–28 days p^3 p^4	6	60.8 (34.7–104.7) ns ns	24.6 (17.8–33.9) <0.001 <0.001	9.0 (6.6–11.9) ns <0.005	13.9 (12.3–15.5) ns <0.005
Adult p^3 p^4	6	4.1 (3.1–5.3) <0.001 <0.001	64.6 (34.7–120.2) <0.005 <0.001	4.7 (3.6–6.0) <0.005 <0.001	17.1 (14.5–19.0) ns <0.001

¹ Each value represents the antilog of log mean and range of $\pm$ S.E. Disaccharidase activities are expressed as μ moles of substrate hydrolyzed per min per g of protein, thymidine kinase activity as pmoles of thymidine phosphate per min per mg of protein, and (Na^+K^+) adenosine triphosphate (ATPase) activity as nmoles of inorganic phosphate per min per mg of protein.² n , number of animals.³ Compared to preceding younger age group.⁴ Compared to 3- to 5-day group.

5 days, remained high at 10 to 14 and 24 to 28 days, but was significantly decreased in adult animals. Sucrase activity was low in the younger age groups increased significantly between 10 to 14 days and 24 to 28 days and again after 24 to 28 days to the adult value.

DISCUSSION

Our data show that important alterations in intestinal Na^+ transport, a major determinant of fluid absorption in the intestine, occur during postnatal development in the rabbit. Studied in isolated jejunal villus cells and across short-circuited intestinal segments, transcellular and paracellular Na^+ fluxes change with age. The isolated cell Na^+ efflux data indicate that whereas jejunal villus enterocytes from suckling animals can actively transport Na^+ under glucose-free conditions and can respond to glucose, the total efflux rate constant is less than in mature animals. Epithelial Na^+K^+ ATPase activity, a reflection of the Na^+ pump in the basolateral membrane, also increases in villus cells as the animal matures. However, in the Ussing chamber studies, active trans-

epithelial net Na^+ absorption could not be demonstrated in the suckling group in response to glucose. This lack of response to glucose in younger animals can not be attributed to tissue death in the chambers because histologic sections taken at the completion of the experiments showed the epithelium to be intact. Electrical data were stable throughout, and flux data from the individual 10-min periods were consistent in each experiment. Glucose did cause an increment in I_{sc} in tissue from suckling animals even though there was no measurable impact on net Na^+ transport. In adult rat jejunum and guinea pig ileum, glucose has been demonstrated to stimulate neutral NaCl secretion simultaneously with electrogenic Na^+ absorption (2, 17, 20). If present in suckling rabbit jejunum, such a mechanism could account for the apparent lack of glucose stimulated Na^+ absorption. An alternative explanation may be the increased permeability of intestine from suckling animals. Unidirectional fluxes and conductance were greatly increased in intestine from young compared with adult animals and active ion fluxes may have been overshadowed by marked paracellular shunting. In adult animals, 80 to 90% of the total conductance is accounted for by a diffusional movement

via a shunt pathway (6), the electrophysiologic counterpart of the intercellular junctional complexes (18). If a similar proportion of shunting occurs in younger animals, our data indicate that these intercellular junctions are less "tight" in infant compared with adult intestine. Cooke and Dawson (4), studying ileum from 1- to 6-day old rabbits in Ussing chambers, also found increased unidirectional ion fluxes, high conductance, and failure of Na⁺ absorption to respond to an actively transported nonelectrolyte alanine, even though there was an effect on I_{sc}.

As expected, enterocytes from suckling animals were rich in total lactase activity and low in sucrase activity; after weaning, lactase activity decreased whereas sucrase activity increased (10, 13, 15). In suckling animals, villus enterocytes were also relatively rich in thymidine kinase, an enzyme of DNA replication normally present in immature crypt cells (9, 21). The enzyme and ion transport characteristics of villus cells from suckling animals therefore are in some respects similar to those of normal crypt cells from mature animals, although in other respects they appear well differentiated with well-developed brush borders as evidenced by high lactase activity and the presence of glucose-coupled Na⁺ transport (9).

Intestinal Na⁺ transport is clearly less efficient in the suckling rabbit. The capacity for active Na⁺ transport is limited in early postnatal life and is associated with diminished activity of villus cell Na⁺K⁺ ATPase, the enzyme system associated with active Na⁺ transport in the basolateral membrane. Furthermore, the infant rabbit epithelium is comparatively leaky; transcellular passive Na⁺ flux measured in isolated enterocytes is similar at all ages, but unidirectional Na⁺ fluxes measured in intact tissue are increased, and conductance is greater in younger animals, strongly suggesting a comparatively large, low-resistance, extracellular ion flux. During the suckling period, the villus epithelium retains thymidine kinase activity and Na⁺K⁺ ATPase activity is less. On the other hand, glucose-coupled Na⁺ transport, a characteristic of differentiated villus epithelium, is intact early in life, suggesting that different aspects of intestinal epithelial function mature at different rates.

Because healthy young rabbits like healthy young humans, do not suffer excessive ion and fluid losses from their intestines, these immaturities of Na⁺ transport appear not to be an important consideration for normal nutrition in early postnatal life. However, faced with enteric disease, like an enteritis in which Na⁺ transport is compromised, limited transport function could become a serious handicap.

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