A cortisol surge mediates the enhanced polyamine synthesis in porcine enterocytes during weaning

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Wu, Guoyao, Nick E. Flynn, Darrell A. Knabe, and Laurie A. Jaeger. A cortisol surge mediates the enhanced polyamine synthesis in porcine enterocytes during weaning. Am J Physiol Regulatory Integrative Comp Physiol 279: R554–R559, 2000.—This study was conducted to determine whether a cortisol surge mediates the enhanced expression of intestinal ornithine decarboxylase (ODC) in weanling pigs. Piglets were nursed by sows until 21 days of age, when 40 pigs were randomly assigned into one of four groups (10 animals/group). Group 1 continued to be fed by sows, whereas groups 2–4 were weaned to a corn and soybean meal-based diet. Weaning pigs received intramuscular injections of vehicle solvent (sesame oil), RU-486 (a potent blocker of glucocorticoid receptors; 10 mg/kg body wt), and metyrapone (an inhibitor of adrenal cortisol synthesis; 5 mg/kg body wt), respectively, 5 min before weaning and 24 and 72 h later. At 29 days of age, pigs were used to prepare jejunal enterocytes for ODC assay and metabolic studies. To determine polyamine (putrescine, spermidine, and spermine) synthesis, enterocytes were incubated for 45 min at 37°C in 2 ml Krebs-bicarbonate buffer containing 1 mM [U-14C]arginine, 1 mM [U-14C]ornithine, 1 mM [U-14C]glutamine, or 1 mM [U-14C]proline plus 1 mM glucose. Weaning increased intestinal ODC activity by 230% and polyamine synthesis from ornithine, arginine, and proline by 72–157%. Arginine was a quantitatively more important substrate than proline for intestinal polyamine synthesis in weaned pigs. Administration of RU-486 or metyrapone to weaning pigs prevented the increases in intestinal ODC activity and polyamine synthesis, reduced intracellular polyamine concentrations, and decreased villus heights and intestinal growth. Our results demonstrate an essential role for a cortisol surge in enhancing intestinal polyamine synthesis during weaning, which may be of physiological importance for intestinal adaptation and remodeling.

WEANING IS ASSOCIATED WITH a marked increase in intestinal activity of ornithine decarboxylase (ODC) in animals, including rats (13) and pigs (28). ODC is the first and key regulatory enzyme in the synthesis of polyamines (putrescine, spermidine, and spermine) from ornithine (17). A metabolic source of intestinal ornithine is arginine via type II arginase (a mitochondrial enzyme), which is induced in enterocytes of weanling pigs (7). Polyamines are essential to growth, differentiation, and migration of intestinal epithelial cells and may play an important role in regulating intestinal maturation and remodeling (11). Thus elucidating the mechanism responsible for the enhanced expression of intestinal ODC during weaning will increase our understanding of intestinal adaptation under stress conditions.

Plasma concentrations of glucocorticoids are markedly increased in pigs and rats during weaning (10, 13, 22). Hydrocortisone (Hyd; cortisol) is the major circulating glucocorticoid in pigs and humans (2, 22). The administration of cortisol to 12-day-old suckling rats (15), adult rats (21), or 21-day-old suckling pigs (26) has been reported to increase intestinal ODC activity. These findings have led to the suggestion that glucocorticoids play a crucial role in mediating the enhanced expression of intestinal ODC in weanling mammals (13, 28). However, as pointed out by Lin et al. (13), further studies using glucocorticoid antagonists are necessary to firmly establish this purported role of glucocorticoids. Thus an objective of this study was to employ the piglet model to test the hypothesis that a glucocorticoid surge plays an important role in regulating the expression of intestinal ODC during weaning. Our strategy for testing this hypothesis involved the administration of RU-486 [a potent blocker of glucocorticoid receptors (1)] or metyrapone [an inhibitor of adrenal cortisol synthesis (18)] to weaning piglets. Antagonizing the action of elevated plasma cortisol and preventing a cortisol surge will provide useful tools to evaluate a role for cortisol in mediating the induction of intestinal ODC during weaning.

An increase in enzyme activity measured under in vitro assay conditions does not necessarily indicate an enhanced metabolic flux or product formation in cells (6). At present, little information is available on intestinal polyamine synthesis in weaned animals. Additionally, the metabolic sources of ornithine for intestinal polyamine synthesis remain to be identified. Thus another objective of this study was to determine intestinal polyamine synthesis from potential substrates (arginine, ornithine, proline, and glutamine) in weaned pigs.
pigs. Proline and glutamine were used as potential substrates for polyamine synthesis, because recent studies have shown that mitochondria of intestinal epithelial cells are capable of converting these two amino acids into ornithine via proline oxidase and pyrroline-5-carboxylate synthase, respectively (24, 27).

**MATERIALS AND METHODS**

**Chemicals.** HPLC-grade methanol and water were purchased from Fisher Scientific (Houston, TX). L-[U-14C]ornithine, L-[U-14C]arginine, L-[U-14C]proline, and L-[U-14C]glutamine were obtained from American Radiolabeled Chemicals (St. Louis, MO). Hydrocortisone-21-acetate, RU-486 [mifepristone; 17-β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(prop-1-ynyl)estra-4,9-dien-3-one], metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone), sesame oil, and all other chemicals were purchased from Sigma Chemicals (St. Louis, MO).

**Animals.** Pigs were offspring of Yorkshire × Landrace sows and Duroc × Hampshire boars and were maintained at the Texas A&M University Veterinary Research Park. Piglets were freely nursed by their mothers until 21 days of age, when 40 pigs were randomly assigned into one of four groups (10 animals/group). Group 1 continued to be fed by sows, whereas groups 2–4 were weaned to a corn and soybean meal-based diet (29). Groups 2–4 received intramuscular injections of vehicle solvent (sesame oil), RU-486 (10 mg/kg body wt), and metyrapone (5 mg/kg body wt), respectively, 5 min before weaning and 24 and 72 h later. This period of RU-486 or metyrapone administration corresponded to the cortisol surge exhibited in weaning pigs (3). The dose of RU-486 was based on previous studies with a number of species, including rats, humans, and guinea pigs (1), as well as piglets (7–9). The dose of metyrapone was chosen on the basis of previous studies with piglets (16, 18). Immediately before metyrapone or RU-486 administration, and at days 2 and 8 postadministration, blood samples (3 ml) were obtained from the external jugular vein for measuring cortisol concentrations using a cortisol kit (9). After blood collection at 29 days of age, pigs were killed between 10:00 and 11:00 AM to obtain the whole small intestine, as previously described (27–29). Small intestine weights and lengths were measured after intestinal contents were thoroughly removed with saline. This study was carried out in accordance with the guidelines of the United States Research Council for the care and use of animals and was approved by the Texas A&M University Institutional Animal Care and Use Committee.

**Preparation and incubation of jejunal enterocytes.** The jejunum was washed three times with saline to remove luminal content and then used for preparing enterocytes with use of oxygenated (95% O2/5% CO2) KHB buffer containing 1 mM glucose, as previously described (27, 28). Cells (25 mg protein/ml) were incubated for 0 or 45 min at 37°C in 2 ml of oxygenated (95% O2/5% CO2) KHB buffer containing 1 mM L-methionine and one of the following: 1) 1 mM L-arginine plus 2 μCi L-[U-14C]arginine, 2) 1 mM L-ornithine plus 2 μCi L-[U-14C]ornithine, 3) 1 mM L-glutamine plus 2 μCi L-[U-14C]glutamine, or 4) 1 mM L-proline plus 2 μCi L-[U-14C]proline plus 1 mM L-glutamine. Methionine was used as the precursor for S-adenosylmethionine and subsequently S-decarboxylated S-adenosylmethionine for spermidine and spermine synthesis (17). Glutamate was required to convert proline-derived P5C into ornithine by ornithine aminotransferase (24); therefore, glutamine, which is metabolized to glutamate by mitochondrial phosphate-dependent glutaminase (27), was added to the incubation medium containing L-[U-14C]proline. [14C]-labeled substrates were used to improve the sensitivity of detecting polyamine synthesis in enterocytes. Incubations were terminated by addition of 0.2 ml of 1.5 M HClO4, and the acidified medium was neutralized with 0.1 ml of 2 M KOH (28). The neutralized extracts were used for analysis of amino acids and [14C]polyamines by HPLC methods (30). Net production of [14C]ornithine was measured by determining the accumulation of [14C]ornithine in cells plus incubation medium, as previously described (24). Incubated enterocytes remained viable for 45 min on the basis of linear O2 consumption, as determined with the use of Clark-type polarographic oxygen probes (27).

**Analysis of polyamines and [14C]polyamines.** Polyamines were determined in freshly isolated enterocytes by an HPLC method involving precolumn derivatization with o-phthalaldehyde as described by Wu et al. (30). For determination of [14C]putrescine, [14C]spermidine, and [14C]spermine in extracts of incubated enterocytes, fractions containing [14C]putrescine, [14C]spermidine, and [14C]spermine were separately collected from the HPLC column. Their radioactivities were measured by a liquid scintillation counter (24). Blank (0 min incubation) radioactivities were subtracted from sample values. Rates of production of putrescine, spermidine, and spermine after the 45-min incubation period were calculated on the basis of intracellular specific activities of [14C]ornithine, which were measured as described by Wu (24).

**Determination of ODC activity.** The cytosolic fraction of enterocytes was prepared and used for measuring ODC activity using 0.2 mM [1-14C]ornithine, as previously described (28).

**Examination of intestinal morphology.** Villus height, crypt depth, and lamina propria depth in jejunum were measured as previously described (29), except that intestinal tissues were fixed with 4% paraformaldehyde.

**Determination of protein.** Protein in enterocytes and the cytosolic fraction were determined by a modified Lowry procedure using BSA as a standard (27).

**Statistical analysis.** Data were analyzed by one- or two-way ANOVA and the Student-Newman-Keuls multiple comparison test (20). Probability values <0.05 were taken to indicate statistical significance.

**RESULTS**

**Plasma cortisol concentrations.** Plasma cortisol concentrations in pigs are shown in Table 1. Early weaning of piglets at 21 days of age resulted in an increase in cortisol concentrations as compared to those of unweaned pigs, whereas plasma cortisol concentrations increased on day 8 postadministration of RU-486 and metyrapone.

**Table 1. Effects of RU-486 and metyrapone administration on plasma concentrations of cortisol in weaned piglets**

<table>
<thead>
<tr>
<th>Age of Piglets, days</th>
<th>Unweaned</th>
<th>Weaned</th>
<th>Weaned + RU-486</th>
<th>Weaned + metyrapone</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>20.7 ± 2.8</td>
<td>21.9 ± 2.8</td>
<td>21.4 ± 2.5</td>
<td>23.1 ± 3.0</td>
</tr>
<tr>
<td>23</td>
<td>21.3 ± 2.4</td>
<td>82.6 ± 7.7</td>
<td>83.0 ± 11.2</td>
<td>24.6 ± 4.3</td>
</tr>
<tr>
<td>29</td>
<td>21.9 ± 2.6</td>
<td>22.5 ± 2.8</td>
<td>87.5 ± 9.4</td>
<td>20.7 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE in μg/l; n = 10 piglets. Plasma was obtained from 21-, 23-, and 29-day-old unweaned piglets and from age-matched weaned piglets treated with vehicle solvent, RU-486, or metyrapone. *P < 0.01, different from the values for unweaned and weaned + metyrapone groups; †P < 0.01, different from the values for all other three groups; ‡ and §, means within a row with different symbols are different (P < 0.05).
piglets decreased ($P < 0.05$) jejunal villus heights and crypt and lamina propria depth compared with untreated weaned pigs and with unweaned pigs.

**ODC activity and polyamine synthesis.** Enterocyte ODC activity (Table 4) and polyamine synthesis from ornithine, arginine, and proline (Table 5) were 230% and 72–157% higher (Table 4), respectively, in 29-day-old weaned pigs compared with age-matched unweaned pigs. Arginine was a quantitatively more important substrate than proline for intestinal polyamine synthesis in weaned pigs. Administration of RU-486 or metyrapone to weanling pigs attenuated the increase in intestinal ODC activity and polyamine synthesis. There was no detectable synthesis of polyamines from arginine in enterocytes of suckling pigs or from glutamine in enterocytes of unweaned and weaned pigs.

**Polyamine concentrations.** Enterocyte polyamine concentrations did not differ ($P > 0.05$) between 29-day-old weaned pigs and age-matched unweaned pigs (Table 6). Administration of RU-486 or metyrapone to weanling pigs decreased ($P < 0.05$) enterocyte concentrations of putrescine, spermidine, and spermine compared with untreated weaned pigs and unweaned pigs.

**Production of ornithine from arginine, proline, and glutamine in enterocytes.** Large amounts of ornithine were produced from proline in pig enterocytes (Table 7). However, substantial amounts of ornithine were produced from arginine in enterocytes of weaned pigs, but not unweaned pigs. Net production of ornithine from glutamine was much lower ($P < 0.01$) than that from proline in all groups of pigs. Administration of RU-486 or metyrapone to weanling pigs attenuated...
Table 5. Effects of RU-486 and metyrapone administration on polyamine synthesis in enterocytes of weaned piglets

<table>
<thead>
<tr>
<th>Addition to Incubation Medium (1 mM each)</th>
<th>Unweaned Piglets</th>
<th>Weaned Piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>RU-486</td>
</tr>
<tr>
<td>Putrescine</td>
<td>9.1 ± 1.5†</td>
<td>23.4 ± 2.1†</td>
</tr>
<tr>
<td>Ornithine</td>
<td>7.1 ± 0.67†</td>
<td>2.4 ± 0.22†</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.2 ± 0.37†</td>
<td>5.5 ± 0.60†</td>
</tr>
<tr>
<td>Proline</td>
<td>24.0 ± 2.8†</td>
<td>59.6 ± 6.3*</td>
</tr>
<tr>
<td>Ornithine</td>
<td>19.3 ± 2.4*</td>
<td>5.3 ± 0.46†</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.5 ± 0.72†</td>
<td>12.4 ± 1.7*</td>
</tr>
<tr>
<td>Proline</td>
<td>26.7 ± 3.2†</td>
<td>54.2 ± 6.7*</td>
</tr>
<tr>
<td>Ornithine</td>
<td>16.3 ± 1.9*</td>
<td>4.2 ± 0.44†</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.8 ± 0.57†</td>
<td>10.2 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol/mg protein. Values differing by at least 30% compared with age-matched weaned piglets treated with vehicle solvent, RU-486, or metyrapone are designated with a star (*). Values differing by at least 60% compared with age-matched weaned piglets treated with vehicle solvent, RU-486, or metyrapone are designated with a double star (**). Values differing by at least 90% compared with age-matched weaned piglets treated with vehicle solvent, RU-486, or metyrapone are designated with a triple star (***) (P < 0.01) the increase in ornithine production from arginine.

**DISCUSSION**

Role of glucocorticoids in mediating the enhanced intestinal ODC activity and polyamine synthesis during weaning. Our results demonstrate increases in both ODC activity and polyamine synthesis in enterocytes of weaning pigs. To provide direct evidence for glucocorticoids in mediating the induction of intestinal ODC activity for polyamine synthesis during weaning, we employed RU-486 and metyrapone to block the action of cortisol and prevent a cortisol surge, respectively. Because a cortisol surge occurs in weaning piglets on day 2 postweaning (3), RU-486 or metyrapone was administered to piglets 5 min before weaning and 24 and 72 h later to prevent the action of elevated plasma cortisol or a cortisol surge, respectively. As reported for humans (2), RU-486 administration increased plasma cortisol concentrations in piglets on days 2 and 8 posttreatment compared with untreated weaned pigs (Table 1), likely due to hypersecretion of cortisol from the adrenal cortex (2). A novel, important finding of this study is that the administration of RU-486 to weaning pigs abolished the increases in intestinal ODC activity and polyamine synthesis, regardless of plasma cortisol concentrations. These results indicate that cortisol plays an important role in regulating enterocyte ODC activity and polyamine synthesis via a glucocorticoid receptor-mediated mechanism in weaning pigs.

Although rates of intestinal polyamine synthesis were higher in 29-day-old weaned pigs (not treated with RU-486 or metyrapone) than in age-matched sowfed pigs (Table 5), intracellular polyamine concentrations did not differ between these two groups of pigs (Table 6). This result may be explained, in part, by the contribution of milk-born polyamines to intestinal polyamine concentrations in suckling pigs (14). Among weaned pigs, polyamine concentrations were lower in RU-486- or metyrapone-treated pigs than in untreated pigs (Table 6). These results indicate that changes in intestinal ODC activity result in altered polyamine synthesis and suggest that de novo synthesis plays a role in modulating intestinal polyamine concentrations during weaning.

Role of amino acids in intestinal polyamine synthesis. Another important novel finding of this study is the relative importance of potential substrates for polyamine synthesis in enterocytes during weaning. Ornithine is the immediate precursor for the synthesis of putrescine by ODC. However, because the conventional weaning diet for piglets contains little or no ornithine (9) and because there is little uptake of arginine into enterocytes of weaned piglets treated with vehicle solvent, RU-486, or metyrapone. Means sharing different symbols within a row are different (P < 0.01).

Table 7. Effects of RU-486 and metyrapone administration on net production of ornithine in enterocytes of weaned piglets

<table>
<thead>
<tr>
<th>Addition to Incubation Medium (1 mM each)</th>
<th>Unweaned Piglets</th>
<th>Weaned Piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>RU-486</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.34 ± 0.03‡</td>
<td>12.8 ± 1.5*</td>
</tr>
<tr>
<td>Proline</td>
<td>5.89 ± 0.62</td>
<td>6.18 ± 0.67</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.32 ± 0.04†</td>
<td>0.68 ± 0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol·mg protein⁻¹·45 min⁻¹; n = 10. Jeunal enterocytes were prepared from 29-day-old unweaned piglets and from age-matched weaned piglets treated with vehicle solvent, RU-486, or metyrapone. Means with different symbols within a row are different (P < 0.01).
amines from glutamine in enterocytes of unweaned and weaned pigs (Table 5) because of the low rate of net ornithine formation (Table 7). This finding does not negate an important role for glutamine in intestinal polyamine synthesis, because glutamine stimulates the expression and activity of ODC in intestinal epithelial cells (12), and glutamine is required for the conversion of proline-derived P5C into ornithine (24). Collectively, our data suggest 1) that mitochondrial-derived ornithine is available for the cytosolic synthesis of putrescine, spermidine, and spermine in enterocytes and 2) that arginine and proline are the major metabolic sources of ornithine for intestinal polyamine synthesis in weaned animals. Because there is little uptake of arterial arginine, proline, and glutamate by the small intestine (25), enteral provision of amino acids is crucial for maintaining optimal polyamine synthesis in intestinal mucosa.

Beneficial effect of a cortisol surge on the small intestine during weaning. Burrin et al. (4) recently reported that daily administration to neonatal pigs of a pharmacological dose of dexamethasone (a more potent synthetic glucocorticoid than cortisol) for 7 days decreased intestinal protein synthesis and mucosal mass. In contrast, a natural cortisol surge during the perinatal period is associated with rapid intestinal growth and maturation in piglets (19). We recently showed that daily intramuscular administration of cortisol (25 mg Hyd/kg body wt) to 21-day-old suckling pigs for 2 days increased intestinal villus heights and small intestine growth by 13–14% (26). Similarly, Chappel et al. (5) found that single administration of Hyd (25 mg/kg body wt) to suckling piglets increased intestinal growth, reduced postweaning mortality, and improved growth rate of piglets weaned at 14 days of age. These results suggest an anabolic effect of physiological concentrations of cortisol on intestinal growth in neonatal and weanling piglets. This notion is further supported by our findings that small intestine weights were 11% lower in RU-486- or metyrapone-treated weaned pigs than in untreated weaned pigs (Table 2), which is consistent with the decreases in intestinal villus height, crypt depth, and lamina propria in the former (Table 3). The decreases in the jejunal villus height as well as small intestine and body weights in weaned pigs most likely resulted from low feed intake during first days postweaning (29) due to the separation of piglets from the sow and being maintained separately.

Perspectives

Polyamines are essential for proliferation, differentiation, migration, and repair of intestinal epithelial cells (11). On the basis of our finding that an increase in circulating cortisol levels mediated the enhanced ODC activity and polyamine synthesis in enterocytes of weanling piglets, we speculate that a cortisol surge may be beneficial for intestinal maturation, adaptation, and remodeling during weaning. In addition, it is conceivable that weaning and the increased plasma concentration of cortisol may directly affect intestinal mucosal cell kinetics and structure, independent of changes in enterocyte ODC activity or polyamine concentrations. This is evident by our findings that intestinal polyamine concentrations were similar between 29-day-old vehicle-treated weaned pigs and age-matched unweaned pigs, and yet there was a decrease in villus height and an increase in crypt depth in weaned pigs than in unweaned pigs. These possible roles of cortisol and weaning in intestinal physiology would need to be addressed in future studies with the piglet model. Such work would involve the measurements of crypt cell proliferation in suckling piglets treated with or without cortisol and in weanling piglets treated with or without RU-486 or metyrapone in combination with 2-difluoromethylornithine (an inhibitor of ODC) (17). Collectively, these studies may greatly expand our knowledge of mammalian intestinal adaptation and its regulation during weaning.

In conclusion, results of this study demonstrate that arginine and proline are the major metabolic sources of ornithine for intestinal polyamine synthesis during weaning. Antagonizing the action of glucocorticoids or preventing an increase in plasma cortisol concentrations through the administration of RU-486 or metyrapone to weaning pigs prevented the induction of intestinal ODC and polyamine synthesis, decreased intestinal polyamine concentrations, and reduced jejunal villus heights and small intestine growth. We suggest that a cortisol surge is beneficial for intestinal adaptation and remodeling during weaning.

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