Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig

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Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, Blikslager AT. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol Gastrointest Liver Physiol 292: G173–G181, 2007. First published August 10, 2006; doi:10.1152/ajpgi.00197.2006.—Weaning in the piglet is a stressful event associated with gastrointestinal disorders and increased disease susceptibility. Although stress is thought to play a role in postweaning intestinal disease, the mechanisms by which stress influences intestinal pathophysiology in the weaned pig are not understood. The objectives of these experiments were to investigate the impact of weaning on gastrointestinal health in the pig and to assess the role of stress signaling pathways in this response. Nineteen-day-old pigs were weaned, and mucosal barrier function and ion transport were assessed in jejunal and colonic tissues mounted on Ussing chambers. Weaning caused marked disturbances in intestinal barrier function, as demonstrated by significant (P < 0.01) reductions in transepithelial electrical resistance and increases in intestinal permeability to [3H]mannitol in both the jejenum and colon compared with intestinal tissues from age-matched, unweaned control pigs. Weaned intestinal tissues exhibited increased intestinal secretory activity, as demonstrated by elevated short-circuit current that was sensitive to treatment with tetrodotoxin and indomethacin, suggesting activation of enteric neural and prostaglandin synthesis pathways in weaned intestinal tissues. Western blot analyses of mucosal homogenates showed increased expression of corticotrophin-releasing factor (CRF) receptor 1 in the jejunum and colon of weaned intestinal tissues. Pretreatment of pigs with the CRF receptor antagonist α-helical CRF(9–41), which was injected intraperitoneally 30 min prior to weaning, abolished the stress-induced mucosal changes. Our results indicate that weaning stress induces mucosal dysfunction mediated by intestinal CRF receptors and activated by enteric nerves and prostanoid pathways.

barrier function; secretion

WEANING is one of the most stressful events a pig encounters in commercial swine production. At this time, the newly weaned pig encounters psychosocial and physical stressors including maternal and littermate separation, abrupt changes in diet and environment, and comingling and establishment of social hierarchy with unfamiliar pigs from different litters (11, 46). In disease challenge models, it has been shown that the incorporation of weaning stressors is often required to initiate the development of enteric diseases, suggesting that stress plays a critical role in disease susceptibility (15, 23). However, the pathophysiological mechanisms that link weaning stress and intestinal disease are unknown.

One reported deleterious effect of weaning is the breakdown of intestinal barrier function (5, 39). The intestinal barrier is composed of the single layer of columnar epithelial cells that line the intestinal tract and serves as the body’s first line of defense against potentially harmful microorganisms and antigens residing within the intestinal lumen (13, 18, 27). Intestinal barrier breakdown is characterized by increased intestinal permeability, which allows luminal antigenic agents (e.g., bacteria, toxins, and feed-associated antigens) to “leak” across the epithelium and gain access to subepithelial tissues, resulting in inflammation, malabsorption, diarrhea, and potentially systemic disease (4, 8, 17). Breaches in intestinal barrier function have been shown to be a pathophysiological event in several postweaning swine enteric diseases such as Escherichia coli edema disease, Clostridium difficile infections, and transmissible gastroenteritis (10, 29, 44). It is also becoming increasingly recognized that stress-induced breakdown of the intestinal barrier is central to several important stress-associated gastrointestinal disorders in humans, including inflammatory bowel diseases and irritable bowel syndrome (2, 9, 38).

Rodent stress models have indicated that stress-induced alterations in intestinal function are mediated by the actions of corticotrophin-releasing factor (CRF) and subsequent activation of CRF receptors expressed locally in the gut (35, 37, 41). For example, restraint stress or peripheral administration of CRF (intraperitoneally) in rodents increased colonic permeability and ion secretion that was prevented by pretreatment with the CRF receptor antagonist drug α-helical CRF(9–41) (31). CRF mediates its actions via binding to two G protein-coupled receptor subtypes: CRF receptor 1 (Crf-r1) and CRF receptor 2 (Crf-r2) (41). Expression patterns and tissue distribution differ between the two receptor subtypes in the gastrointestinal tract, which likely explains the different physiological properties associated with their respective activation (6, 7, 16). However, it appears that stress-induced intestinal disturbances such as intestinal secretion, visceral hypersensitivity, and motility changes are predominantly mediated through CRF-r1 activation (19, 24, 34, 36, 40).

The role of weaning stress and CRF signaling pathways on intestinal function in the weaned pig has not been previously reported. Therefore, the objective of the present study was to investigate the role of stress and CRF receptor signaling pathways in weaning-induced intestinal dysfunction in the weaned pig.

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Animals and weaning protocol. All studies were approved by the North Carolina State University Institutional Animal Care and Use Committee. Yorkshire cross-bred pigs of either sex were housed in standard farrowing crates with sows and subjected to routine management practices. At 19 days of age, two to three pigs from each of three different litters were removed from the sow (weaned) and placed in nursery pens in a nearby nursery facility. This weaning age (19 days of age) was selected because this represents the current average weaning age in United States swine production systems (http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/swine/index.htm). The remaining pigs from each litter were handled briefly (sham stress) but were returned to the sow to continue to nurse and served as unweaned controls. Weaned pigs were offered ad libitum access to water and standard nursery diet (Renaissance Nutrition, Roaring Spring, PA). Twenty-four hours after being weaned, weaned (n = 8) and unweaned pigs (n = 8) with equal numbers of males and females within each group were sedated with a combination of xylazine (1.5 mg im) and ketamine (11 mg/kg im) followed by euthanasia with an overdose of pentobarbital intravenously via a catheterized ear vein. Initial sedation was used to minimize stress prior to the intestine being obtained for subsequent intestinal studies. Segments of the midjejunum and ascending colon were immediately harvested following euthanasia and prepared for Ussing chamber studies. For studies investigating changes in intestinal function over the first week postweaning, intestinal tissues were harvested from pigs on days 1, 2, and 7 postweaning.

Ussing chamber experiments. Segments of the midjejunum and ascending colon were harvested from the pig, and the mucosa was stripped from the seromuscular layer in oxygenated (95% O2-5% CO2) Ringer solution. Tissues were then mounted in 1.13-cm2 aperture Ussing chambers, as described in a previous study (3). Tissues were bathed on the serosal and mucosal sides with 10 ml Ringer solution. The serosal bathing solution contained 10 mM glucose, which was osmotically balanced on the mucosal side with 10 mM mannitol. Bathing solutions were oxygenated (95% O2-5% CO2) and circulated in water-jacketed reservoirs maintained at 37°C. The spontaneous potential difference (PD) was measured using Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through Ag-AgCl electrodes using a voltage clamp that corrected for fluid resistance. Transepithelial electrical resistance (TER; in Ω·cm2) was calculated from the spontaneous PD and short-circuit current (ISC) as previously described.

Mucosal-to-serosal fluxes of [3H]mannitol. To assess the mucosal permeability after experimental treatments, 0.2 μCi/ml [3H]mannitol was placed on the mucosal side of Ussing chamber-mounted tissues. After a 15-min equilibration period, standards were taken from the mucosal side of each chamber, and a 60-min flux period was established by taking 0.5-nl samples from the serosal compartment. The presence of [3H] was established by measuring the β-emission in a liquid scintillation counter (LKB Wallac, model 1219 Rack Beta, Perkin-Elmer Life and Analytical Sciences, Boston, MA). Unidirectional [3H]mannitol mucosal-to-serosal fluxes were evaluated by determining the mannitol specific activity added to the mucosal bathing solution and calculating the net appearance of [3H] over time in the serosal bathing solution on a chamber unit area basis.

TTX and indomethacin experiments. For experiments assessing the role of enteric nerves and prostanoids on Isc in weaned intestinal tissues, jejunal and colonic tissues from weaned pigs (24 h postweaning) were treated (time = 30 min) with the neural inhibitor TTX (10−7 M) or the nonsteroidal anti-inflammatory drug indomethacin (Indo; 10−6 M) on the serosal side of the tissues. For each in vitro experiment, a total of six pigs was used (n = 6). For each pig, multiple mucosal samples were harvested and mounted in Ussing chambers, with each tissue exposed to a distinct treatment.

CRF and cortisol ELISA. Blood samples were obtained from pigs via venipuncture on days 1, 2, and 7 postweaning and prior to euthanasia. Pigs were sedated prior to blood collection to minimize the stress of sampling procedures. All samples were taken at the same time of day to minimize the effects of diurnal rhythms. Serum was separated by centrifugation (20 min, 10,000 g), and the serum was stored at −80°C until analysis. Serum levels of CRF and cortisol were determined using commercial ELISA kits (CRF: Phoenix Pharmaceuticals, Belmont, CA; and cortisol: R&D Systems, Minneapolis, MN). For CRF ELISA analyses on the intestinal mucosa, intestinal samples were prepared according to the manufacturer’s instructions.

RT-PCR. RNA was extracted from the colonic mucosa using a RNeasy tissue extraction kit (Qiagen) with on-column DNase digestion. First-strand cDNA synthesis was performed using 2 μg RNA with the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer’s protocol. One microliter of cDNA was used to perform PCR for CRF. The amplification cycle was 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s with a final extension step of 72°C for 2 min. Primers were designed using Primer 3 software. The primers sequences were as follows: forward 5′-GTCCTGTGGTGTC3CTCTTT-3′ and reverse 5′-GGAGCAGGGAGACTCTTATT-3′. The 218-bp product was confirmed by electrophoresis of the PCR product on a 2% agarose gel stained with ethidium bromide.

CRF receptor antagonist experiments. Nineteen-day-old pigs were injected intraperitoneally with either saline or the CRF receptor antagonist drug α- helical CRF(9–41) (250 μg/kg) 30 min prior to being weaned. Pigs were redosed with saline and α-helical CRF(9–41) treatments at 12 h postweaning. Twenty-four hours after pigs had been weaned, jejunal and colonic tissues were mounted on Ussing chambers for measurements of TER and Isc as described above.

Histological examination. Tissues were taken after pigs had been euthanized for routine histological evaluation. Jejunal and colonic tissues were sectioned (5 μm) and stained with hematoxylin and eosin.

Gel electrophoresis and Western blot analysis. Jejunal and colonic mucosal scrapings from weaned and unweaned pigs were snap frozen and stored at −70°C before SDS-PAGE was performed. Tissue aliquots were thawed at 4°C and added to 3 ml of chilled lysis buffer, including protease inhibitors at 4°C, as previously described. This mixture was homogenized on ice and then centrifuged at 4°C, and the supernatant was saved. Protein analysis of extract aliquots was performed (DC protein assay, Bio-Rad, Hercules, CA). Tissue extracts (amounts equalized by protein concentration) were mixed with an equal volume of 2× SDS-PAGE sample buffer and boiled for 4 min. Lysates were loaded on a 10% SDS-polyacrylamide gel, and electrophoresis was carried out according to standard protocols. Proteins were transferred to a nitrocellulose membrane (Hybond ECL, Amersham Life Science, Birmingham, UK) using an electroblotting mini-transfer apparatus. Membranes were blocked at room temperature for 60 min in Tris-buffered saline plus 0.05% Tween 20 and 5% dry powdered milk. Membranes were washed and incubated with primary antibody (goat CRF-r1 polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA); β-Actin antibody (Abcam, Cambridge, MA) was used as a control for protein loading. After an additional wash, membranes were incubated with horseradish peroxidase-conjugated secondary antibody and developed for visualization of protein by the addition of enhanced chemiluminescence reagent (Amersham, Piscataway, NJ) as previously described.

Immunofluorescence labeling. Immunofluorescence labeling was performed on colonic tissues that had been embedded in optimal cutting temperature medium, frozen, cut into 5-μm sections, and fixed in cold acetone. Tissue sections were blocked with 2% BSA prior to being incubated with goat anti-CRF polyclonal antibody (1:250) in BLOTTO for 2 h at 4°C. Sections were washed with BLOTTO and incubated for 45 min with Texas red-conjugated anti-goat secondary antibody (1:300; Santa Cruz Biotechnology) in the dark. Sections
were mounted, and well-oriented villi were examined with a Vanox AH5-3 photomicroscope linked to a Spot RT Slider cooled-charge-coupled device digital camera. 

Chemicals. Indo, TTX, and [3H]mannitol were all purchased from Sigma Chemical (St. Louis, MO). α-Helical CRF(9–41) was obtained from Bachem (Torrance, CA).

Statistical analyses. Data are reported as means ± SE based on the experimental number (n). All data were analyzed by using a standard one-way ANOVA (Sigmastat, Jandel Scientific, San Rafael, CA). A post hoc Tukey’s test was used to determine differences between treatments following ANOVA.

RESULTS

Impact of weaning on intestinal barrier health and secretory activity. Weaning induced marked disturbances in intestinal barrier function in pigs, as demonstrated by significant reductions in jejunal and colonic TER by 59 ± 5% (TER: 64 ± 7 and 26 ± 3 Ω·cm² in the unweaned and weaned jejunum, respectively) and 34 ± 3% (TER: 86 ± 12 and 57 ± 5Ω·cm² in the unweaned and weaned colon, respectively) compared with unweaned littermate controls (Figs. 1 and 2). In accordance with TER values, weaned jejunal and colonic tissues exhibited significant (P < 0.01) increases in intestinal permeability to the paracellular probe [3H]mannitol (Figs. 1 and 2). Increased secretory activity in the piglet jejunum and colon was observed in weaned intestinal tissues, as indicated by significant (P < 0.05) elevations in baseline Isc (Fig. 3).

Influence of enteric nerve and prostaglandin synthesis blockade on weaning-stimulated Isc. To determine if the elevated Isc observed in weaned intestinal tissues was attributable to the activation of enteric nerves or prostaglandin synthesis pathways, the neural inhibitor TTX or the nonselective nonsteroidal anti-inflammatory drug Indo were applied to the serosal surfaces of the weaned jejunum and colon mounted on Ussing chambers. In the weaned jejunum, Isc increased gradually over time on Ussing chambers, resulting in a positive ∆Isc of 20 ± 6 μA/cm². This was in contrast to unweaned tissues, in which ∆Isc decreased over time (∆Isc of −6 ± 2 μA/cm²). The application of serosal TTX (10−7 M) or Indo (10−6 M) to weaned tissues resulted in significant (P < 0.05) reductions in Isc, indicating that the enhanced baseline secretory tone in the weaned piglet jejunum and colon was attributed to both enteric neural and prostaglandin pathways, respectively (Fig. 4). In unweaned jejunal tissues, TTX reduced baseline Isc, whereas Indo was without effect in these tissues (∆Isc: −6 ± 2, −16 ± 2.3, and −2 ± 3 μA/cm² for unweaned control, TTX treatment, and Indo treatment; data not shown). The application of TTX or Indo had no effect on TER in these experiments.

Histological analysis of weaned intestinal tissues. Histological analysis of tissues did not reveal significant changes in intestinal morphology or any identifiable histopathological lesions in the jejunum or colon between treatment groups, although a trend (P = 0.09) was observed for decreased villus height (villus height: 341.9 ± 16 and 291.9 ± 23 μm in unweaned and weaned tissues, respectively) and increased crypt depth (crypt depth: 53.8 ± 0.5 and 76.8 ± 10.4 μm in unweaned and weaned tissues, respectively) in the jejunum (data not shown). An increased number of goblet cells was also observed in the weaned piglet jejunum compared with unweaned controls (P < 0.05).

Serum CRF and cortisol levels in weaned pigs. Weaning induced significant (P < 0.05) elevations in serum CRF (by 114%) and cortisol (by 95%) compared with unweaned control levels (P < 0.01) measured 24 h postweaning, thus indicating the activation of central stress pathways in the weaned pig (Fig. 5A). Measurements of CRF and cortisol over the first week postweaning displayed different time-course kinetics. For example, CRF levels were peaked in weaned pigs on day 1 postweaning and were significantly (P < 0.05) elevated on day 2 postweaning but returned to unweaned control levels by day 7 postweaning. In contrast, serum cortisol concentrations remained elevated throughout the 7-day postweaning period. To determine if the changes in CRF or cortisol over the first week of postweaning reflected changes in intestinal function, intestinal barrier function and secretory activity in the jejunum of weaned pigs were measured over a 7-day postweaning period. These data showed that weaning-induced reductions in TER
CREATED VOL 292 • JANUARY 2007 • www.ajpgi.org

Effect of CRF receptor antagonist on weaning-induced intestinal dysfunction. To definitively assess the role of intestinal CRF receptor activation in weaning-induced changes in epithelial physiology, we performed experiments to assess the role of CRF antagonism. Initially, we pretreated pigs with the nonselective CRF-r1/CRF-r2 antagonist α-helical CRF(9–41) (250 µg/kg ip) prior to the pigs being weaned and measured its effect on intestinal barrier function and secretory activity. As shown in Fig. 8, the administration of the CRF receptor

CRF mRNA analysis and CRF peptide levels in the weaned pig mucosa. Although serum CRF levels corresponded with intestinal dysfunction, it was not clear at this point whether changes in intestinal physiology were a result of the central activation of CRF or local synthesis within the intestinal mucosa. Therefore, to determine if CRF is synthesized locally in the weaned pig intestine, we initially performed mRNA analysis of CRF gene transcripts in the porcine intestinal mucosa. CRF mRNA analysis showed that CRF mRNA was expressed in the weaned pig jejunal and colonic mucosa (Fig. 6A). In addition, ELISA experiments were performed for CRF levels in both the unweaned and weaned pig jejunal mucosa. These experiments showed a significant increase (P < 0.05) in CRF peptide concentrations in the weaned jejunal mucosa compared with unweaned tissues (Fig. 6B).

Expression of CRF receptors in the weaned piglet intestine. There is mounting evidence for a critical role of CRF receptors in intestinal function. CRF-r1 activation is involved in stress-induced intestinal disturbances such as hypersecretion, increased colonic motility, and visceral hypersensitivity (24, 30, 34), whereas CRF-r2 activation results in inhibition of gastric motility and antinociceptive effects (21, 25, 30). Western blot analyses from the pig jejunal mucosa revealed that CRF-r1 and CRF-r2 were expressed as proteins of ~60 kDa in molecular weight (Fig. 7A). Furthermore, the present study revealed increased expression of CRF-r1 and CRF-r2 in the jejunum of weaned pigs compared with unweaned control tissues. Western blot analyses of CRF-r1 were also performed in the colonic mucosa and revealed similarly increased expression in weaned tissues (Fig. 7B). CRF-r1 immunolocalization experiments performed in colonic tissues revealed that CRF-r1 was expressed predominantly in the subepithelium and cells within the lamina propria (Fig. 7C).

Fig. 2. Impact of weaning on TER and [3H]mannitol permeability in the piglet ascending colon. Values represent means ± SE; n = 8 animals. Pigs were either subjected to weaning at 19 days of age (weaned) or left with the sow to continue to nurse (unweaned). Twenty-four hours postweaning, segments of the ascending colon from weaned and unweaned pigs were mounted in Ussing chambers for measurements of TER and [3H]mannitol flux over a 1-h experimental period. A: weaning induced significant reductions in TER in the piglet colon compared with unweaned littermate controls (**P < 0.01). B: weaning induced elevations in [3H]mannitol flux across the colon of weaned pigs compared with unweaned littermate controls (*P < 0.01 vs. unweaned controls).

Fig. 3. Impact of weaning on intestinal ion transport in 19-day-old pigs. Values represent means ± SE; n = 8 animals. Pigs were either subjected to weaning or left undisturbed with the sow to continue to nurse. Weaning stimulated increases in $I_{sc}$ in the jejunum (A) and colon (B) of pigs compared with unweaned controls. *P < 0.05 vs. unweaned controls.
Antagonist prior to the pigs being weaned prevented the weaning-induced reductions in TER (Fig. 8A) and increases in mucosal-to-serosal \(^{3}H\)mannitol fluxes (Fig. 8C) in the jejunum compared with weaned control animals administered a saline vehicle (Fig. 8A). In contrast to our initial experiments assessing the impact of weaning on colonic TER (Fig. 2A), weaning or CRF receptor antagonist treatment had no significant impact on TER in the colon in these experiments (Fig. 8B). However, \(^{3}H\)mannitol fluxes were significantly elevated in weaned colonic tissues, and these elevations were prevented by CRF antagonist treatment (Fig. 8D). The lack of correlation between TER and flux measurements suggests some disparity in what these techniques assess in terms of barrier function. However, mannitol flux is likely of more clinical relevance because of its assessment of permeability rather than the calculated TER measurement of barrier function. CRF receptor antagonist treatment significantly \((P < 0.05)\) attenuated the weaning-induced elevations in \(I_{Sc}\) in the jejunum and partially inhibited baseline colonic \(I_{Sc}\) (Fig. 8E and F). The inability of the CRF receptor antagonist to fully inhibit elevations in baseline colonic \(I_{Sc}\) indicates that alternative (non-CRF receptor dependent) pathways are contributing to weaning-induced elevations in \(I_{Sc}\).

**DISCUSSION**

In the present study, we have shown that weaning induces disturbances in intestinal mucosal health, as characterized by
the elevated secretory activity and increased intestinal permeability in the pig jejunum and colon. The combined disturbances in mucosal function were due to the activation of CRF receptor pathways expressed in the intestine.

In line with findings from the present study, others have documented the deleterious impact of weaning on intestinal barrier function and secretory activity in the pig intestine. Boudry et al. (5) reported transient reductions in jejunal TER but not colonic TER and increased baseline $I_{sc}$ in the jejunum and colon of weaned pigs. These authors also observed that the $I_{sc}$ responsiveness to the secretagogues serotonin (5-HT) and cAMP was decreased when $I_{sc}$ was measured at 2 wk postweaning, a response that likely reflects part of normal intestinal maturation in pigs. In our laboratory studies, we have shown that by delaying weaning to 28 days of age, reductions in TER and increases in $I_{sc}$ were abolished, coinciding with decreased serum CRF and CRF-r1 protein expression in the intestine (unpublished results). Taken together, this suggests that the intestine undergoes normal maturation processes such as reductions in secretory capacity; however, the mucosal disturbances, including barrier disruption and enhanced baseline intestinal secretion, in the weaned pig appear to be pathophysiological consequences of stress signaling pathways activated by weaning.

Circulating levels of stress hormones CRF and cortisol in response to weaning. CRF and cortisol were significantly elevated in the weaned pig, suggesting that weaning induced activation of central stress pathways. Measurement of stress hormones over a 7-day postweaning period revealed that serum CRF levels mirrored disturbances in intestinal function (in terms of TER and $I_{sc}$), whereas cortisol levels did not correlate with intestinal function. This suggests that CRF may be a more sensitive stress indicator of stress-induced intestinal dysfunction. Previous reports have shown that the central administration of CRF (intracisternally) stimulates colonic motor function, indicating that central activation of CRF signaling pathways can influence intestinal function. However, it has also been clearly shown that peripheral CRF pathways influence intestinal physiology. For example, the administration of CRF (either intraperitoneally or intravenously), which does cross the blood-brain barrier from the systemic circulation (22), induces epithelial disturbances, including increased colonic permeability, ion secretion, and diarrhea (32, 34). Furthermore, in vitro studies have demonstrated that CRF applied directly to colonic...
Fig. 8. Effect of the CRF receptor antagonist α-helical CRF(9–41) on weaning-induced increases in jejunal and colonic TER, \([^{3}H]\)mannitol flux, and \(I_{sc}\). Values represent means ± SE; \(n = 6\) animals. Pigs were injected with α-helical CRF(9–41) (250 μg/kg ip) or saline vehicle 30 min before and 12 h after being weaned. Twenty-four hours postweaning, intestinal tissues were mounted on Ussing chambers. α-Helical CRF(9–41) treatment prevented the reductions in TER in the weaned piglet jejunum (A) compared with pigs treated with saline vehicle, whereas no differences in TER were observed in the colon (B) \(^{*}P < 0.05\) vs. α-helical CRF(9–41) treatment. Reductions in mucosal-to-serosal \([^{3}H]\)mannitol fluxes were observed in both weaned jejunal (C) and colonic (D) tissues of pigs treated with α-helical CRF(9–41) compared with saline vehicle \(^{*}P < 0.05\) vs. α-helical CRF(9–41) treatment. α-Helical CRF(9–41) treatment prevented the increases in \(I_{sc}\) in the weaned jejunum (E) and partially inhibited \(I_{sc}\) in colonic tissues (F) compared with saline treatment. \(^{*}P < 0.05\) significantly different from saline treatment; \(^{#}P < 0.05\), significantly different from each other.)
tissues increases colonic motility, permeability, and ion secretion, suggesting a direct action of CRF on effector cells within the intestine (19, 20, 35). In the present study, we detected mRNA expression of CRF gene transcripts in the pig intestinal mucosa, indicating that CRF is synthesized locally in the intestine. Increased levels of mucosal CRF in the weaned jejunal mucosa compared with unweaned tissues were also observed overall, suggesting that CRF can be synthesized locally within the intestinal mucosa of the weaned pig. It remains unknown which cell population is responsible for the synthesis CRF in the porcine mucosa. CRF mRNA has been detected in other species in both epithelial and subepithelial cell types within the intestinal mucosa, including crypt epithelium, enterochromaffin cells, and enteric nerves (1, 43). Further studies are needed to determine the cell type that expresses CRF in the porcine intestinal mucosa.

Peripheral CRF receptor blockade with the nonselective CRF-r1/CRF-r2 antagonist α-helical CRF(9–41) in the present study prevented weaning-induced reductions in barrier function and increased secretory activity in the pig intestine, suggesting that CRF-CRF receptor systems are activated within the intestinal mucosa in response to weaning stress. Rodent stress models have demonstrated similar findings in that psychological (water avoidance stress) and physical (cold restraint stress) stressors stimulated increases in intestinal permeability and $I_{sc}$ that were abolished with an intraperitoneal injection of α-helical CRF(9–41) (32, 34, 35). Recently, Gareau et al. (12) showed that rat pups subjected to early life stress in the form of intermittent maternal separation resulted in increased colonic permeability and bacterial adherence and penetration that was prevented by the administration of α-helical CRF(9–41) prior to stress. Although the present findings demonstrate a clear role of CRF receptor signaling pathways in the pathophysiological response to weaning in the gut, the role of individual CRF receptor types in this response is unknown. CRF mediates its central and peripheral actions via binding to two G protein-coupled receptor subtypes: CRF-r1 and CRF-r2 (41). In the literature, stress-induced alterations in colonic motility, secretion, and visceral hypersensitivity appear to be mediated through CRF-r1 (19, 24, 34, 36, 42), whereas the actions of CRF-r2 are less known but have shown to inhibit gastric motility and confer antinociceptive properties (21, 25, 30). Given the nonselective nature of the CRF receptor antagonist used in the present study, it is not clear from the present study which role each CRF receptor type (CRF-r1 or CRF-r2) plays in weaning-induced intestinal dysfunction in the pig. More studies are required with selective CRF receptors antagonists to determine which receptors are involved in this response.

Inhibitor experiments showed that the application of the neural inhibitor TTX inhibited $I_{sc}$ in both unweaned and weaned tissues, suggesting the role of enteric nerves in mediating baseline secretory tone in the piglet intestine. Inhibition of prostaglandin synthesis with Indo did not influence baseline $I_{sc}$ in the unweaned jejunum but significantly decreased baseline $I_{sc}$ in weaned tissues, suggesting that activation of prostaglandin pathways are contributing to the elevated secretion in the weaned intestine. The link between CRF-CRF receptor systems and activation of enteric neural and prostanoi synthesis pathways in the weaned intestine is not entirely clear. Immunofluorescence analyses in the present study revealed that CRF-r1 was localized predominantly to the lamina propria of the porcine colonic mucosa. Others have reported varying localization patterns of CRF-r1 in the gastrointestinal tract. For example, CRF-r1 was expressed in colonic epithelia (goblet cells and stem cells), lamina propria cells, and the myenteric and submucosal nervousplexuses in the rat (6). In the guinea pig small and large intestines, CRF-r1 expression was detected in the myenteric and submucosal nervousplexuses (16). In human colonic biopsies, CRF-r1 and CRF-r2 mRNA were detected in isolated lamina propria mononuclear cells (28). Recently, we have reported colocalization of CRF-r1 to porcine intestinal mast cells (26). The latter finding may provide insight into the effects of CRF on the intestinal barrier and secretory disturbances observed in this study as mast cells are recognized as a critical cell population involved in stress-induced intestinal disturbances. For example, the increased ion secretion and permeability observed in stressed rodents can be prevented by preadministration of the mast cell stabilizer drug doxantrazole (31). Additionally, mast cell-deficient mice have been shown to be resistant to stress-induced intestinal dysfunction (33). Mast cells stimulate epithelial secretion by activating enteric nerves or by releasing prosecretory mediators such as prostaglandins and histamine (33, 45, 47), which could likely explain the results from our inhibitor studies. Mast cells can also influence barrier properties of the intestinal epithelium, possibly via the release of mast cell tryptase and tight junction protein disruption (14). The interaction between CRF-CRF receptor and mast cells pathways requires more investigation.

The results from this study show that weaning induces marked disturbances in intestinal function in the pig. The enhanced intestinal permeability and secretory tone observed in response to weaning is mediated through the activation of peripheral CRF receptors. Our hypothesis is that the stress of weaning activates CRF-CRF receptor signaling pathways in the gut resulting in intestinal dysfunction, which may be an important mechanism of stress-associated enteric disease in weaned pigs. Further elucidation of the stress signaling pathways in the weaned pig may also provide insight into the mechanisms of stress-induced intestinal disorders in humans.

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