Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets

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Wu, Guoyao, Nick E. Flynn, and Darrell A. Knabe. Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets. Am J Physiol Endocrinol Metab 279: E395–E402, 2000.—This study was conducted to determine a role for cortisol in regulating intestinal ornithine decarboxylase (ODC) activity and to identify the metabolic sources of ornithine for intestinal polyamine synthesis in suckling pigs. Thirty-two 21-day-old suckling pigs were randomly assigned to one of four groups with eight animals each and received daily intramuscular injections of vehicle solution (sesame oil; control), hydrocortisone 21-acetate (HYD: 25 mg/kg body wt), RU-486 (10 mg/kg body wt, a potent blocker of glucocorticoid receptors), or HYD plus RU-486 for two consecutive days. At 29 days of age, pigs were killed for preparation of jejunal enterocytes. The cytosolic fraction was prepared for determining ODC activity. For metabolic studies, enterocytes were incubated for 45 min at 37°C in 2 ml of Krebs-bicarbonate buffer (pH 7.4) 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 glutamine. Cortisol administration increased intestinal ODC activity by 230%, polyamine (putrescine, spermidine, and spermine) synthesis from ornithine and proline by 75–180%, and intracellular polyamine concentrations by 45–83%. Polyamine synthesis from arginine was not detected in enterocytes of control pigs but was induced in cells of cortisol-treated pigs. There was no detectable synthesis of polyamines from glutamine in enterocytes of all groups of pigs. The stimulating effects of cortisol on intestinal ODC activity and polyamine synthesis were abolished by coadministration of RU-486. Our data indicate that an increase in plasma cortisol concentrations stimulates intestinal polyamine synthesis via a glucocorticoid receptor-mediated mechanism and that proline (an abundant amino acid in milk) is a major source of ornithine for intestinal polyamine synthesis in suckling neonates.

Polyamines (putrescine, spermidine, and spermine) are essential to the proliferation, differentiation, and migration of mammalian cells, including intestinal epithelial cells (17). Ornithine decarboxylase (ODC) is the first and key regulatory enzyme in polyamine synthesis from ornithine (23). Intestinal ODC expression is induced in early weaned animals, including rats (19) and pigs (37), which may play a role in intestinal maturation and remodeling. Glucocorticoids, whose plasma concentrations are markedly increased in weanling animals [e.g., rats (16) and pigs (31)], may play an important role in regulating intestinal ODC activity during weaning. To test this hypothesis, Nasi-Emvo et al. (22) determined the effect of cortisol (hydrocortisone) administration on intestinal ODC activity in 12-day-old suckling rats so as to eliminate the confounding effects of weaning, stress, and feeding. Despite the foregoing, little information is available on the effect of glucocorticoids on intestinal polyamine synthesis in the neonatal pig, an excellent animal model for studying infant intestinal physiology and metabolism (6, 25, 37). Also, we were not aware of studies to determine the effects of glucocorticoids on intestinal ODC activity or polyamine synthesis in pigs.

An increase in enzyme activity measured under in vitro assay conditions does not necessarily indicate an enhanced metabolic flux or product formation in intact cells (12). Thus both ODC activity and polyamine synthesis should be determined in enterocytes of cortisol-treated suckling animals so as to establish its role in enhancing intracellular provision of polyamines. Arginase is generally assumed to be a major source of ornithine for polyamine synthesis in mammalian cells (39). However, intestinal arginase activity is negligible in suckling animals, including pigs (37). In addition, ornithine is negligible in milk, including human and sow’s milk (9, 34), and there is little uptake of ornithine from arterial blood by the small intestine (33). Thus potential metabolic sources of the ornithine for intestinal polyamine synthesis remain unknown. Our recent studies have identified proline and glutamine [abundant amino acids in milk (9, 34)] as major substrates for ornithine synthesis in enterocyte mitochondria of suckling pigs (32, 36). However, it is not known whether ornithine generated from proline and glutamine in mitochondria can be used for polyamine synthesis in the cytosol.

In view of the foregoing, the objectives of this study were to determine whether glucocorticoids increase intestinal ODC activity and polyamine synthesis in suckling pigs and to identify potential metabolic

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sources of ornithine for polyamine synthesis in enterocytes. Our results demonstrate that an increase in plasma cortisol concentrations stimulates intestinal polyamine synthesis via a glucocorticoid receptor-mediated mechanism and that proline is the major source of ornithine for intestinal polyamine synthesis in suckling neonates.

MATERIALS AND METHODS

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 Committee.

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 (HYD), RU-486 [4-(4-dimethylaminophenyl)17α-(prop-1-ynyl)estra-4,9-dien-3-one], and all other chemicals used were purchased from Sigma Chemical (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. At 21 days of age, 32 suckling pigs (5.5 kg) were randomly assigned within litter to one of four groups with eight animals each and received daily intramuscular injections of vehicle (sesame oil; control), HYD (25 mg/kg body wt), RU-486 (25 mg/kg body wt), or HYD plus RU-486 (same doses) for 2 consecutive days. The HYD administration was chosen to mimic a cortisol surge in piglets during the first 2 days postweaning (15, 31). Cortisol was used because it is the major circulating glucocorticoid in pigs (31). This dose of HYD was selected because it has been reported to increase 1) disaccharidase activities and growth of the small intestine (8) and 2) intestinal glutamine and arginine metabolism (14) in suckling pigs. The dose of RU-486 [a potent blocker of glucocorticoid receptors (3)] was based on previous in vivo studies with a number of species, including rats, humans, and guinea pigs (3) as well as pigs (13–15). Immediately before cortisol or RU-486 administration, and at days 2 and 8 after the administration, blood was obtained from the jugular vein for measuring cortisol concentrations using a cortisol kit (15).

After blood collection at 29 days of age, pigs were killed between 10:00 and 11:00 AM to obtain the whole small intestine. Small intestine weights and lengths were measured after intestinal contents were thoroughly removed with saline. Preliminary studies indicated that the responses of ODC activity and polyamine synthesis to cortisol treatment were similar between jejunal and ileal enterocytes in suckling pigs. Thus, because the present work was labor and resource intensive, we concluded that it was not necessary to study all segments of the small intestine from control and cortisol-treated piglets. We chose jejunal enterocytes in this study, because most of the previous studies on intestinal amino acid metabolism were performed with jejunal enterocytes (18, 35–37) and jejenum constitutes most of the small intestine. During the entire experimental period, suckling piglets were nursed by sows and did not consume the feed provided for the sows. The feeder design prevented the access of piglets to sow’s diet.

Preparation and incubation of jejunal enterocytes. The jejenum was washed three times with saline to remove luminal contents and then was used for preparing enterocytes with the use of oxygenated (95% O2, 5% CO2) Ca2+-free Krebs-Henseleit bicarbonate (KHB) buffer (pH 7.4) containing 5 mM glucose as previously described (35–37). 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 t-methionine and one of the following: 1) 1 mM t-arginine plus 2 µCi L-[U-14C]arginine, 2) 1 mM t-ornithine plus 2 µCi L-[U-14C]ornithine, 3) 1 mM t-glutamine plus 2 µCi L-[U-14C]glutamine, or 4) 1 mM t-proline plus 2 µCi L-[U-14C]proline plus 1 mM glutamine. Methionine was used as the precursor for S-adenosylmethionine, and subsequently S-decarboxylated 5-adenosylmethionine was used for spermidine and spermine syntheses (23). Glutamate, derived from glutamine by mitochondrial pH-dependent glutaminase, was required to convert proline-derived pyrrolidine-5-carboxylate (P5C) into ornithine by ornithine aminotransferase (32); therefore, glutamate was added to the incubation medium containing L-[U-14C]proline. In keeping with this notion, we found in our preliminary studies that there was little synthesis of [14C]putrescine, spermidine, or spermine from L-[U-14C]proline in pig enterocytes when glutamine or glutamate was not added to the incubation medium. 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 K2CO3 (35). The neutralized extracts were used for analyses of amino acids by an HPLC method involving precolumn derivatization with o-phthalaldehyde (34) and of 14C-labeled polyamines (see below). Net production of [14C]ornithine was measured by determining the accumulation of [14C]ornithine in cells plus incubation medium, as previously described (32). Incubated enterocytes remained viable for 45 min on the basis of linear consumption of O2, as determined with the use of Clark-type polarographic O2 probes (35–37).

Analysis of polyamines and 14C-polyamines. Polyamine concentrations were determined in freshly isolated enterocytes as described by Wu et al. (40). Briefly, cells (10 mg protein) were acidified with 1 ml of 1.5 M HClO4 and were neutralized with 0.5 ml of 2 M K2CO3. The neutralized extracts were used for polyamine analysis by an ion-pairing HPLC method involving precolumn derivatization with o-phthalaldehyde. The assay mixture contained 150 µl sample and 10 µl of 1.2% benzoic acid (in 40 mM sodium borate, pH 9.5). An aliquot (100 µl) of the assay mixture was derivatized in an autosampler (model 712 WISP, Waters, Milford, MA) with 100 µl of 30 mM o-phthalaldehyde (in 3.1% Brij-35, 50 mM 2-mercaptoethanol, and 40 mM sodium borate, pH 9.5), and 100 µl of the derivatized mixture was injected in a Supelco 3-µm reversed-phase C18 column (150 × 4.6 mm ID). Polyamines were separated using a solvent gradient consisting of solution A (0.1 M sodium acetate, 2 mM SDS, 0.5% tetrahydrofuran, and 9% methanol, pH 7.2) and solution B (methanol and 2 mM SDS). Putrescine, spermidine, and spermine in samples were quantified on the basis of authentic standards. In our preliminary studies, we found that, in pig enterocytes incubated at 37°C in the presence of 1 mM ornithine or 1 mM proline plus 1 mM glutamine, cellular concentrations of putrescine were decreased by 12–15% at the end of a 45-min incubation period compared with the values for freshly isolated cells, and there were few differences in cellular concentrations of spermidine or spermine between freshly isolated and incubated cells.

For determining [14C]putrescine, [14C]spermidine, and [14C]spermine, neutralized extracts (2 ml) of enterocytes plus incubation medium were freeze-dried and suspended in 0.3 ml of HPLC H2O. Polyamines were separated by the HPLC method as described above, and the fractions containing [14C]putrescine, [14C]spermidine, and [14C]spermine were...
collected from the HPLC column for measuring radioactivities by a Packard liquid scintillation counter (Meriden, CT). Blank (0 min incubation) radioactivities were subtracted from sample values. Rates of production of putrescine, spermidine, and spermine were calculated on the basis of intracellular specific activities of [14C]ornithine, which were measured as described by Wu et al. (32).

Determination of ODC activity. The cytosolic fraction of enterocytes was prepared and used for measuring ODC activity using 0.2 mM [1-14C]ornithine (37). Briefly, enterocytes (20 mg protein) were homogenized with the use of a glass homogenizer in 2 ml of 50 mM sodium phosphate buffer (pH 7.2) containing 0.2 mM pyridoxal 5-phosphate, 1 mM EDTA, 2.5 mM dithiothreitol, 150 mM sucrose, and protease inhibitors (5 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml apro tinin, 5 μg/ml chymostatin, and 5 μg/ml pepstatin A). The homogenates were centrifuged at 13,000 g for 15 min at 4°C. The supernatant (free of mitochondria) was used for ODC assay. The assay mixture (0.5 ml) consisted of 0.2 mM L-[1-14C]ornithine (106 dpm/nmol), 0.2 mM pyridoxal 5-phosphate, 0.2 mM EDTA, 0.5 mM dithiothreitol, enzyme preparations (~1 mg protein), and 50 mM sodium phosphate buffer (pH 7.2). Radioactivity blanks containing [1-14C]ornithine but no enzyme preparations were run along with the samples. After incubation at 37°C for 1 h, 14CO2 was collected in 0.2 ml NCS-II, a tissue solubilizer (Amersham, Arlington Heights, IL), and its radioactivity was measured by a liquid scintillation counter.

Examination of intestinal morphology. Villus height, crypt depth, and lamina propria depth in jejunum and ileum were measured in a masked manner, as described by Wu et al. (38), except that intestinal tissues were fixed with 4% paraformaldehyde. Briefly, jejunal samples (3–5 cm long) were split along the mesentery, pinned flat with toothpicks (lumen facing up) to balsa wood, and immersed (lumen facing down) in a 4% paraformaldehyde solution. After 24 h, jejunal samples were removed from the fixative and washed three times with 70% ethanol before being embedded in paraffin. Four cross-sections (6 μm thick) per sample were stained with hematoxylin and eosin. Villus height, crypt depth, and lamina propria depth were measured in villi with well-defined tips and well-attached lamina propria. The lamina propria is an underlying connective tissue layer on which the epithelium of the small intestine rests. The lamina propria depth measurements extended from the base of the villus to the muscularis mucosae.

Milk consumption. Milk consumption was estimated by the weigh-suckle-weigh technique (21) using an additional 24 21-day-old sucking piglets. Piglets were randomly assigned to one of four groups (6 piglets/group) and were treated with vehicle solution, cortisol, RU-486, or cortisol plus RU-486 as described above. At days 1, 4, and 7 postcortisol or -RU-486 administration, milk consumptions were measured before and after sucking every 1.5 h during a 12-h period from 8:00 AM to 8:00 PM to estimate milk intake.

Determination of protein. Protein in enterocytes and the cytosolic fraction was determined by a modified Lowry procedure with BSA as a standard (36). Briefly, 1 ml of diluted enterocyte suspensions (25–100 μg protein/ml) or BSA standard (0–100 μg/ml) was incubated with 3 ml of an alkali solution (2% Na2CO3, 0.4% NaOH, 0.16% potassium sodium tartrate, and 0.026% CuSO4) for 3 min at room temperature, followed by addition of 0.3 ml of the diluted (1:1) phenol reagent (Sigma Chemical). After incubation for 45 min at room temperature, absorbance of the solution at 660 nm was measured by a UV/VIS spectrophotometer.

Statistical analysis. Data were analyzed by one-way ANOVA and the Student-Newman-Keul’s multiple comparison test (28). Probability values < 0.05 were taken to indicate statistical significance.

RESULTS

Milk consumption. At days 1, 4, and 7 postcortisol or -RU-486 administration, milk consumption did not differ (P > 0.05) among the four groups of pig. Thus data from each day of the measurements were pooled. Milk consumption by pigs was 189 ± 15, 183 ± 12, and 177 ± 14 ml·kg body wt−1·day−1 (means ± SE, n = 24), respectively, at days 1, 4, and 7 postcortisol or -RU-486 treatment.

Plasma cortisol concentrations. Figure 1 illustrates plasma cortisol concentrations in sucking piglets treated with or without cortisol or RU-486. Cortisol administration to 21-day-old sucking pigs for two consecutive days markedly increased (P < 0.01) plasma cortisol concentrations at day 2 posttreatment. At day 8 postcortisol administration, plasma concentrations of cortisol did not differ (P > 0.05) between control and HYD-treated piglets. Administration of RU-486 alone increased (P < 0.01) plasma cortisol concentrations at day 2 posttreatment compared with control pigs. At day 8 post-RU-486 administration, plasma cortisol concentrations were higher (P < 0.01) in RU-486-treated piglets compared with pigs not treated with RU-486.

Body and small intestine weights. Cortisol treatment increased (P > 0.05) on body weights in 29-day-old sucking piglets (Table 1). However, small intestine weights were 14% greater (P < 0.05) in cortisol-treated piglets compared with control piglets. Coadministration of RU-486 prevented the stimulating effect of cortisol on small intestine growth.

Jejunal morphology. Cortisol treatment increased (P < 0.05) jejunal villus heights by 13% but had no significant effect (P > 0.05) on jejunal crypt depth or lamina propria depth (Table 2). Coadministration of RU-486 prevented the cortisol-induced increase in jejunal villus heights and had no effect (P > 0.05) on jejunal crypt depth and lamina propria depth.

Fig. 1. Effects of cortisol and RU-486 administration on plasma concentrations of cortisol in sucking piglets. Values are means ± SE; n = 8. Twenty-one-day-old sucking pigs were treated daily with cortisol or RU-486 for two consecutive days. Means sharing different letters (a–c) are different (P < 0.01). *P < 0.01, different from the values for days 0 and 8. †P < 0.01, different from the values for day 0. Brackets denote concentration.
Effects of cortisol administration on ODC activity. Cortisol treatment increased \( P < 0.01 \) enterocyte ODC activity by 230\% (Fig. 2). RU-486 administration alone had no effect \( P > 0.05 \) on ODC activity compared with control pigs. However, coadministration of RU-486 with cortisol abolished the stimulating effect of cortisol on enterocyte ODC activity.

Effects of cortisol administration on polyamine synthesis. Synthesis of putrescine, spermidine, and spermine in enterocytes is shown in Table 3. Cortisol treatment increased \( P < 0.01 \) the synthesis of putrescine, spermidine, and spermine from ornithine by 75–180\% in enterocytes of suckling piglets. Polyamine synthesis from proline was also enhanced \( P < 0.01 \) in enterocytes of cortisol-treated piglets compared with control pigs. Polyamine synthesis from arginine was not detectable in enterocytes of suckling piglets but was induced in cells of cortisol-treated pigs. In pig enterocytes, spermidine was the major polyamine formed from ornithine, arginine, and proline. There was no detectable synthesis of polyamines from glutamine in enterocytes of all groups of pigs studied. Coadministration of RU-486 abolished the stimulating effect of cortisol on intestinal polyamine synthesis from ornithine, arginine, and proline in piglets.

Effects of cortisol administration on polyamine concentrations. Figure 3 summarizes intracellular concentrations of putrescine, spermidine, and spermine in enterocytes of pigs treated with or without cortisol or RU-486. In enterocytes of all groups of pigs studied, the concentration of spermidine was highest, followed by spermine and putrescine. Cortisol treatment increased \( P < 0.05 \) polyamine concentrations by 45–83\%. RU-486 administration alone had no effect \( P > 0.05 \) on intestinal polyamine concentrations. However, coadministration of RU-486 prevented the increase in enterocyte polyamine concentrations in cortisol-treated piglets. The ratios of putrescine to spermidine to spermine were 1:4.2:2.9, 1:3.2:2.5, 1:4.4:3.1, and 1:4.9:3.4 for control, cortisol-treated, RU-486-treated, and cortisol- plus RU-486-treated pigs, respectively.

Production of ornithine from arginine, proline, and glutamine in enterocytes. Large amounts of ornithine were generated from proline in enterocytes of both control and cortisol-treated piglets (Fig. 4). Net production of ornithine from arginine was low in enterocytes of control piglets but was enhanced ~10-fold in cortisol-treated piglets. Net production of ornithine from glutamine was much lower \( P < 0.01 \) than that from proline in pig enterocytes. RU-486 treatment prevented the increase in ornithine production from arginine, but not from proline, in enterocytes of cortisol-treated pigs.

**DISCUSSION**

Effect of cortisol administration on intestinal polyamine synthesis. Results of this study demonstrate that daily administration of cortisol to 21-day-old suckling pigs for 2 days enhanced intestinal ODC activity (Fig. 2) and polyamine synthesis (Table 3). This is the first report of increases in both ODC activity and polyamine synthesis in enterocytes of cortisol-treated neonatal pigs. In these cells, induction of both arginase (14) and ODC (Fig. 2) results in polyamine synthesis.
Table 3. Effects of cortisol and RU-486 administration on polyamine synthesis in enterocytes of suckling pigs

<table>
<thead>
<tr>
<th>Addition to Incubation Medium</th>
<th>Vehicle</th>
<th>Cortisol</th>
<th>RU-486</th>
<th>Cortisol + RU-486</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ornithine</td>
<td>9.8 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.1 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.8 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proline&lt;sup&gt;†&lt;/sup&gt;</td>
<td>3.5 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2 ± 0.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.7 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine&lt;sup&gt;ND&lt;/sup&gt;</td>
<td>4.0 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ornithine&lt;sup&gt;ND&lt;/sup&gt;</td>
<td>21.4 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.4 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.1 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proline&lt;sup&gt;ND&lt;/sup&gt;</td>
<td>5.7 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.1 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.4 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine&lt;sup&gt;ND&lt;/sup&gt;</td>
<td>10.6 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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Values are means ± SE in pmol·mg protein<sup>−1</sup>·45 min<sup>−1</sup>; n = 8. Concentrations for ornithine, proline, and arginine were 1 mM. Means sharing different superscript letters (a-b) within a row are different (P < 0.01).<sup>†</sup>P < 0.01, different from the ornithine (1 mM) and proline (1 mM) groups.<sup>ND</sup>ND, not detectable.

Fig. 3. Effects of cortisol and RU-486 administration on polyamine concentrations in enterocytes of suckling piglets. Polyamines were determined in freshly isolated enterocytes. Values are means ± SE; n = 8. Means sharing different letters (a-b) are different (P < 0.05).

Fig. 4. Effects of cortisol and RU-486 administration on net production of ornithine (Orn) in enterocytes of suckling piglets. Values are means ± SE; n = 8. Means sharing different letters (a-b) are different (P < 0.01). Pro, proline; Arg, arginine; Gln, glutamine.

from arginine, whereas increased ODC activity (Fig. 2) accounts for the enhanced synthesis of polyamines from proline. Spermidine was the major polyamine formed from ornithine in pig enterocytes, followed by putrescine and spermine (Table 3). Interestingly, intracellular concentrations of putrescine were the lowest among all three polyamines measured (Fig. 3). This result may be explained by the contribution of milk-born polyamines to intestinal concentrations in suckling neonates, as spermine is more abundant than spermidine in sow’s milk (20) as in human and rat milk (24), and sow’s milk contains little putrescine (20). RU-486 administration increased plasma cortisol concentrations in piglets (Fig. 1; see Ref. 15), as reported for humans (5). This result may be explained by the hypersecretion of cortisol from the adrenal cortex (5) and possibly decreased cellular uptake of cortisol. Administration of RU-486 alone had no effect on intestinal ODC or polyamine synthesis (Fig. 2 and Table 3) or intestinal arginine metabolism (14) in suckling pigs, suggesting that basal, endogenous glucocorticoid levels do not play a role in these metabolic pathways. There are circadian variations of plasma cortisol concentrations (6–24 μg/l) in pigs, with the highest and lowest values found at 8:00 AM and 12:00 AM, respectively (30). An important finding of this study is that coadministration of RU-486 abolished the stimulating effect of exogenous cortisol on intestinal ODC activity and polyamine synthesis in neonatal pigs, regardless of plasma cortisol concentrations (Fig. 2). These results indicate that an increase in plasma cortisol concentrations enhances enterocyte ODC activity and polyamine synthesis via a glucocorticoid receptor-mediated mechanism, as we previously reported for the induction of intestinal arginase in suckling pigs (14).

Role of amino acids in intestinal polyamine synthesis. Glucocorticoid treatment decreases intestinal lactase activity and increases intestinal sucrase and maltase activities in suckling rats and pigs (8, 16). In addition, cortisol administration increases arginine and glutamine metabolism in pig enterocytes (14). Our current study extends the regulatory role of glucocorticoids in intestinal digestive enzymes and amino acid metabolism (13–16) to polyamine synthesis in suckling pigs. Another important, novel finding of this study is the relative importance of potential substrates for polyamine synthesis in enterocytes (Fig. 5). Ornithine is the immediate precursor for the synthesis of putrescine by ODC. However, because milk contains only a negligible amount of ornithine (9, 34) and because there is little uptake of arterial ornithine by the small intestine (33), it is important to identify the metabolic sources of ornithine for intestinal polyamine synthesis in suckling neonates. Although arginine is often assumed to be the major precursor of ornithine in mammalian cells (39), this pathway is insignificant for enterocytes of suckling mammals because of a low or negligible activity of intestinal arginase (37).
Our recent studies have shown that ornithine can be produced from both glutamine and proline [abundant amino acids in milk (9, 34)] in enterocyte mitochondria via P5C synthase and proline oxidase, respectively (32, 36). There was substantial synthesis of polyamines from proline in enterocytes of all groups of pigs studied. However, there was no detectable synthesis of polyamines from glutamine in pig enterocytes (Table 3), which may result from low rates of net production of ornithine from glutamine, which were only 5–10% of those from proline (Fig. 4). It is possible that very small amounts of [14C]polyamines were formed from [U-14C]glutamine, but they were below the detection limit (35 dpm) of our liquid scintillation counter. Our finding, however, does not negate an important role for glutamine in intestinal polyamine synthesis, because glutamine markedly stimulates the expression and activity of ODC in intestinal epithelial cells (18) and because glutamine is required for the conversion of proline-derived P5C into ornithine (32). Taken together, our data suggest 1) that mitochondria-derived ornithine is available for cytosolic polyamine synthesis by ODC, spermidine synthase, and spermine synthase in enterocytes and 2) that proline (an abundant amino acid in milk) is the major source of ornithine for intestinal polyamine synthesis in suckling neonates. In view of our novel findings, it would be of great interest to determine the effect of artificial feeding with a low-proline diet on the intestinal polyamine-synthetic pathway in neonates.

**Anabolic effect of cortisol on intestinal growth.** Daily intramuscular administration of cortisol (25 mg HYD/kg body wt) to 21-day-old suckling pigs for 2 days resulted in elevated plasma concentrations of cortisol at day 2 posttreatment (Fig. 1) to values similar to those observed in newborn piglets (27). The daily dose of HYD used in our study is equivalent to 1 mg dexamethasone · kg body wt⁻¹ · day⁻¹ (1). It is noteworthy that this cortisol treatment increased intestinal polyamine synthesis, villus height, and small intestine growth in suckling piglets (Tables 1 and 3). These beneficial effects of cortisol administration were not due to an altered supply of dietary nutrients because milk consumption did not differ between control and cortisol-treated piglets. Similarly, Chappel et al. (8) found that a single administration of HYD (25 mg/kg

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**Fig. 5. Polyamine synthesis from amino acids in enterocytes.** DCAM, decarboxylated 5-adenosylmethionine; Glu, L-glutamate; α-KG, α-ketoglutarate; MTA, methylthioadenosine; OAT, ornithine aminotransferase; PO, proline oxidase; P5CS, pyrroline-5-carboxylate synthase; SAMS, S-adenosylmethionine; PDG, phosphate-dependent glutaminase; SAMD, S-adenosylmethionine decarboxylase; PPi, inorganic pyrophosphate.
body wt) to suckling piglets increased small-intestinal growth, reduced postweaning mortality, and improved growth rate of piglets weaned at 14 days of age. A cortisol surge during the perinatal period is also associated with intestinal maturation and growth in piglets (27). Collectively, these results suggest an anabolic effect of physiological concentrations of cortisol on the small intestine in neonatal pigs. Interestingly, Rhoads et al. (26) showed that glucocorticoid treatment (30 mg methylprednisolone \(\cdot\) kg body wt \(^{-1}\) \(\cdot\) day \(^{-1}\) for 2 days) had no effect on jejunal villus height or crypt depth in healthy piglets weaned at 17–21 days of age but increased jejunal villus height by 120% in weaned piglets infected with a gastroenteritis virus. In contrast, Wang and Johnson (29) reported that multiple glucocorticoid administrations (5 mg corticosterone/kg body wt, 3 times daily for 3 days) to 22-h-fasted young rats caused significant damage to duodenal mucosa. Burrin et al. (1) also observed that daily administration of dexamethasone (1 mg/kg body wt) to 2-day-old pigs for 7 days decreased intestinal protein synthesis and mucosal mass. Whether glucocorticoids promote intestinal anabolism or catabolism likely depends on the following factors: (1) the type of corticosteroids administered (e.g., natural or synthetic), (2) the frequency of corticosteroid administration, (3) the concentrations of circulating corticosteroids, (4) the tissue sensitivity to corticosteroids, and (5) the species and developmental stages studied. We suggest that an increase in plasma cortisol concentrations within physiological ranges may be beneficial for stimulating small intestine growth and development in suckling neonates.

**Physiological and nutritional significance.** Polyamines are essential for protein synthesis as well as intestinal maturation and function (11, 17, 23). Thus results of this study have important implications for intestinal physiology and nutrition in neonates. First, enhanced intestinal synthesis of polyamines may help explain the beneficial effect of prenatal or postnatal administration of glucocorticoids on improving gut maturation and function (1) as well as decreasing the incidence of necrotizing enterocolitis (2) in premature infants. Our results may also provide a metabolic basis for the more rapid intestinal villus recovery in piglet viral enteritis after treatment with glucocorticoid (26). Second, reduced intestinal synthesis of polyamines may be a biochemical basis for the gut atrophy frequently observed in neonates maintained on glutamine-free parenteral nutrition solutions (10). This is likely due to a limited supply of arterial amino acid substrates for polyamine synthesis in intestinal mucosa. Third, enteral provision of amino acids, including proline, glutamine, glutamate, arginine, and ornithine, may be critical for optimizing intestinal polyamine synthesis and therefore intestinal development and integrity. This may help explain the previous findings that early introduction of enteral feeding promotes intestinal maturation and motility in preterm infants (4). We thank Wene Yan, E. Lichar Dillon, Erin Hibun, Sean Flynn, and Edward Gregg for technical assistance, Tony Haynes for preparing figures, and Frances Mutscher for secretarial support. This research was supported by United States Department of Agriculture Grant 97–35206–5096 (to G. Wu) and by Hatch Projects HS200 (G. Wu) and H6601 (D. A. Knabe) from the Texas Agricultural Experiment Station.

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