Early Weaning Induces Jejunal Ornithine Decarboxylase and Cell Proliferation in Neonatal Rats

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ABSTRACT Increased ornithine decarboxylase (ODC) activity is associated with rapid cell proliferation in many cell types. The cellular effects of early weaning on intestinal development are not well established. To investigate whether ODC is involved in intestinal growth after early weaning, we precociously weaned suckling rats on postnatal d 15 and followed through d 21 (6 d after early weaning). Age-matched suckling pups served as controls. Rat pups were killed 1, 2, 3 and 6 d after early weaning and jejunal mucosa was assayed for ODC and sucrase activities, and protein and DNA contents. Jejunal cell proliferation was monitored by bromodeoxyuridine immunohistochemistry. Elevated jejunal ODC activity 1 d after early weaning was the earliest cellular event that was detected in the current study. ODC activity peaked at d 3 (about 15-fold greater than age-matched unweaned suckling controls). Sucrase activity was elevated at d 2 after weaning and peaked at d 3 (about 10-fold greater than controls). Greater bromodeoxyuridine immunostaining in early weaned rats occurred on d 3. Protein and DNA contents were greater in jejunal mucosa of early weaned rats at d 6. Serum corticosterone levels were elevated on d 1 and d 2 after early weaning compared to controls. To explore whether the intake of nonpurified diet played a role, we also compared the induction of jejunal ODC activity in early weaned pups and pups that were food-deprived for 1 d. ODC activity was not greater in the food-deprived group compared to suckling controls while the early weaned group had 6-fold greater activity 1 d after early weaning. Early weaning stimulates jejunal cell proliferation and differentiation. The temporal sequence of increased ODC activity followed by increases in other growth variables suggests that the induction of ODC activity may act as an early marker of intestinal growth during early weaning. J. Nutr. 128: 1636–1642, 1998.

KEY WORDS: rats • weaning • ornithine decarboxylase • intestinal growth • cell proliferation

Polyamines (putrescine, spermidine and spermine) are required for initiation of rapid cellular growth in many cell types (Jänne et al. 1978, Pegg and McCann 1982, Tabor and Tabor 1984, Williams-Ashman and Canellakis 1979). These compounds have been shown to facilitate DNA, RNA and protein synthesis in vitro (Jänne et al. 1978, Williams-Ashman and Canellakis 1979). The basal activity of ornithine decarboxylase (ODC, EC 4.1.1.17), the first and rate-limiting enzyme in polyamine biosynthesis, is low in quiescent cells and tissues. Its activity increases markedly during the initial stage of cell proliferation (Jänne et al. 1978, McCann 1980, Pegg and McCann 1982, Tabor and Tabor 1984). The increase of ODC activity is usually associated with a rapid accumulation of cellular polyamines (Jänne et al. 1978, McCann 1980, Oka and Perry 1976, Pegg and McCann 1982, Tabor and Tabor 1984). The induction of ODC activity and concomitant accumulation of cellular polyamines have been found to be critical and necessary during the process of adaptation/growth in the small intestine mucosa (Luk et al. 1980, McCann 1980, Yang et al. 1984). The rat small intestine undergoes major developmental changes during the third postnatal week, which corresponds to the time of weaning (Henning 1981). The major structural and functional changes of the rat small intestine during weaning have been related to dietary changes from high-fat maternal milk to high-carbohydrate nonpurified diet (Cox and Mueller 1937, Henning 1981). Previous studies showed an increase in intestinal sucrase and maltase activities in early weaned rats (Boyle and Koldovsky 1980, Lee and Lebenthal 1983). It is not clear whether the precocious expression of intestinal sucrase after early weaning involves only cell differentiation or both cell proliferation and differentiation. A recent study by Wu et al. (1996) showed increases in ODC activity of jejunal enterocytes in early weaned pigs. However,
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Information regarding the markers for cell proliferation such as mitotic index or DNA synthesis in intestine of early weaned rats is lacking. Since ODC activity increases markedly during the initial stage of cell proliferation and differentiation (Jänne et al. 1978, Pegg and McCann 1982, Russell and Durie 1978, Tabor and Tabor 1984, Williams-Ashman and Canellakis 1979), we hypothesize that it may act as an early marker of intestinal growth in early-weaning induced precocious maturation in rats. To understand the process of intestinal maturation in the early weaned rats, we investigated whether increased cellular proliferation occurred and whether changes in ODC activity were involved in the process. In the current study, we evaluated the sequential enzymatic changes of the rat small intestine in early weaned pups and simultaneously assessed proliferating activity by using 5-bromo-2'-deoxyuridine (BrdU) immunohistochemistry. In rats, glucocorticoids are important regulatory hormones for intestinal development because circulating concentrations of corticosterone (the principal glucocorticoid in this rodent) increase before the third postnatal week when rat intestine undergoes major developmental changes (Redman and Sreebny 1976). In the current study, we also measured serum corticosterone concentration in early weaned rats to assess its relationship with early weaning.

MATERIALS AND METHODS

Animals. Timed-pregnant Sprague-Dawley rats (Charles River, Wilmington, MA) were obtained on the 15th d of gestation and housed in individual cages with a 12-h light-dark cycle. The day of birth was regarded as d 0. On the second postnatal day, all pups were pooled and redistributed such that each dam had nine pups. Each dam with her group of pups was housed separately. Dams were provided with free access to nonpurified diet (Rodent Laboratory Chow 5001, Purina Mills, Inc., St. Louis, MO) and water. The proximate nutrient composition of this nonpurified diet is 30% of protein, 6% of fat and 64% of carbohydrate. Pups of both sexes were used in the experiments. This animal study was approved by the Animal Investigation Committee of Wayne State University and complied with the Guide for the Care and Use of Laboratory Animals (1985) of the National Institutes of Health.

Procedures. Six litters of rats were used for the early weaning study. On postnatal d 15, one pup from each litter was decripated to measure ODC and sacrose activities. The remaining pups (eight in each litter) were divided into two groups: i) early weaning and ii) unweaned suckling control. Pups in each group were redistributed into three litters with eight pups in each litter. The rat pups from the early weaning group were removed from their nursing dams and housed in special cages. Special care was taken in early weaned rats and included the following: Short cages (15 × 45 × 22 cm) were used to house early weaned rats so that the pups had easy access to food and water. Solid nonpurified diet was ground to a mixture of tiny pellets and powder and placed on the floor of the cages in containers that were readily accessible to the pups. Two drinking bottles with long nozzles were provided in each cage for easy water access. Heating pads were placed under the floor of the cages to provide warmth. Age-matched unweaned control pups with one nursing dam in each litter were housed in tall cages (20 × 45 × 22 cm). The tall cage was used to prevent the pups from reaching the nonpurified diet placed on top of the cage. Control pups were allowed to suckle freely until the time of decapitation. Daily body weight of pups was measured during the study period. Since there is no accurate way of quantifying the intake of small pellet/powdered nonpurified diet that is scattered over the cage in early weaning group, nor the intake of breast milk in control group, no attempt was made to measure food intake.

To determine whether the induction of ODC activity after early weaning is from the decreased food intake alone or a combination of other factors, we also conducted a food-deprivation study. In this experiment, two litters of 15-d-old suckling rats (nine pups per litter) were divided into three groups (n = 6): i) food-deprived, ii) early weaning and iii) unweaned suckling control. In the food-deprived group, pups were separated from their dams and placed in short cages without nonpurified diet. Other experimental conditions were the same as previously described in the early weaning study. The food-deprivation study lasted for 1 d. Intestinal ODC activities from each group were measured.

Tissue preparation. Rat pups (two pups from each litter with three litters in each group) from each group were decapitated on postnatal d 16, 17, 18 and 21 (i.e., 1, 2, 3 and 6 d after early weaning). One pup from each litter was injected intraperitoneally with BrdU at a dose of 45 mg/kg body weight 1 h before decapitation. In the food-deprivation study, all pups were decapitated on postnatal d 16 (i.e., 1 d after food-deprivation). Animals were decapitated between 11:00 and 12:00 h after at least a 3-h isolation to minimize stress. Immediately after death, trunk blood was collected, and the small intestine (from the ligament of Treitz to the ileocecal junction) was removed, placed immediately on ice, trimmed of excess fat and mesentery and weighed. The intestinal contents were removed by gentle flushing with iced saline. The proximal half of the small intestine was defined as the jejenum and used for the experiments. The intestinal segment was opened and the mucosa was scraped and weighed. The mucosal preparations were placed in liquid nitrogen immediately after food deprivation and stored at −80°C for enzyme and biochemical analyses.

In rats injected with BrdU, a 0.5-cm segment located 2 cm distal to the ligament of Treitz was circumferentially cut, fixed in Hollan’s fixative for 2 h, stored in 70% ethanol overnight, embedded in paraffin, cut into histological sections (4 μm thick) and processed for BrdU immunohistochemistry. Trunk blood samples were centrifuged at 5,000 × g and serum samples were stored at −20°C for the measurement of corticosterone.

Biochemical determinations. All reagents were of reagent grade and obtained from Sigma Chemical Co. (St. Louis, MO). ODC activity was measured in intestine mucosal homogenates by the quantification of 14CO2 liberated from [carboxyl-14C]ornithine according to Seely and Pegg (1983) with slight modifications as described previously (Lin et al. 1991). Intestinal mucosal tissues were homogenized 1:10 (wt/vol) in 45 mmol/L phosphate buffer (pH 7.2) containing 0.1 mmol/L EDTA and 3 mmol/L dithiothreitol and then centrifuged at 100,000 g for 1 h at 37°C. Enzyme activity was expressed as pmol·mg protein −1·h −1. To verify the validity of ODC assay from frozen tissue, we performed the experiment to compare intestinal ODC activities between fresh tissues and frozen tissues of 15-d-old rats. ODC activity did not differ between fresh and frozen tissues (data not shown).

Sucrase (EC 3.2.1.48) activity was assayed by the method of Dahlqvist (1965), which uses sucrose as the substrate. Sucrase activities were expressed as μmol sucrose hydrolyzed/mg protein. Serum corticosterone was measured by competitive protein-binding assay as described by Henning (1980). Triplicate aliquots of serum (0.05 mL) were extracted with 0.35 mL of absolute ethanol at room temperature. Precipitated proteins were removed by centrifugation, and supernatant extract was pipetted into a test tube. After complete evaporation, 0.25 mL of the 1H-corticosterone/phosphat/corticosterone-binding globulin (CBG) mixture was added to each tube. Serum for the use as a source of CBG was obtained from an adult female rat. All tubes were incubated in ice water for 60 min. Dextran-coated charcoal (0.03 mL) was added to each tube. The test tube rack was shaken 25 times by hand. Exactly 5 min after shaking began, samples were centrifuged at 300 × g at 4°C for 5 min. Protein-bound radioactivity was determined by counting 0.2 mL of the supernatant fluid in 2 mL of scintillation fluid.

Protein was determined by the Bradford procedure (Bradford 1976) using the Bio-Rad protein reagent (Bio-Rad Laboratories, Hercules, CA) and bovine γ-globulin as the standard. DNA was measured by a fluorometric method (Prasad et al. 1972) using highly purified calf thymus DNA as the standard.

**Determination of BrdU immunohistochemistry.** After deparaffinization and hydration, sections (4 μm) of proximal small intestine...
were incubated at ambient temperature with anti-BrdU monoclonal antibody (Amersham) for 1 h. The avidin-biotin technique was then performed with matched components (secondary-biotinylated antibody and avidin-peroxidase complex) of the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to manufacturers instructions. Diaminobenzidine, together with cobalt and nickel ions to intensify and increase the contrast of the resulting stain, was used as chromogen to localize BrdU immunoreactivity. Sections were counterstained with methyl green. Cells were considered labeled when nucleus showed dense reaction products. For morphometric analysis, the enterocyte nuclei labeled with BrdU were counted. Five slides from each sample were examined under high-powered field. Ten well-oriented crypts and villi from each slide were examined. A minimum of 600 enterocytes per slide was counted.

**Statistical analyses.** Results are reported as the means ± SEM. Differences in mean values between any two groups were evaluated by the Student’s t-test with \( P < 0.05 \) considered as statistically significant (Armitage and Berry, 1994).

**RESULTS**

**Effect of early weaning on body weight.** In the early weaning group, pups lost weight 1 d after early weaning. Their weights were lower than age-matched suckling controls from 1 to 4 d after early weaning (Fig. 1). Gradual weight gain was observed after d 2 of early weaning. By d 5 and 6 after early weaning, no difference in body weight was observed between suckling control and early weaning groups.

**Effect of early weaning on jejunal development.** One day after early weaning, the early-weaned pups had lower jejunal mucosal weight and DNA content than unweaned controls although the protein content was not significantly different (Fig. 2). Jejunal growth occurred from d 2 of early weaning because mucosal weight, protein and DNA contents were not different from those of suckling controls on d 2 and 3. Jejunal growth continued to accelerate as indicated by the significantly higher jejunal mucosal weight and protein and DNA contents in pups 6 d after early weaning compared to suckling controls (Fig. 2).

Significantly greater jejunal sucrase activity was observed 2 d after early weaning and persisted throughout subsequent days of the study compared to age-matched suckling controls (Fig. 3). A maximal rise in sucrase activity (13-fold) was observed 3 d after early weaning. Six days after early weaning sucrase activity had declined about 50% but still remained higher than in the suckling controls.

**Effect of early weaning on jejunal ODC activities.** Jejunum mucosal ODC activity on d 1–6 after early weaning was significantly greater than in age-matched suckling controls, with the maximal difference occurring on d 3 (Fig. 4).

**BrdU immunostaining after early weaning.** In the presence of cobalt and nickel, immunostaining for BrdU resulted in blue-black staining in the nucleus of positive enterocytes in the proliferative crypt compartment (Fig. 5). The number of BrdU-positive cells (expressed as BrdU-labeled cells/300 enterocytes) in early weaned rats was significantly higher 3 d after early weaning than in suckling controls (Fig. 6).

**Effect of early weaning on serum corticosterone levels.** Serum corticosterone levels were significantly higher in early weaned pups than in suckling controls on d 1 and 2 after early weaning.
weaning with the greatest difference (2.5-fold) on d 1 (Table 1). Concentrations did not differ on d 3 after weaning and by d 6, serum corticosterone was greater in controls than in early weaned pups.

Comparison of jejunal ODC activity between early weaning and food-deprived groups. Early weaning pups lost weight 1 d after early weaning (Fig. 1). To determine whether the induction of ODC activity after early weaning is from the decreased food intake alone or a combination of other factors, we conducted a food-deprivation study. Mucosal ODC activity was significantly higher (6-fold) in early weaning group than in suckling controls. However, no difference in ODC activity was observed between the food-deprived group and suckling controls (Fig. 7).

DISCUSSION

Infant feeding practices and timing of weaning vary greatly in the United States and other countries. Indeed, recommendations for this important aspect of infant care differ consid-
Early weaning in rats induces precocious expression of sucrase activity (Boyle and Koldovsky 1980, Lee and Leman 1983). However, it is unclear whether the precocious expression of intestinal sucrase results from cell differentiation alone or both cell proliferation and differentiation. Traditionally, the study of cell proliferation involves the use of 3H-thymidine to monitor DNA synthesis in individual cells by autoradiography. Recently, a rapid and nonradioactive technique has been developed in which a thymidine analog, BrdU, is incorporated into the replicating DNA and subsequently localized using a specific monoclonal antibody in histological sections (Grazner 1982, Wilson et al. 1985). This technique of labeling cells in the synthesis (S) phase has been applied to intestinal tissues, and the result is comparable to that of 3H-thymidine labeling (Lacy et al. 1991). In the current study, we utilized BrdU immunostaining and the measurement of ODC activity to examine jejunal cell proliferation after early weaning. Our results showed higher cell proliferation in early weaned rats as indicated by the greater number of BrdU-positive cells, mainly located in the crypt compartment, 3 d after early weaning (Figs. 5 and 6). Jejunal ODC activity was significantly greater than in controls as early as d 1 after weaning (Figs. 5 and 6). Significantly different from suckling controls, *P < 0.05, **P < 0.005.

Both hormonal factors such as glucocorticoids and thyroxine (Boyle and Koldovsky 1980, Lee and Leman 1983, Lerman and Koldovsky 1979) and nutritional factors (Castillo et al. 1990, Raul et al. 1978) have been implicated in the maturation processes of weaning and early weaning. Glucocorticoids play an important role in the induction of sucrase activity in suckling rats (Boyle and Koldovsky 1980, Lebenthal et al. 1972). Administration of cortisone resulted in a precocious induction of jejunal sucrase activity 24 h after injection (Herbst and Koldovsky 1972). Fujimoto et al. (1978) reported that the administration of hydrocortisone to adult rats resulted in the induction of small intestinal ODC activity 6–7 h after injection. Nsi-Envo et al. (1996) also reported that treatment of 12-d-old suckling rats with hydrocortisone resulted in an increase of ODC mRNA 4 h after treatment. The results from jejunal enterocytes in early weaned pigs. The temporal sequence of cellular events after early weaning is consistent with the mucosal hyperplasia model outlined by Luk and Baylin (1984). Within 24 h after early weaning, mucosal ODC activity increased. This is closely followed by the increase in DNA synthesis/cell proliferation on d 3 and finally, the increase in DNA contents and mucosal hyperplasia by d 6. Since the induction of intestinal ODC activity precedes changes in sucrase, BrdU immunostaining, DNA and protein, the induction of intestinal ODC activity may act as an early marker of intestinal growth during early weaning.

**TABLE 1**

<table>
<thead>
<tr>
<th>Days (after weaning)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckling control</td>
<td>240 ± 88</td>
<td>160 ± 47</td>
<td>237 ± 33</td>
<td>422 ± 154</td>
</tr>
<tr>
<td>Early weaned</td>
<td>863 ± 82**</td>
<td>519 ± 146*</td>
<td>232 ± 72</td>
<td>113 ± 49*</td>
</tr>
</tbody>
</table>

1 Suckling pups were early weaned at d 15 and followed through d 21.
2 Values are means ± SEM, (n = 6). Significantly different from suckling controls, *P < 0.05, **P < 0.005.

**FIGURE 7** Jejunal ornithine decarboxylase (ODC) activity in control, early weaned and food-deprived neonatal rats. Suckling rats (d 15) were divided into three groups: C—suckling controls, F—food-deprived group and W—early weaning group. In food-deprived group and early weaning group, the pups were separated from nursing dams at d 15. No food was provided in the food-deprived group, while drinking water was consumed ad libitum. Intestinal samples were obtained at d 16 (1 d after early weaning). Values are means ± SEM, n = 6. **P < 0.001, significantly different from age-matched suckling controls.
our sequential studies of the serum corticosterone levels in early weaned rats showed an early surge of serum corticoste-
ron on d 1 and 2 after weaning (Table 1). These results suggest a possible involvement of corticosterone in the initial stage of early weaning. Further studies applying glucocorticoid antagonist (RU-38486) in evaluating the cellular events after early weaning are needed to confirm the possible role of ODC and corticosterone in early weaning.

Early weaning represents an abrupt change in the compo-
sition of the diet, i.e., a shift from high-fat maternal milk (Cox and Mueller 1937) to high-carbohydrate nonpurified diet (5× energy %). The type of carbohydrate also differs, i.e., glucose polymers and sucrose in nonpurified diet vs. lactose in rat milk (Lee and Lebenthal 1983). These dietary constituents might also be important factors affecting mucosal changes. Other possible dietary factors are the dietary amines (dimethylamine, ethylamine and methylamine) in the nonpurified diet. These dietary amines are trophic stimuli for gastrointestinal tract mucosa (Lichtenberger et al. 1982) and have been shown to induce intestinal ODC activity (Tabata and Johnson 1986a). Besides the possible effect of corticosterone on the induction of intestinal ODC activity, the dietary differences in the differing dietary carbohydrate in milk and non-purified diet may also be responsible for the stimulation of ODC activity following early weaning. Dietary factors probably played a more prominent role in the stimulation of intestinal ODC activities at the later stage (3–6 d after early weaning) when serum corticosterone concentrations were not elevated (Table 1) and the diet was the only factor differing in early weaned and suckling control groups.

Our results from the food deprivation study demonstrated that the induction of intestinal ODC activity in the early weaned group was not due to lower food intake (Fig. 7). The lack of elevation in intestinal ODC activity in food-deprived rats is in agreement with other studies (Luk and Yang 1988, Moore and Swenseid 1983, Tabata and Johnson 1986a, 1986b), which all showed a reduction in ODC activity in rats deprived of food for 18–48 h. Tabata and Johnson (1986a) reported 15-fold increases in intestinal ODC activity 2 h after food-deprived rats began feeding normally and concluded that activation of intestinal ODC following feeding was by both direct stimulation from luminal contents and by humoral effects. They suggested that direct effects provided more stimulation than humoral factors. Possibly the small amount of diet also be important factors affecting mucosal changes. Other dietary factors are the dietary amines (dimethylamine, ethylamine and methylamine) in the nonpurified diet. These dietary amines are trophic stimuli for gastrointestinal tract mucosa (Lichtenberger et al. 1982) and have been shown to induce intestinal ODC activity (Tabata and Johnson 1986a). Besides the possible effect of corticosterone on the induction of intestinal ODC activity, the dietary differences in the differing dietary carbohydrate in milk and non-purified diet may also be responsible for the stimulation of ODC activity following early weaning. Dietary factors probably played a more prominent role in the stimulation of intestinal ODC activities at the later stage (3–6 d after early weaning) when serum corticosterone concentrations were not elevated (Table 1) and the diet was the only factor differing in early weaned and suckling control groups.

In summary, our findings demonstrate that early weaning stimulates jejunal cell proliferation and differentiation as in-
dicated by increased BrdU immunostaining and increased su-
crease activity. Induction of jejunal ODC activity after early weaning was the earliest cellular event measured in the current study, followed by an increase in BrdU incorporation and an increase in the DNA and protein contents. The temporal sequence of ODC increase followed by increases in other growth variables suggests that the induction of ODC activity may act as an early marker of intestinal growth during the process of early weaning.

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LITERATURE CITED


Boyle, J. T., & Koldovsky, O. (1989) Critical role of adrenal glands in precocious increase in jejunal sucrase activity following premature weaning in rats: neg-


Gratzner, H. G. (1982) Monoclonal antibody to 5-bromo- and 5-iododeoxyurini-


Moore, P. & Swenseid, M. E. (1983) Dietary regulation of the activities of orni-


Oka, T. & Perry, J. W. (1976) Studies on regulatory factors of ornithine decar-