Intestinal Mucosal Ornithine Decarboxylase and Brush Border Membrane Vesicle Na^+-H^+ Exchange Activities in Diabetic Rats (43647)

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Abstract. **To determine the possibility that intestinal mucosal ornithine decarboxylase activity can modulate mucosal brush border membrane Na^+-H^+ exchange activity, we studied the relationship between jejunal mucosal ornithine decarboxylase activity and mucosal brush border membrane Na^+-H^+ exchange activity in adolescent streptozotocin-diabetic and normal control rats. Diabetes was associated with enhanced intestinal mucosal ornithine decarboxylase and Na^+-H^+ exchange activities. Groups of diabetic and control rats were given difluoromethylornithine in drinking water to suppress intestinal mucosal ornithine decarboxylase activity. As expected, 10 days after induction of diabetes, intestinal mucosal weight (67.7 mg/cm vs 56.1 mg/cm), DNA (47.3 μg/mg protein vs 32.7 μg/mg protein), ornithine decarboxylase activity (1107 units/hr vs 654 units/hr), and brush border membrane vesicle Na^+-H^+ exchange activity, assessed as V_{max} of $^{22}Na^+$ uptake (32.5 nmol/mg protein/15 min vs 15.2 nmol/mg protein/15 min), were significantly greater in diabetic than in control rats. Treating diabetic and control rats with difluoromethylornithine suppressed jejunal mucosal growth by over 30%, ornithine decarboxylase activity by over 80%, and brush border membrane vesicle $^{22}Na^+$ uptake by over 60%. Highly significant direct correlations ($r > 0.900$) were observed between jejunal DNA content, mucosal ornithine decarboxylase activity, and brush border membrane vesicle Na^+-H^+ exchange activity. The above findings suggest that jejunal mucosal ornithine decarboxylase activity can modulate mucosal epithelial proliferation and mucosal brush border membrane Na^+-H^+ exchange activity.**

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Ornithine decarboxylase activity and contents of intracellular polyamines (putrescine, spermidine, and spermine) are elevated during initiation of rapid cellular replication and are thought to be required for proliferation in many diverse organs (1–3). We have recently shown that the marked intestinal epithelial hyperplasia noted in streptozotocin-diabetic rats was also associated with increased enterocyte activity of ornithine decarboxylase and contents of putrescine and spermidine (4). Suppression of enterocyte ornithine decarboxylase activity (needed for the conversion of ornithine to putrescine) by α-difluoromethylornithine, a specific nonreversible inhibitor of ornithine decarboxylase, administered in drinking water, prevented the intestinal epithelial hyperplasia in the diabetic rats and also the normal epithelial growth in control rats (4).

Intestinal mucosal brush border membrane Na^+-H^+ exchange activity seems also to play an important role in enterocyte proliferation. The dramatic trophic change in the gastrointestinal mucosa in response to refeeding of 48-hr fasted rats was inhibited by amiloride, an inhibitor of Na^+-H^+ exchange activity, with prevention of DNA synthesis (5). In another study, Na^+-H^+ exchange activity was found to be significantly greater in 48-hr fasted refed rats than in 48-hr fasted control rats (6). Administration of difluoromethylornithine to the 48-hr fasted refed rats prevented the enhancement in brush border membrane Na^+-H^+ exchange activity, suggesting a close relationship between the polyamines and Na^+-H^+ exchange activity (6). Contents of the polyamines and ornithine decarboxylase activity in the

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intestinal epithelium were not determined in the above study.

In the present study, we evaluated the possibility that intestinal epithelial ornithine decarboxylase activity could modulate intestinal mucosal brush border membrane Na^+-H^+ exchange activity. Jejunal mucosal hyperplasia (assessed from mucosal DNA content and wt:cm ratio), ornithine decarboxylase activity, and brush border membrane vesicle Na^+-H^+ exchange activity were compared and correlated in diabetic, control, and difluoromethylornithine-treated diabetic and control rats.

Materials and Methods

Rats. Male albino Sprague-Dawley 140–150 g rats (Herlemn, Indianapolis, IN) were divided into weight-matched diabetic and control groups. Regular rat chow (Purina, Indianapolis, IN) and water were allowed *ad libitum*. Rats were housed individually in raised wire mesh-bottom cages in a room at 25 ± 1°C with a 12:12-hr light:dark cycle. Amounts of food and water consumed by the rats and their body weight were measured daily.

Induction of Diabetes Mellitus. Diabetes was induced by intraperitoneal injection of streptozotocin (Sigma Chemical Co., St. Louis, MO). 100 mg/kg body wt dissolved in citrate buffer (pH 4.3) as described before (7). Control rats were injected with a similar volume of vehicle. Diabetes was confirmed from hyperglycemia (blood glucose >250 mg/dl), glucosuria (4+), polyphagia, and polydipsia. Blood glucose was determined at the end of the study using a Glucometer II (Ames, Miles, NJ). Urine glucose was determined every other day using Strip Test (Ames).

Administration of Difluoromethylornithine. Diabetic and control rats were divided into two groups each. One group of diabetic and control rats received difluoro-α-methyl ornithine (a gift from Merrell Dow Pharmaceuticals, Cincinnati, OH) as a 2 g/100 ml solution in drinking water. Because of the large volume of water consumed by the diabetic rats, the concentration of difluoromethylornithine was reduced to around 0.7 g/100 ml in the drinking water so that the diabetic rats consumed a similar daily amount of difluoromethylornithine as the control rats. Difluoromethylornithine was administered 24 hr after induction of diabetes and continued for the duration of the study.

Rats were studied on the 10th day after induction of diabetes. At the time of study, nonfasted rats were anesthetized with intraperitoneal injection of sodium pentobarbital, 20 mg/100 g body wt. After a midline abdominal incision, 60 cm of the proximal small bowel (jejunum) distal to the ligament of Treitz were washed with ice-cold 0.9% NaCl solution and then flushed with 100 ml of room air. The jejunal segment was cut open along the mesenteric line and the mucosa was scraped lightly with the edge of a microscope slide. The mucosa was weighed and then frozen at -70°C before analysis for ornithine decarboxylase activity. DNA was assayed as described by Burton (8).

Determination of Ornithine Decarboxylase. Ornithine decarboxylase specific activity was determined in the scraped jejunal mucosa by a method similar to that described by Yang *et al.* (9). The scraped mucosa was homogenized in 2 ml of 100 m*M* phosphate buffer (pH 7.4), then sonicated for 60 sec and centrifuged for 60 min at $1 \times 10^5 g$. The supernatant was added to 500 μl of 0.1 *M* phosphate buffer (pH 7.2) containing 20 mg/dl of 23-laurylether, 5 m*M* NaF, 10 μ*M* EDTA, 2 m*M* dithiothreitol, and 100 μ*M* pyridoxal phosphate. The above methods and reactions were performed at 4°C. Ornithine decarboxylase activity was measured by release of CO_2 from L-(1-^{14}C)-ornithine (58 mCi/mmol; New England Nuclear, Boston, MA) and expressed as dpm of $^{14}CO_2$ released/hr/mg protein content. Supernatant (300 μl) was incubated with 15 μl of ^{14}C-labeled ornithine, 150 μl of 100 m*M* phosphate buffer (pH 7.30), 25 μl of 5 m*M* pyridoxal phosphate, and 10 μl of 10 μmol/ml L-ornithine. The incubation took place in glass scintillation vials, at 37°C for 120 min in a shaker water bath. The reaction was stopped by the addition of 300 μl of 5 N sulfuric acid. The $^{14}CO_2$ liberated by the decarboxylation of ornithine was trapped in 200 μl of 1 N hyamine hydroxide (Sigma) placed in a center well. Blanks were run simultaneously by using the vehicle instead of the supernatant. The hyamine hydroxide with the trapped $^{14}CO_2$ was placed in a scintillation vial and 10 ml of Packard Permafluor V scintillation fluid were added, and radioactivity was counted in a Packard liquid scintillation spectrometer (Packard 1600CA).

Isolation of Brush Border Membrane Vesicles. Brush border membrane vesicles were prepared in freshly scraped jejunal mucosa by a Mg^{2+} precipitation technique (10) at 4°C. Briefly, the removed jejunal segment was everted and gently scraped with the edge of a microscope glass slide. The scrapings were homogenized for 5 min in 50 ml of homogenizing buffer (300 m*M* mannitol, 5 m*M* EGTA, and 12 m*M* Tris HCl, pH 7.2) and the final volume was made to 200 ml with the addition of ice-cold double-distilled water. The homogenate was treated with 4 ml of 1 *M* $MgCl_2$ and centrifuged (Jouan model MR14.11) at 1,000*g* for 15 min. The supernatant was then recentrifuged at 31,000*g* for 30 min (Sorval OTD60B). The resulting pellet was resuspended in a Potter Elvehjem tube with 25 ml of the homogenizing buffer, and homogenized for approximately 10 strokes at medium speed and 15 strokes at high speed. The homogenate was then treated with 1 ml of 1 *M* $MgCl_2$ and centrifuged again at 3,000*g* for 12 min. The pellet was resuspended in 25 ml of 300 m*M* mannitol and 20 m*M* Hepes Tris (pH 7.2) and

centrifuged at 51,000*g* for 30 min (Sorval centrifuge model OTD 60 B). Using a 1-ml syringe, the pellet was resuspended in the desired volume of 50 m*M* 2-(*N*-morpholino)-ethane sulfonic acid and 50 m*M* Tris. They were briefly sonicated for 5 sec.

Determination of Brush Border Membrane Na^+-H^+ Exchange Activity. Na^+-H^+ exchange activity of the brush border membrane vesicles was assessed from their ability to transport sodium against an H^+ ion gradient. Uptake of $^{22}Na^+$ from the incubation media was measured by a rapid filtration technique (6). All incubations were done at 25°C and were initiated by the addition of 25 μl of the brush border membrane vesicle suspension to 75 μl of incubation solution. The composition of the incubation solution is noted in the Figure Legends. At each desired time interval, 1 ml of ice-cold stop solution, which consisted of 145 m*M* NaCl, 20 m*M* Tris, 16 m*M* Hepes, and 1.5 m*M* amiloride, was used to terminate the reaction. The cold diluted reaction mixture was immediately pipetted onto prewetted filter (0.45 μ*M* Millipore HAWP; Millipore Corp., Bedford, MA) kept under suction. The filter was rinsed three times with 5 ml of ice-cold stop solution and then placed in a scintillation vial and 10 ml of Packard Permafluor E scintillation fluid were added and radioactivity was counted in a Packard liquid scintillation spectrometer (Packard 1600CA). Radioactivity remaining in the filters after pipetting incubation medium in the absence of vesicles was considered as background and was accounted for in the calculations.

Statistics. Mean and SE were calculated for all parameters determined. Data from at least five separate rats were used to calculate mean ± SE values for a given parameter. When indicated, data were expressed graphically. Significance of differences among the groups was determined using analysis of variance. Values of $P < 0.05$ were considered to indicate statistical significance between the corresponding mean values.

Results

Data pertaining to the rats studied are shown in Table I.

Body Weight. Diabetic and difluoromethylornithine-treated diabetic rats lost weight while control and difluoromethylornithine-treated control rats gained weight during the 10 days of the experiment. Treatment with difluoromethylornithine did not alter change in body weight significantly in either diabetic or control rats. Blood glucose was around 400 mg/dl in diabetic rats and <250 mg/dl in control rats and not significantly altered by the treatment with difluoromethylornithine in either diabetic or control rats.

Food and Water Consumption. Daily food consumption was 1.5-fold and daily water consumption was over 5-fold greater in diabetic than in control rats. Administration of difluoromethylornithine did not significantly affect food or water consumption in either diabetic or control rats.

Jejunal Weight. Weight of the 60-cm jejunum and wt:cm length ratio of the jejunal mucosa were greater in diabetic than in control rats ($P < 0.01$). Jejunal weight and mucosal wt:cm ratio were significantly lower in the difluoromethylornithine-treated diabetic and control rats than in the corresponding nontreated rats ($P < 0.05$).

Jejunal Mucosa DNA Content. Mucosal DNA content expressed as μg/mg was significantly greater in diabetic than in control rats and significantly lower in the difluoromethylornithine-treated than in the corresponding nontreated diabetic and control rats ($P < 0.05$).

Jejunal Mucosa Ornithine Decarboxylase Activity. As shown in Table I, activity of ornithine decarboxylase in jejunal mucosal scraping was enhanced almost 2-fold in diabetic as compared with control rats ($P < 0.001$). Administration of difluoromethylornithine reduced ornithine decarboxylase activity in the mucosal scrapings by over 80% in both the diabetic and the control rats.

Brush Border Membrane Na^+-H^+ Exchange Activity. Figure 1 depicts Na^+ uptake by the brush border membrane vesicles in the presence of H^+ ion gradient (pH 5.5 inside, pH 7.5 outside) over time in the diabetic and control and the difluoromethylornithine-treated diabetic and control rats. Na^+ uptake rates were significantly greater at all time points studied in the diabetic than in the control rats ($P < 0.05$–0.001) and significantly lower in the difluoromethylornithine-treated than in the corresponding nontreated diabetic and control rats ($P < 0.05$–0.001). Equilibrium values at 60–90 min were similar, indicating similar intravesicular volume. Na^+ uptake rates were linear up to 15 sec in all groups.

Na^+ uptake over time by brush border membrane vesicles in the absence of a hydrogen ion gradient (7.5 inside, 7.5 outside) were less than one fourth those noted in the presence of H^+ gradient indicated in Figure 1 and not statistically significantly different in the diabetic and control and in the difluoromethylornithine-treated diabetic and control rats. Na uptake did not exceed equilibrium values at 60 min during any time interval studied.

Figure 2 indicates kinetics of $^{22}Na^+$ uptake by the brush border membrane vesicles from the incubation medium with varying Na^+ ion concentrations in the presence of H^+ ion gradient (pH 5.5 inside, pH 7.5 outside) in diabetic, control, and the difluoromethylornithine-treated diabetic and control rats. $^{22}Na^+$ uptake rates were concentration dependent and appeared to be linear up to incubation medium concentration of 30 mmol sodium. At all incubation medium sodium con-

Table I. Change in Body Weight, Blood Glucose, Consumption of Food and Water, Jejunal Weight, and Jejunal Mucosal Wt:Cm Ratio, Its DNA Content, and Ornithine Decarboxylase Activity in the Diabetic, Difluoromethylornithine (DFMO)-Treated Diabetic, Control, and DFMO-Treated Control Rats Studied[a]

Rats	D	D + DFMO	C	C + DFMO
Body wt (g/day)	−1.0 ± 0.2	−1.6 ± 0.4	7.6 ± 0.4[b]	7.0 ± 0.3[c]
Blood glucose (mg/dl)	400.5 ± 8.0	398.7 ± 7.1	165.6 ± 4.0[b]	168.4 ± 5.0[c]
Food consumed (g/day)	39.01 ± 3.2	35.0 ± 3.6	24.2 ± 3.0[b]	26.3 ± 2.0[c]
Water consumed (ml/day)	110.2 ± 3.3	101.6 ± 3.1	40.1 ± 2.5[b]	41.3 ± 2.7[c]
Jejunal wt (g)	5.8 ± 0.3	4.4 ± 0.4[d]	4.9 ± 0.1[b]	4.2 ± 0.1[e]
Mucosal wt (mg/cm)	72.7 ± 3.1	51.2 ± 3.8[d]	59.0 ± 3.7[b]	47.8 ± 3.3[e]
Mucosal DNA (μg/mg)	47.3 ± 6.8	20.7 ± 4.8[d]	32.7 ± 6.1[b]	16.4 ± 5.1[e]
Mucosal ODC activity[f]	1107.0 ± 63.0	193.0 ± 63.0[d]	654.0 ± 73.0[b]	142.0 ± 27.0[e]

[a] Values are means ± SE. D, diabetic; D + DFMO, difluoromethylornithine-diabetic; C, control; C + DFMO, difluoromethylornithine-treated control; ODC, ornithine decarboxylase.
[b] Mean values in control rats significantly different from corresponding mean values in diabetic rats, $P < 0.05$.
[c] Mean values in C + DFMO rats significantly different from corresponding mean values in D + DFMO, $P < 0.05$.
[d] Mean values in D + DFMO rats significantly different from corresponding mean values in diabetic rats, $P < 0.05$.
[e] Mean values in C + DFMO rats significantly different from corresponding values in control rats, $P < 0.05$.
[f] Dpm of $^{14}CO_2$ released/mg protein/hr when incubated in media containing 20 mg/dl laurylether, 5 m*M* NaF, 10 μ*M* EDTA, 2 m*M* dithiothreitol, 10 μ*M* pyridoxal phosphate, 10 m*M* L-ornithine, and L-[1-^{14}C]ornithine tracer.

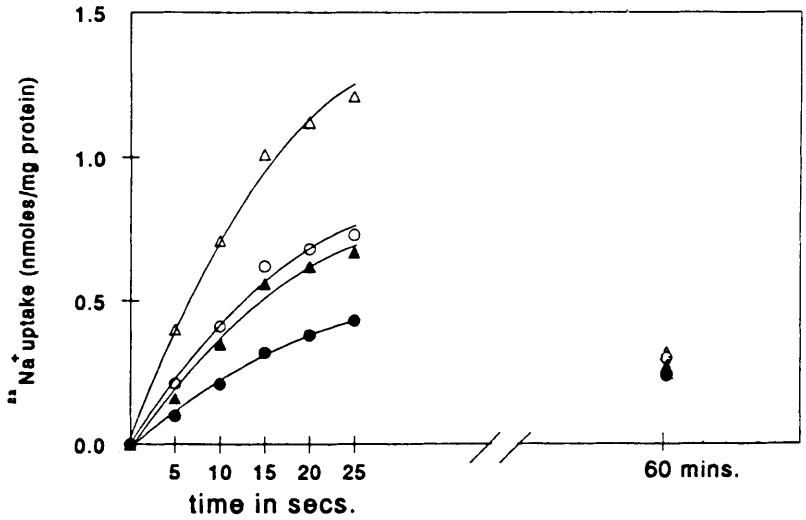

Figure 1. $^{22}Na^+$ uptake overtime by jejunal brush border membrane vesicles of streptozotocin-diabetic (△), control (○), and difluoromethylornithine-treated streptozotocin-diabetic (▲) and control (●) rats. $^{22}Na^+$ uptake rates by brush border membrane vesicles were measured at 25°C against a H^+ ion gradient (pH_i 5.5/pH_o 7.5) at the various time intervals shown. Brush border membrane vesicles were preincubated for 1 hr at 25°C with 40 m*M* Hepes and 90 m*M* 2-(*N*-morpholino)ethane sulfonic acid (pH 5.5). The reaction was started with the addition of 25 μl of brush border membrane vesicles to 75 μl of medium containing 85 m*M* Hepes, 45 m*M* Tris (pH 7.5), 1 m*M* sodium chloride, and ^{22}NaCl tracer (New England Nuclear). Each point represents mean value of eight experiments. Standard errors were less than 1% of the mean values, and are not shown in the Figure. At each time interval, mean values in diabetic and control rats were significantly greater than corresponding mean values in the difluoromethylornithine-treated diabetic and control rats ($P < 0.05$–0.001).

centrations, uptake rates were significantly greater in the diabetic than in the control rats ($P < 0.01$). Sodium uptake rates were significantly lower in the difluoromethylornithine-treated than in the corresponding nontreated animals ($P < 0.01$). V_{max} for Na^+ transport was 32.6 ± 2.5 nmol/mg protein in the diabetic rats, significantly greater than that in control rats (15.2 ± 0.8 nmol/mg protein; $P < 0.001$). V_{max} for Na^+ transport in the difluoromethylornithine-treated diabetic (11.7 ± 0.9) and control (5.4 ± 0.5) rats were statistically significantly lower than the corresponding values in the nontreated rats ($P < 0.001$). K_m values were not statistically significantly different in the control (24 ± 4) and diabetic (31 ± 4) and in the difluoromethylornithine-treated diabetic (25 ± 4) and difluoromethylornithine-treated control (21 ± 6) rats.

Correlation Between Mucosal Ornithine Decarboxylase and Brush Border Membrane Na^+-H^+ Exchange Activities. As shown in Figure 3, a highly significant direct correlation was observed between mu-

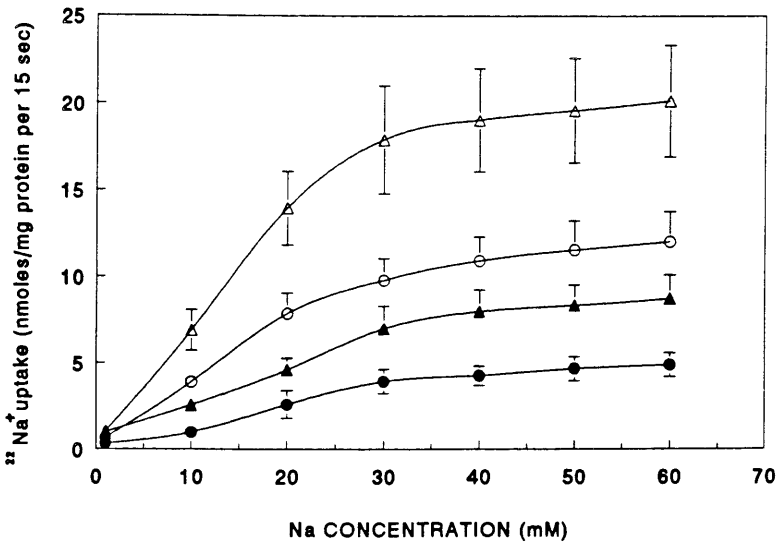

Figure 2. $^{22}Na^+$ uptake rates by jejunal brush border membrane vesicles of streptozotocin-diabetic (△), control (○), and difluoromethylornithine-treated diabetic (▲) and control (●) rats. $^{22}Na^+$ uptake rates by brush border membrane vesicles were measured at 25°C against a H^+ ion gradient (pH_i 5.5/pH_o 7.5) from media containing different sodium concentrations. Incubation time was 15 sec for all determinations. Brush border membrane vesicles were preincubated for 1 hr at 25°C with 40 mmol Hepes, 90 mmol morpholino-ethane-sulfonic acid buffer (pH 5.5). The reaction was started by the addition of 25 μl of brush border membrane vesicles to 75 μl of medium containing 85 mmol Hepes, 45 mmol Tris (pH 7.5), and various concentrations of sodium chloride (1–60 mmol) and $^{22}NaCl$ tracer. Each point represents mean (±SE) values of five to eight experiments. Mean values in diabetic and control rats were significantly greater than corresponding mean values in difluoromethylornithine-treated diabetic and control rats ($P < 0.01$–0.001).

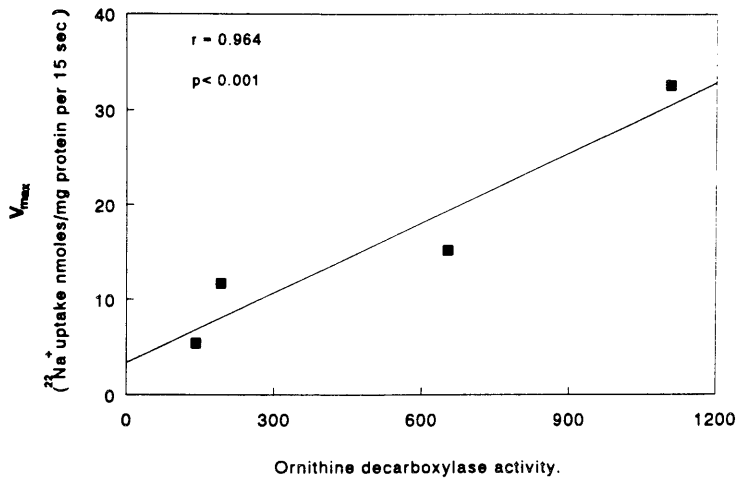

Figure 3. Correlation between jejunal mucosal ornithine decarboxylase activity and jejunal mucosal brush border membrane vesicle Na^+-H^+ exchange activity determined as V_{max} for $^{22}Na^+$ uptake by the brush border membrane vesicles in the diabetic, control, and difluoromethylornithine-treated diabetic and control rats.

cosal ornithine decarboxylase activity and brush border membrane vesicle V_{max} for Na^+ uptake. Similar highly significant correlations were observed between mucosal ornithine decarboxylase activity and DNA content ($r > 0.900$, $P < 0.001$)

Discussion

A close relationship between intestinal epithelial proliferation and ornithine decarboxylase activity has been shown in many situations in which intestinal epithelium undergoes hyperplasia, such as after refeeding of 48-hr fasted rats (5), during lactation (9), and weaning (11). We have recently shown this to be true during mucosal hyperplasia in streptozotocin-diabetic rats as well (4). The small intestinal brush border membrane Na^+-H^+ exchange activity is also closely associated with proliferation of intestinal epithelial cells of weaning (12) and of refed fasted rats (6) and has also been reported in streptozotocin-diabetic rats (13). The interrelations between ornithine decarboxylase activity

and brush border membrane Na^+-H^+ exchange activity and the mechanism(s) by which enhancements in brush border membrane Na^+-H^+ exchange and intracellular ornithine decarboxylase activity influence cell proliferation are not well understood.

Proliferation of many types of cells appears to be stimulated by increases in Na^+ influx (14) and intracellular pH (15). However, the concept that alkalinization of cells serves as a stimulus for mitogenesis is controversial (16), and a direct relationship between increased Na^+-H^+ exchange activity and hypertrophic stimuli could not be established in a previous study (17). In diabetic rats, the intestinal hyperplasia was dissociated from the enhanced brush border membrane Na^+-H^+ exchange activity by administration of $1,25(OH)_2D_3$, which corrected the transport abnormality, yet had no effect on intestinal mucosal mass (13). In the above study, intestinal mucosal ornithine decarboxylase activity was not measured.

Increase in activity of ornithine decarboxylase seems to be an intermediate event to the ionic and intracellular pH changes and the enhanced rate of DNA synthesis when cells undergo proliferation. However, it seems possible that under certain conditions, the enhancement in Na^+-H^+ exchange activity may be secondary to or be influenced by increased epithelial ornithine decarboxylase activity. The finding of the very strong positive correlation between ornithine decarboxylase activity and V_{max} for Na^+ uptake by brush border membrane vesicles in the four groups of rats in the present study supports the above hypothesis.

It has been thought that the stimulation of ornithine decarboxylase activity is linked to Na^+-H^+ exchange activity only when nutrients enter through the apical membrane, the location of the Na^+-H^+ antiport (5). In diabetic rats, intestinal absorption of glucose (18), amino acids (19, 20), and sodium (21) are increased while absorption of calcium is decreased (22, 23). In the present study diabetic rats consumed significantly more food than control rats and manifested higher ornithine decarboxylase and Na^+-H^+ exchange activity. However, in the difluoromethylornithine-treated diabetic rats, where mucosal growth and Na^+-H^+ exchange activity were suppressed, food consumption remained high, suggesting that Na^+-H^+ activity was related to ornithine decarboxylase activity rather than to food consumption.

Our finding of increased Na^+-H^+ exchange activity in intestinal brush border membrane vesicles of diabetic rats is similar to that reported previously (13). In agreement with the previous findings, kinetic analysis of the data showed increases in V_{max}, but not in K_m of $^{22}Na^+$ uptake by the brush border membrane vesicles. The factor(s) responsible for increased sodium uptake by brush border membranes of diabetic rats is not known. Diabetic rats are known to have increased serum corticosterone levels, but insulin treatment of diabetic rats failed to influence corticosterone levels, while Na^+-H^+ exchange activity was restored to normal (13). Dudeja *et al.* (13) also indicated that brush border membrane fluidity and metabolic acidosis did not seem to play major roles in the enhanced brush border membrane Na^+-H^+ exchange activity in diabetic rats. Treating diabetic rats, in whom $1,25(OH)_2D_3$ levels are low, with $1,25(OH)_2D_3$ restored the enhanced V_{max} of $^{22}Na^+$ uptake by the brush border membrane vesicles to normal (13). The modulation of brush border membrane Na^+-H^+ exchange activity in diabetic rats by $1,25(OH)_2D_3$ could be via alterations in enterocyte calcium concentrations brought about from the amount of calcium absorbed or that released from intracellular calcium stores. A close relationship between Na^+-H^+ exchange activity and intracellular calcium levels has been described previously (24, 25).

The findings of the present study showing significant positive correlations between intestinal mucosal hyperplasia, ornithine decarboxylase, and brush border membrane Na^+-H^+ exchange activity may be explained by changes in tyrosine kinase activity in the enterocytes brought about by enhancement in levels of polypeptide growth factor(s) such as enteroglucagon in the diabetic state.

Mitogenic polypeptides are known to enhance cell membrane Na^+-H^+ exchange activity (26). This enhancement seemed to depend upon the tyrosine kinase activity associated with the mitogen receptor (27). The intracellular polyamines, by reducing activity of the cellular protein tyrosine kinase phosphatases toward endogenous substrates, may allow protein tyrosine kinase activity to increase (28) and serve as a stimulus for the enhancement in membrane Na^+-H^+ exchange activity (25). Increased ornithine decarboxylase activity in the enterocytes of the diabetic rat is associated with increased enterocyte concentrations of the polyamines (4), which by reducing enterocyte protein tyrosine kinase phosphatase activity could enhance the activity of brush border membrane Na^+-H^+ exchanger. The enhancement in cell membrane Na^+-H^+ exchange activity by mitogens seemed to be associated with changes in intracellular pH that appear to be permissive, but not causal, in allowing cells to proceed through the cell cycle (27).

Cell membrane Na^+-H^+ exchange activity may have different isoforms. The function of the exchanger at the brush border membrane could be mainly to enhance sodium absorption from the intestinal lumen, while the isoform at the basolateral membrane may be more important in the regulation of intracellular pH and cell volume. Further studies are needed to determine whether indeed the jejunal enterocyte brush border membrane and basolateral membrane Na^+-H^+ exchange activity is modulated by the concentration of

the polyamines in the enterocytes via their effect on protein tyrosine kinase activity.

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1. Das R, Kanungo MS. Activity and modulation of ornithine decarboxylase and concentrations of polyamines in various tissues of rats as a function of age. Exp Gerontol **17**:95–103, 1982.
2. Fozard JR, Koch-Weser J. Pharmacological consequences of inhibition of polyamine biosynthesis with α-difluoro-methylornithine. Trends Pharmacol Sci **3**:107–110, 1982.
3. Russell DH. Roles of the polyamines, putrescine, spermidine and spermine in normal and malignant tissues. Life Sci **13**:1635–1647, 1973.
4. Younoszai MK, Parekh VV, Hoffman JL. Polyamines and intestinal epithelial hyperplasia in streptozotocin-diabetic rats. Proc Soc Exp Biol Med **202**:206–211, 1993.
5. Ulrich-Baker MG, Wang P, Fitzpatrick L, Johnson LR. Amiloride inhibits rat mucosal ornithine decarboxylase activity and DNA synthesis. Am J Physiol **254**:G408-G415, 1988.
6. Kikuchi K, Kikuchi T, Miwa T, Ghishan FK. Effect of dietary polyamines and α-difluoromethylornithine on regulation of intestinal Na^+/H^+ exchanger. J Dev Physiol **12**:163–167, 1989.
7. Younoszai MK, Schedl HP. Effects of diabetes on intestinal disaccharidase activities. J Lab Clin Med **79**:579–581, 1972.
8. Burton K. A study of conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J **62**:315–323, 1956.
9. Yang P, Baylin SB, Luk GD. Polyamines and intestinal growth: Absolute requirement for ODC activity in adaptation during lactation. Am J Physiol **247**:G553-G557, 1984.
10. Murer H, Hopfer U, Kinne R. Sodium/proton antiport in brush border membrane vesicles isolated from rat small intestine and kidney. Biochem J **154**:597–604, 1976.
11. Luk GD, Marton LJ, Baylin SB. Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. Science **210**:195–198, 1980.
12. Kikuchi K, Kikuchi T, Ghishan FK. Ontogeny of Na^+-H^+ exchanger in rat ileal brush border membrane vesicles. J Membr Biol **114**:257–263, 1990.
13. Dudeja PK, Ramesh KW, Klitzke A, Sitrin MD, Brasitus TA. Correction of enhanced Na^+/H^+ exchange of rat small intestinal brush-border membranes in streptozotocin-induced diabetes by insulin or 1,25-dihydroxycholecalciferol. J Clin Invest **87**:1755–1762, 1991.
14. Koch KS, Leffert HL. Increased sodium ion influx is necessary to initiate rat hepatocyte proliferation. Cell **18**:153–163, 1979.
15. Moolenaar WH, Tsien RY, Van der Saag PT, de Laat SW. Na^+/H^+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. Nature **304**:645–648, 1983.
16. Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes: Role in cellular proliferation. Biochem Biophys Acta **988**:73–97, 1989.
17. Harris RC, Brenner BM, Seifter JL. Sodium-hydrogen exchange and glucose transport in renal microvillus membrane vesicles from rats with diabetes mellitus. J Clin Invest **77**:724–733, 1986.
18. Schedl HP, Wilson HD. Effect of diabetes on intestinal growth and hexose transport in the rat. Am J Physiol **220**:1739–1745, 1971.
19. Younoszai MK, Schedl HP. Intestinal amino acid transport and tissue concentrations in diabetes. Am J Physiol **223**:828–831, 1972.
20. Lal D, Schedl HP. Intestinal adaptation in diabetes: Amino acid absorption. Am J Physiol **227**:827–831, 1974.
21. Schedl HP, Wilson HD. Jejunal sodium transport: Effects of alloxan diabetes. Biochim Biophys Acta **367**:225–231, 1974.
22. Schneider LE, Schedl HP. Diabetes and intestinal calcium absorption in the rat. Am J Physiol **223**:1319–1323, 1972.
23. Younoszai MK, Nathan R. Intestinal calcium absorption is enhanced by D-glucose in diabetic and control rats. Gastroenterology **88**:933–938, 1985.
24. Semrad CE, Chang EB. Calcium-mediated cyclic AMP inhibition of Na^+-H^+ exchange in small intestine. Am J Physiol **252**:C315-C322, 1987.
25. Donowitz M, Wicks J, Cusolito S, Sharp GWG. Cytosol free Ca^{++} in the regulation of active intestinal Na and Cl transport. In: Donowitz M, Ed. Mechanisms of Intestinal Electrolyte Transport and Regulation by Calcium. New York: Alan R. Liss, Inc., pp171–189, 1984.
26. Glaser L, Whiteley B. Control of ion fluxes by mitogenic polypeptides. Hypertension **10**:127–131, 1987.
27. Grinstein S, Rothstein A. Mechanisms of regulation of the Na^+-H^+ exchanger. J Membr Biol **90**:1–12, 1986.
28. Oetken C, Pessa-Morikawa T, Autero M, Anderson LC, Mustelin T. Reduced tyrosine phosphorylation in polyamine-starved cells. Exp Cell Res **202**:370–375, 1992.