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Maternal deprivation alters epithelial secretory cell lineages in rat duodenum: role of CRF-related peptides

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Abstract

Objective—Chronic psychological stress is associated with development of intestinal barrier dysfunction and impairs host defence mechanisms. The intestinal epithelium, consisting of enterocytes, endocrine cells, goblet cells and Paneth cells, is an important component of this barrier. In the present study, the impact of maternal deprivation (MD) on secretory lineages of duodenal epithelium and the involvement of the peripheral corticotropin-releasing factor (CRF) pathway were investigated.

Methods—Rat pups were deprived of their dam for 3 h/day (days 5–20). Non-deprived pups served as controls. On days 8, 13, 20, 24, 34, 44 and 84, duodenal tissues were collected for quantitative real-time PCR and immunohistochemistry studies.

Results—MD induced a sustained decrease in the number of Paneth and goblet cells but hyperplasia of endocrine cells. These alterations were associated with a duodenal increase of CRF, urocortin 2 and CRF receptor subtype 2 ($CRFR_2$) mRNA, whereas $CRFR_1$ expression was decreased. The effects of MD on intestinal epithelium were inhibited by the $CRFR_1/R_2$ antagonist astressin injected daily before MD. Studies using specific receptor antagonists in rats subjected to MD revealed that $CRFR_1$ was involved in the hyperplasia of endocrine cells and $CRFR_2$ in the depletion of Paneth cells. Conversely, daily injection of CRF and of the $CRFR_2$ agonist urocortin 2 in control rats resulted in changes in epithelial differentiation similar to MD.

Conclusions—The activation of $CRFR_1$ and $CRFR_2$ induced by MD markedly altered the quantitative distribution of secretory cells of the intestinal epithelium. These alterations, in particular the depletion of Paneth and goblet cells, may create conditions leading to the development of an epithelial barrier defect.

The defensive barrier against damaging agents of the intestinal lumen is conferred by the epithelial layer itself and by factors such as peristalsis, gut microbiota and the pre-epithelial mucus $HCO3^-$ layer. Among these factors, intestinal mucus plays a critical role in the protection against acidic aggression, luminal proteases, mechanical insults, colonisation by

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pathogenic bacteria and their toxins, and potential carcinogens.[1–3] The major component of the intestinal mucus is the gel-forming mucin MUC2, which is produced by goblet cells of the epithelium.[4,5] Numerous studies now indicate that alterations in the rate of MUC2 synthesis/secretion or in the number of goblet cells may be involved in the initiation or maintenance of intestinal diseases.[6,7] For example, Pugh *et al* demonstrated that active duodenal ulcerations were associated with a decreased number of goblet cells.[8] In ulcerative colitis, the mucus layer and goblet cell density are also significantly reduced.[9] Importantly, mice genetically deficient in *Muc2* spontaneously develop colitis and colorectal tumours.[6,10] In spite of the crucial function of goblet cells in intestinal homeostasis and protection, the mechanisms which control or may alter the balance for allocation of stem cells to differentiate into goblet cells or into another cell lineage are not completely understood.

The intestinal epithelium is characterised by its rapid and constant renewal, occurring every 3–5 days under normal homeostasis and increasing after injury. The multipotent stem cells underlying this renewal reside near the base of crypts, on average at the +4 position.[11] They divide and produce an actively proliferating population of transit amplifying cells that differentiate during a series of steps into either absorptive enterocytes, the predominant population, or the secretory lineages (goblet, enteroendocrine and Paneth cells). Enterocytes, goblet cells and enteroendocrine cells differentiate while migrating up the crypt–villus axis, whereas Paneth cells reside in the bottom of crypts.[12]

The processes by which stem cells give rise to transit amplifying precursor cells and then to functionally mature cells of a particular lineage are regulated by complex epithelial–epithelial and epithelial–mesenchymal interactions. Among these, the Notch cascade plays a critical role in cell fate decision between the absorptive and secretory lineages.[13] When the Notch pathway is activated, its downstream target gene, Hes1, represses the expression of the helix–loop–helix (HLH) protein Hath1 and promotes an absorptive cell fate over a secretory cell fate. Neurogenin3 (Ngn3) is then required for endocrine cell specification. Downstream of Ngn3, several transcription factors such as NeuroD/β2, Pdx1, Nkx2.2, Pax4 or Pax6 are responsible for different subtypes of enteroendocrine cells. The differentiation and maturation of goblet and Paneth cells are also controlled by several genes such as Gfi1, Spdef and Klf4.[14] In some other aspects, intestinal epithelial cell renewal and differentiation may also respond to environmental conditions including luminal nutrients[15] or trophic gastrointestinal hormones.[16] Interestingly, psychological stress is now considered as an environmental factor that can influence epithelial functions of the small and large intestine.[17,18] Psychological stress may also contribute to gastric and duodenal ulcer pathogenesis in humans,[19,20] thus suggesting that stress can impair mucosal defences supported by mucus and bicarbonate, as well as by the epithelial lining and by a rapid cell renewal. It was shown in particular that stress induced by early maternal deprivation (MD) in rat pups predisposes to gastric ulcer.[21]

Physiological responses to stress include release of central corticotropin-releasing factor (CRF) by the hypothalamus. Recent studies demonstrated that beside its central function, peripheral CRF plays a key role in intestinal disturbances triggered by stress.[22,23] Teitelbaum et al recently showed that chronic peripheral administration of CRF can mimic the effects of chronic stress on intestinal barrier dysfunction.[24] In the intestine, CRF and CRF-related peptides (urocortin (Ucn) 1, 2 and 3) are produced in enterochromaffin cells, Paneth cells, colonocytes, mast cells, mucosal macrophages and in the myenteric and submucosal plexus.[25–27] Effects of CRF and CRF-related peptides are mediated by two distinct receptors: CRF receptor subtype 1 ($CRFR_1$) and $CRFR_2$. CRF binds either of the two receptors although with a preferential affinity for $CRFR_1$. Ucn 1 binds both $CRFR_1$ and $CRFR_2$, whereas Ucn 2 and Ucn 3 bind exclusively $CRFR_2$.[25,28] Interestingly, $CRFR_1$ and $CRFR_2$ have been localised mainly in myenteric neurons, nerve fibres and isolated cells of

the lamina propria in rat small intestine and colon.[27,29] The presence of CRF receptors has also been demonstrated on goblet and stem cells of rat colonic crypts,[30] suggesting that CRF agonists could act directly on the intestinal epithelium to regulate its functions and renewal of epithelial cells.

In the present study, our aim was first to assess the impact of early MD on goblet, Paneth and enteroendocrine cell numbers during and after this environmental stress. Thereafter, we investigated the mechanisms of MD-induced alteration in epithelial differentiation with a particular emphasis on the role of peripheral CRF signalling pathways.

MATERIALS AND METHODS

Animals

Primiparous timed-pregnant Wistar female rats were purchased (Harlan France, Gannat) on gestational day 14. Dams were individually housed on a 12:12 h light–dark cycle with free access to food and water. The day of birth was considered to be postnatal day 1. All procedures were approved by the regional ethics committee for animal experiment, Rhone Alpes, approval No. 0196.

Experimental protocols

Experiment 1: MD—Dams and their litters were randomly assigned to the MD protocol or to the control non-deprived (CT) protocol. Pups subjected to MD (MD pups) were individually deprived of their dam for 3 h/day from postnatal day 5 to 20 of life, at the same time every day to minimise the effects of circadian rhythm. MD pups were removed from the home cage and taken into a separate room, where they were kept singly in temperature-controlled cages (30±1°C). CT pups remained undisturbed in their home cage with their dam. MD and CT pups were weaned on day 20. Body weight was recorded daily. Six MD rats and six CT rats were deeply anaesthetised with pentobarbital sodium on postnatal days 8, 13, 20, 24, 34, 44 or 84. Samples of proximal duodenum were harvested for immunohistological and real-time PCR (RT-PCR) studies.

Experiment 2: CRF receptor antagonists—In a second set of experiments, rat pups (n=6 per group) were injected subcutaneously with antalarmin (a selective antagonist for $CRFR_1$, 20 mg/kg, Sigma-Aldrich, St Louis, Missouri, USA) or astressin$_2$-B (a selective antagonist for $CRFR_2$, 150 µg/kg, Sigma-Aldrich). or intraperitoneally with astressin (a non-specific CRF receptor antagonist, 60 µg/kg, Bachem, Bibendorf, Switzerland) or vehicle (water), daily 30 min before MD from postnatal day 5 to postnatal day 13. Rats were weighed every morning and then the volume to be injected was calculated accordingly (50–120 µl). These doses have been previously shown to inhibit peripheral injection of CRF-induced barrier dysfunction or other aspects of gut pathophysiology.[31–33] The CT pups were injected with vehicle, and then returned to the home cage. Antalarmin does not dissolve in water or saline and therefore was dissolved in ethanol/cremophor/saline (Sigma-Aldrich).[33] Additional studies validated that a similar quantity of this vehicle alone had no detrimental effects on gut differentiation in vivo. Animals were euthanised by pentobarbital sodium on postnatal day 13. Samples of proximal duodenum were taken for immunohistological studies.

Experiment 3: CRF receptor agonists—To confirm the role of CRF receptors, non-deprived rat pups (n=6 per group) received a daily intraperitoneal injection of rat/human CRF (30 µg/kg/day, Tocris, Ellisville, Missouri, USA) or Ucn 2 (20 µg/kg/day, Bachem) from postnatal day 5 to postnatal day 13. Control pups were injected with vehicle (saline).

Rats were euthanised by pentobarbital on postnatal day 13. Samples of proximal duodenum were taken for immunohistological studies.

Immunohistochemistry

Duodenal tissues were removed, fixed in 90% ethanol for 24 h at −20°C and processed into paraffin. Serial paraffin sections (4 μm) were rehydrated and treated as described in the supplementary data. Primary antibodies are listed in table 1.

The immune reactions were then detected by incubating with a ready-to-use peroxidase-labelled secondary reagent, ImmPRESS (MP-7401 for rabbit antibodies or MP-7402 for mouse antibody, Vector, Abcys, Paris, France) (30 min, room temperature). Control experiments were performed simultaneously omitting the primary antibody or incubating with preimmune rabbit serum. All slides were analysed by a single investigator who was blinded to the treatment groups (see supplementary Materials and methods).

Real-time PCR

Measures were performed with the real-time fluorescence detection method using the LightCycler System with a FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Meylan, France) in 20 μl capillaries (see supplementary Materials and methods). The expression of ARP (acidic ribosomal phosphoprotein P0), a gene widely used in stress studies, was used as a reference.[34] Calculations were performed according to the $2^{-\Delta\Delta C_t}$ method and the final value was adjusted so that controls had a mean relative mRNA level of 1.[35]

Statistical analysis

Data obtained from histological counts after drug treatment were compared by a one-way analysis of variance (ANOVA), followed by Bonferroni test when appropriate. Histological counts or RT–PCR data obtained after MD were compared by two-way ANOVA, followed by Mann–Whitney test when appropriate. All the data are expressed as the mean±SEM. Differences with $p<0.05$ were considered significant. Statistical analyses were performed with XLSTAT, Version 2008.6.06 (Addinsoft).

RESULTS

Effect of MD on secretory cell lineages in rat duodenum

No significant difference in body weight was observed between MD and CT pups. The morphology of the duodenum of rats subjected to MD was also normal, with a typical crypt–villus structure. We assessed mucosal proliferation by determining proliferating cell nuclear antigen (PCNA) in crypt cells on postnatal days 8–34. No alteration in basal level of proliferation was detected in MD pups (data not shown).

Goblet cells

The analysis of MUC2 staining in duodenal mucosa revealed that MD induced a decrease in the number of goblet cells per crypt–villus axis as compared with controls (non-deprived rats) (figure 1A, B). This decrease was present from the first time observation (postnatal day 8) and was significant until postnatal day 24. Later on, the number of goblet cells in MD pups tended to reach that of control animals (figure 1A).

Paneth cells

As previously described in rat,[3637] Paneth cells with lysozyme granules appeared in duodenum around postnatal day 13. MD decreased the percentage of crypts showing Paneth

cells. The effect of MD on the percentage of duodenal crypts exhibiting Paneth cells was retained on postnatal day 20 (figure 2). After weaning, the percentage of crypts with Paneth cells progressively decreased in CT rats down to the level observed in MD rats.

The number of Paneth cells detected in lysozyme-positive crypt cross-sections was not significantly greater in CT than in MD animals (data not shown).

Enteroendocrine cells

The distribution of enteroendocrine cells was examined with the pan-endocrine marker chromogranin A (CGA). MD significantly increased the number of enteroendocrine cells. This effect was significant from postnatal day 8 and persisted until postnatal day 84, that is 64 days after the end of the MD protocol (figure 3A, B).

In addition to an increase in number, the distribution of CGA-positive cells was altered in MD rats. Normally, enteroendocrine cells appear singly and are not in close proximity. In contrast, in MD rats, enteroendocrine cells were frequently clustered (figure 3C), suggesting that neonatal stress may alter the lateral inhibition process that orchestrates the normal pattern of secretory cell distribution.

As shown in supplementary figure 1, enterochromaffin cells, one of the major populations of enteroendocrine cells, were increased in MD rats.

Transcription factor expression

Quantitative RT-PCR analysis indicated that MD did not modify the expression of *Rath1* and *Hes1* (figure 4A). Among transcription factors implicated in secretory epithelial cell differentiation, MD sessions induced a significant increase in *Klf4*, *Gfi1* and *Pdx1* mRNA levels (ANOVA $p<0.05$) but not in *Spdef* and *Nkx2.2* (figure 4B, C). Sessions of MD also induced a weak (−20%) but significant decrease (ANOVA $p<0.05$) in duodenal *Ngn3* mRNA expression over the whole experimental period (from postnatal day 8 to 34).

CRF receptor antagonists inhibited the effects of MD on duodenal epithelial differentiation

During the MD protocol, the level of CRF and Ucn 2 mRNA progressively increased, a maximal effect being observed on postnatal day 20 (175±12 and 212±45% of that of CT pups, respectively) (supplementary figure 2). By postnatal day 24 and thereafter, no significant difference was observed between MD and CT pups. In contrast, MD did not modify the expression of Ucn 1 and Ucn 3 in rat duodenum (data not shown).

In neonatal MD pups, the mRNA level of $CRFR_1$ was also decreased as compared with non-deprived pups (supplementary figure 3). In contrast, $CRFR_2$ mRNA expression was enhanced by MD ($p<0.05$). This effect was maximum on postnatal day 13 (+78%) (data not shown).

As shown in figure 5, repeated injections of astressin before MD significantly prevented the effect of stress on the number of goblet cells, on the percentage of crypts with Paneth cells and on the number of enteroendocrine cells. The selective blockade of $CRFR_2$s with astressin$_2$-B or $CRFR_1$s with antalarmin failed to alter significantly the effect of MD on the number of goblet cells. In contrast, the effect of MD on Paneth cells was abolished by pretreatment with astressin$_2$-B, and antalarmin pretreatment inhibited the effect of MD on enteroendocrine cells.

CRF or Ucn 2 administration and differentiation in rat duodenum

Daily CRF injection was without effect on goblet cells and tended to decrease the percentage of crypts showing Paneth cells in the duodenum of rats (figure 6A, B). In contrast, treatment with CRF increased the number of enteroendocrine cells (figure 6C).

Daily Ucn 2 injection (20 μg/kg) caused a slight and non-significant decrease in the number of goblet cells but significantly reduced the percentage of crypts showing Paneth cells. Ucn 2 did not alter enteroendocrine cell number.

DISCUSSION

In the present study, we showed for the first time that exposing rat pups to neonatal MD increased the number of duodenal enteroendocrine cells whereas there was a depletion in goblet and Paneth cells. Because the number of goblet or Paneth cells was still decreased 4 or 24 days after the end of the MD protocol, this alteration is unlikely to result from a massive secretion. In agreement with our results, Garcia-Rodenas *et al* demonstrated that MD decreased mucin content in the small intestine of rats at postnatal day 35.[38] A depletion of goblet cells was also observed in the ileum and colon of adult rats exposed to water avoidance over 10 days,[39] suggesting that differentiation of goblet cells may be affected by different chronic stresses.

Goblet cells produce the gel-forming mucin MUC2 which represents the main component of the intestinal mucus and thus plays important roles in intestinal protection.[45] The depletion of goblet cells observed in our study could thus be harmful in the host defence against noxious factors (eg, acidity, proteolytic enzyme activities, toxins or pathogens). This loss of goblet cells may also modify the local luminal environment for nutrient transit, increase the intestinal absorption and make easier the passage of protein antigens, including food antigens and microbial toxins. In some other aspects, Paneth cells contribute to innate immunity by sensing bacteria and by discharging antimicrobial peptides including α-defensins. Recently, Fernandez *et al*[40] showed that the maturation of Paneth cells was followed by the death of bacteria reaching the jejunum and that depletion of Paneth cells restored the susceptibility to *Shigella* infection, thus demonstrating the crucial role of these cells in intestinal protection. Analysis of the intestine of Paneth cell-deficient mice also showed an increased penetration of the mucus barrier by commensal bacteria.[41] Interestingly, the depletion of Paneth and goblet cells induced by MD was accompanied by a hyperplasia of enteroendocrine cells that persisted after the end of the MD protocol. A major function of enteroendocrine cells is to coordinate the appropriate physiological response to meal ingestion, to activate local neural circuitry and to elicit secretory, motor and vasodilatory activity. MD, like other chronic exposures to stress, is known to induce several gut dysfunctions in adulthood, including an altered ion secretion, an increase of epithelial permeability and lower visceral pain thresholds. The increased number of enteroendocrine cells observed in our study could thus be one of the mechanisms mediating these effects.

All this indicates that the impact of stress on the quantitative distribution of secretory cells could render the mucosa vulnerable to damaging and ulcerogenic agents of the intestinal lumen. This could participate in the effects of stress as one of the major aetiological factors in susceptibility to development of duodenal ulceration.

The effect of MD on the quantitative distribution of secretory cell lineages of the duodenal epithelium suggests that postnatal stress affects the choice of a bipotential secretory cell to adopt an endocrine rather than a goblet–Paneth cell fate. In the small intestine, enteroendocrine cells, goblet cells and Paneth cells arise from a common secretory progenitor expressing Hath1, an atonal-related basic HLH protein (encoded by *Atoh1*, also

called *Math1* in mouse and *Rath1* in rat). Our results showed that MD did not modify *Rath1* expression in rat duodenum, thus suggesting that MD did not direct the fate of progenitor cells towards the secretory or the absorptive epithelial lineage. After the initial decision has been made between secretory and absorptive cell lineages, the fate of the enteroendocrine cell is further delineated from that of other secretory cells (goblet and Paneth cells) by a second atonal-related basic HLH protein, Ngn3. A previous study demonstrated that overexpression of *Ngn3* under the control of a villin promoter in mice produced an increased number of cells expressing CGA and a decreased number of goblet cells.[42] Surprisingly, we found that MD slightly but significantly decreased the expression of intestinal *Ngn3*. These data suggest that stress induced by MD would act in the course of a downstream stage of the differentiation of secretory progenitors and that the decreased expression of *Ngn3* could take place to limit the expansion of endocrine cells induced by this stress. Interestingly, we observed an overexpression of *Pdx1* in the duodenum of rats subjected to MD, suggesting that this transcription factor may be implicated in the expansion of endocrine cells. In neonatal mice, Pdx1 induces the differentiation of endocrine cells specific for the upper small intestine (GIP, CCK and gastrin-producing cells), whereas in the adult, Pdx1 generates intestinal hormone gene expression.[43–45] Other factors, such as β2/NeuroD, Pax4 or Pax6 may also play a role. Another element essential to the analysis is that the expression of *Gfi1* increased in the small intestinal of MD pups. Gfi1 is a zinc-finger transcriptional repressor that functions downstream of Rath1 to select goblet/Paneth versus endocrine progenitors. Gfi1 is expressed in endocrine precursors and in a subset of mature enteroendocrine cells. Shroyer *et al*[46] proposed that GfI1 stimulates production of goblet/Paneth cells from adjacent progenitors. Thus, the increased expression of *Gfi1* observed at postnatal day 8 may play a role, in concert with the decrease in *Ngn3*, to limit the expansion of the endocrine compartment. Several studies also indicated that Klf4, a Krüppel-like factor, is critically involved in differentiation and maturation of colonic goblet cells.[47] Recent research showed, however, that, in the small intestine, the function of Klf4 was not restricted to differentiation of goblet cells and regulated genes that are differentiation specific.[48] Thus the rise in *Klf4* transcripts observed in our study probably is not related to goblet cells. It is also interesting to note that Klf4 is known to interact with β-catenin and to antagonise Wnt signalling.[49] Most important of all, Wnt signalling, which helps maintain the undifferentiated state of the progenitor cells,[50] also induces differentiation and maturation of Paneth cells in intestinal crypts.[51] The Paneth cell gene programme is thus critically dependent on Tcf-4 in the embryonic mouse intestine.[51] Taken together, these data suggest that the depletion of Paneth cells induced by MD may be driven by overexpression of Klf4 until postnatal day 13, which corresponds to the period during which Paneth cells appear in the rat duodenal epithelium. On the other hand, some studies point to a function for Klf4 as a mediator of external stressors. It has been reported, for example, that Klf4 was upregulated by heat stress in several tissues in vivo and in vitro[52] and that Klf4 can be induced by hydrogen peroxide, interferon γ or lipopolysaccharide.[53–55] Taken together, these results indicate that Klf4 may be a key mediator of intestinal epithelial response to stress induced by MD.

Other information emerging from our study is that peripheral CRF-related peptides play a pivotal role in the alteration of epithelial fate determination induced by repeated MD. Indeed, we showed that intraperitoneal administration of the nonselective receptor antagonist astressin abolished all the stress-induced changes in the quantitative distribution of different cell lineages of the intestinal epithelium. To our knowledge, this is the first report specifically linking CRF peptides to changes in epithelial cell populations. From the use of both the $CRFR_1$-specific receptor antagonist antalarmin and daily CRF injection, we also showed that $CRFR_1$ promotes the expansion of the endocrine cell lineage. This finding is consistent with a recent in vitro study showing that in BON cells, used as a valid model to study serotonin secretion from enterochromaffin cells, CRF modulates endocrine cell activity by a mechanism involving $CRFR_1$s.[56] Moreover, we found that $CRFR_2$ regulated

the differentiation of Paneth cells and probably that both $CRFR_1$ and $CRFR_2$ were involved in the reduction of the goblet cell lineage after MD.

It is interesting to note that after cessation of the intermittent postnatal stress, its effects on goblet and Paneth cells did not exceed the duration of the renewal of cellular populations (2–5 days for mucus cells and 18–23 days for Paneth cells) whereas, in the case of the enteroendocrine cells, the effect was maintained. In our study, we showed that the expression of CRF returned to the level of that of control rats from postnatal day 24. As the renewal of enteroendocrine cells (also 2–3 days) is very rapid, this suggests that the stem cell population was altered by a CRF/$CRFR_1$-dependent mechanism at a critical stage of the intestinal maturation. In a previous study, Bjerknes and Cheng found that intestinal crypts may contain both short-lived (days) as well as long-lived (months) multipotent progenitors that are capable of giving rise to all epithelial cell types.[57] We can thus suppose that neonatal stress can modify these long-lived progenitors by a CRF/$CRFR_1$-dependent mechanism. This hypothesis is supported by the observation that $CRFR_1$s are present on intestinal stem cells.[30]

Alternatively, it has been proposed that the microenvironment around the stem cells, called the ‘niche’, plays an important role in epithelial cell renewal through cell–cell and cell–extracellular matrix interactions. It is thus possible that changes induced during intestinal maturation by postnatal stress are located at the stem cell microenvironment level. The effects of CRF-related peptides on epithelial differentiation in deprived rats would then occur by indirect mechanisms, via receptors localised in the enteric nervous system, as well as in isolated cells of the lamina propria.[30,58,59] This point was not examined in the present study and will be the focus of future experiments.

In conclusion, we show that neonatal MD of rat pups induces alterations in the differentiation of intestinal epithelial cells, resulting in a hyperplasia of enteroendocrine cells and in a depletion of Paneth and goblet cells. As far as we are aware, this is the first report showing that a chronic psychological stress modifies the specification of secretory epithelial lineages in the duodenum. These effects are mediated by CRF pathways acting on both $CRFR_1$ and $CRFR_2$. Such changes in the population of epithelial cells, in particular the depletion of Paneth and goblet cells, could create conditions leading to the development of epithelial barrier defects, perturb mucosal function and promote subsequent exposure to sensitising antigen or to bacterial infection.

Significance of this study

What is already known about this subject?

- Recent studies have suggested a pivotal role for psychological stress in intestinal epithelial functions. In animal experiments, chronic stress induces small and large intestine pathologies with low-grade inflammation, increased epithelial permeability, and altered bacterial–host interactions leading to bacterial translocation. In human, stress also decreases intestinal barrier function and exacerbates symptoms of irritable bowel syndrome and inflammatory bowel disease. The defensive barrier against damaging agents of the intestinal lumen is conferred by the epithelial layer itself and by factors such as peristalsis, mucus produced by goblet cells and antimicrobial peptides produced by Paneth cells.
- However, whether psychological stress modulates the fate of secretory epithelial cells (endocrine, Paneth and goblet cells) during and after stress remains unknown.

What are the new findings?

- Maternal deprivation induces a sustained decrease of Paneth and goblet cells but hyperplasia of endocrine cells.
- These effects are mediated by a CRF pathway acting on both $CRFR_1$ and $CRFR_2$ receptors.
- Some of these disturbances are maintained after the end of stress (depletion of Paneth cells and hyperplasia of endocrine cells).
- Stress induces alterations of transcription factors implicated in epithelial cell specification (Klf4, Pd×1).

How might it impact on clinical practice in the foreseeable future?

- These changes in the population of epithelial cells, in particular the depletion of Paneth and goblet cells, could create conditions leading to the development of epithelial barrier defects and perturb mucosal function. These effects could impair the mucosa and make it vulnerable to luminal aggression. Our data are in line with the emergent role of stress in mucosal barrier dysfunction and development/exacerbation of duodenal ulcers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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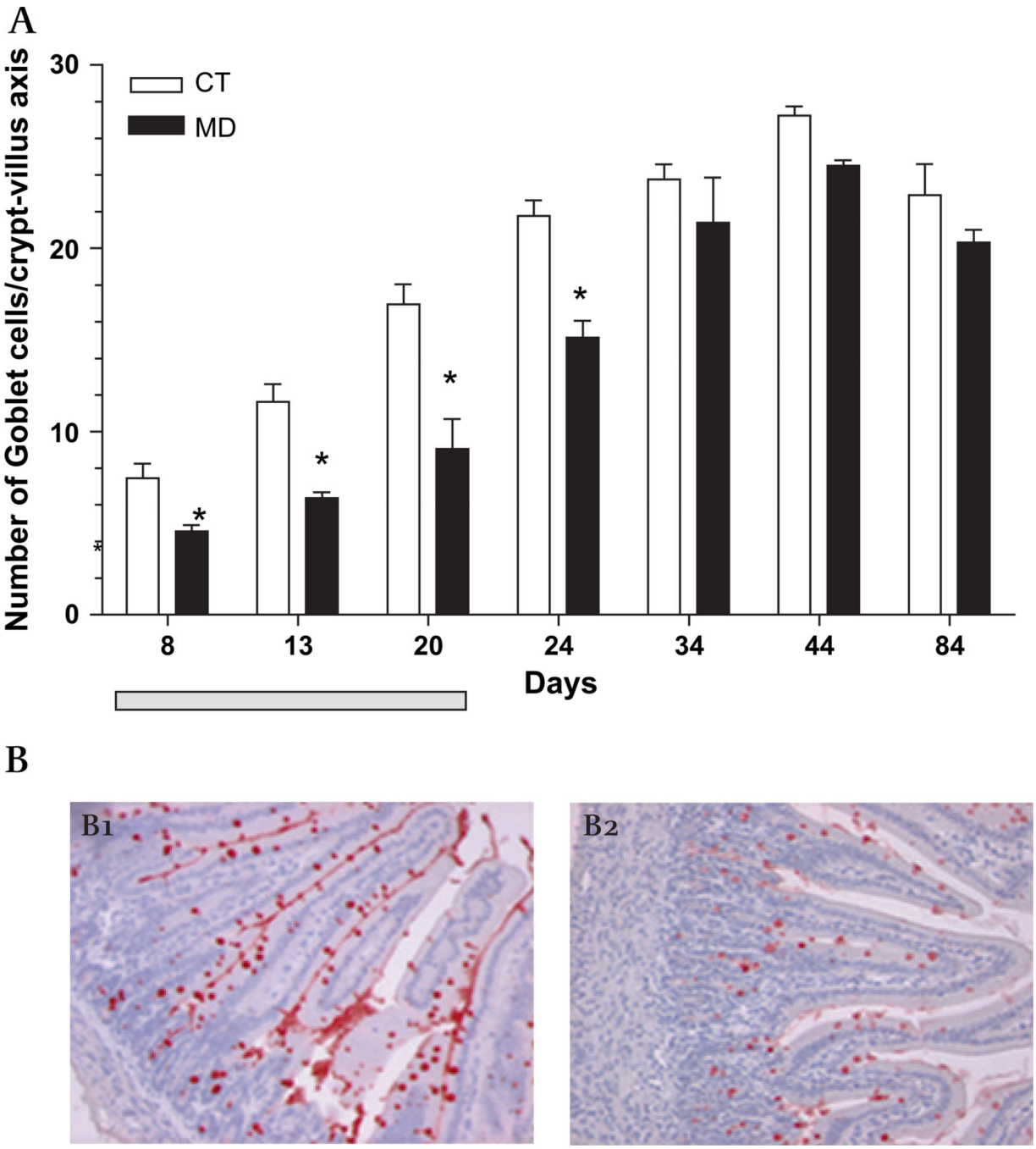

Figure 1.

Effect of maternal deprivation (MD) on goblet cells. (A) Number of goblet cells on postnatal days 8–84. Counts were performed on tissue sections (×20 magnification) and were expressed as the mean number of goblet cells per crypt–villus axis (±SEM, n=6). *p<0.05. CT, controls. The grey area shows the MD protocol. (B) Thin-section histology of rat duodenum (postnatal day 20; ×20 magnification). (B1) Duodenum of a control rat. Numerous goblet cells show MUC2 immunoreactivity (IR). (B2) A photomicrograph showing fewer goblet cells in the duodenum of a maternally deprived rat.

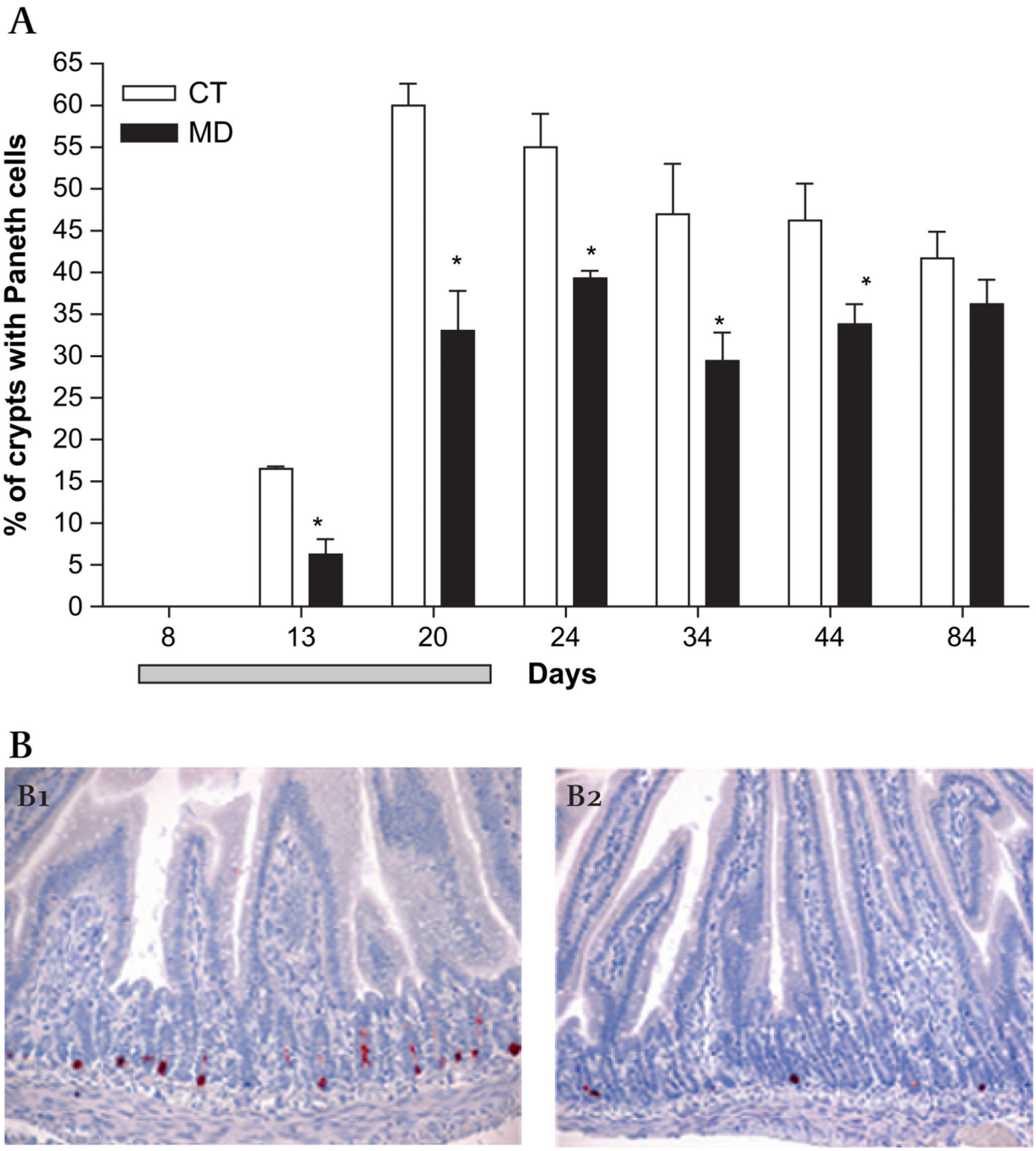

Figure 2.

Effect of maternal deprivation (MD) on Paneth cells. (A) Percentage of Paneth cell-containing crypts (mean±SEM, n=6) determined in the duodenum of control (CT) and MD pups. Paneth cell counts were performed on tissue sections (×20 magnification). *p<0.05. The grey area shows the MD protocol. (B) Immunostaining for lysozyme on paraffin-embedded sections of CT duodenum (B1) and MD duodenum (B2) at postnatal day 20 (×20 magnification). Paneth cells are clearly depleted in the epithelium of MD tissue as compared with controls.

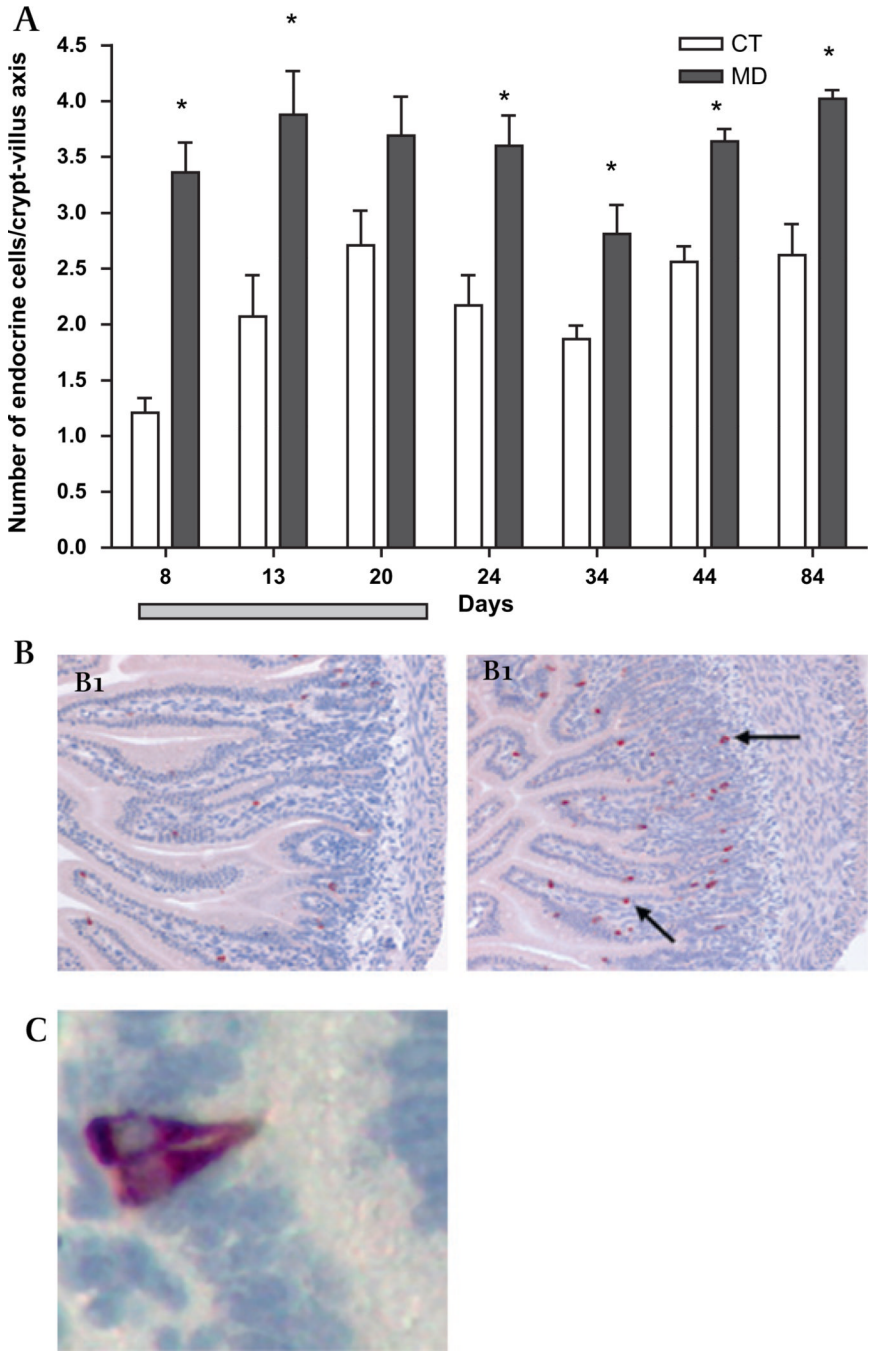

Figure 3.

Effect of maternal deprivation (MD) on enteroendocrine cells. (A) The number of enteroendocrine cells (chromogranin A (CGA) immunostaining) on postnatal days 8–84. Some 50–100 immunoreative (IR) endocrine cells per rat were counted (×20 magnification). Data are the mean±SEM (n=6). *p<0.05 compared with the control (CT) group. The grey area shows the MD protocol. (B) Sections stained with anti-CGA antibody from CT (B1) and MD (B2) rat duodenum showing an increased number of IR endocrine cells (arrows) after MD (×20 magnification). (C) Microscopic analysis shows that endocrine cells in the MD rats frequently lay next to each other (two adjacent cells are shown) instead of the normal pattern of scattered single cells (×40 magnification).

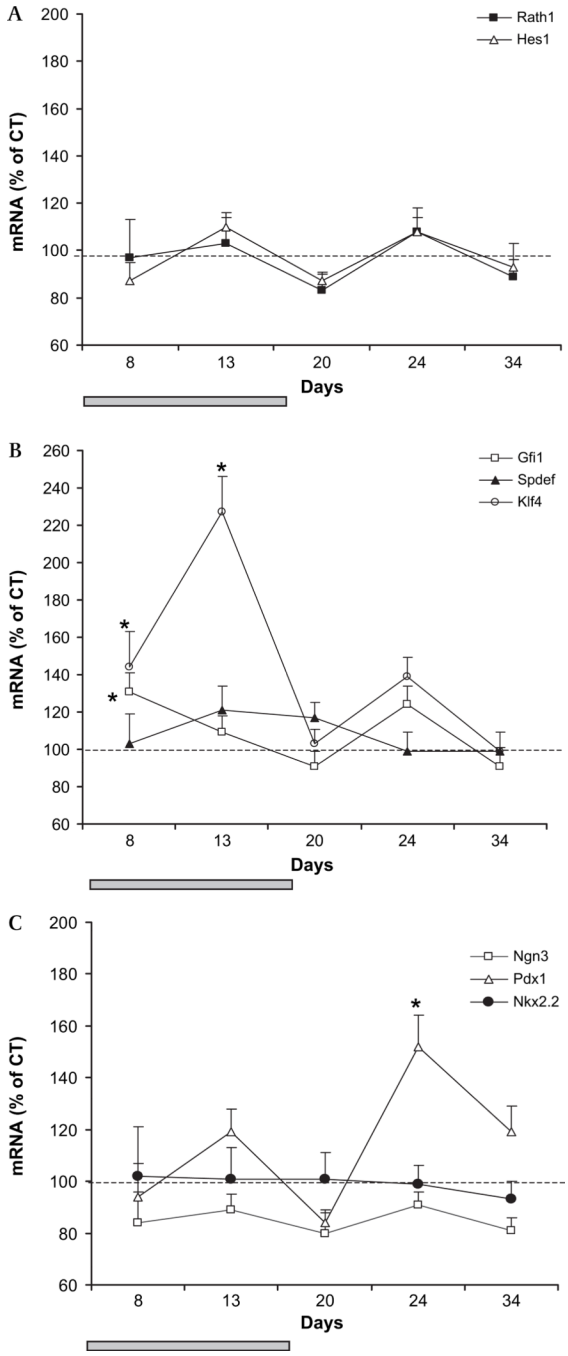

Figure 4.

Effect of maternal deprivation (MD) on transcription factor expression. Real-time RT-PCR analysis of transcription factors in the duodenum from postnatal day 8 to 34. The expression of each gene was normalised to the reference gene level in each sample. The results are expressed as the percentage of control unstressed rats of the same age (means±SEM, n=6 per group). *p<0.05 versus controls. The grey area shows the MD. (A) Transcription factors involved in the initial specification between absorptive and secretory lineages. (B) Transcription factors involved in the differentiation of goblet and Paneth cells. (C) Transcription factors involved in the specification of enteroendocrine lineages. CT, control.

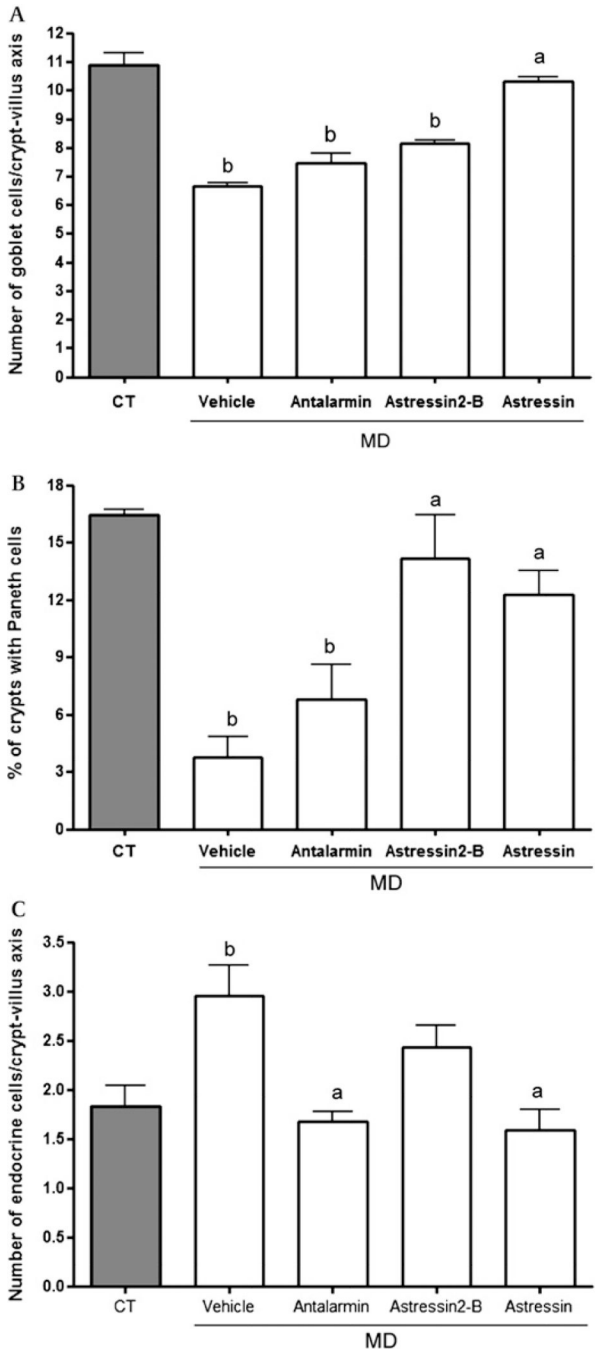

Figure 5.

Effect of corticotropin-releasing factor (CRF) receptor antagonists on maternal deprivation (MD)-induced alterations of intestinal secretory cell lineages in rats. Rats were injected with antalarmin ($CRFR_1$ antagonist, 20 mg/kg subcutaneously), $astressin_2$-B ($CRFR_2$ antagonist 150 μg/kg subcutaneously), astressin (non-specific CRFR antagonist, 60 μg/kg intraperitonally) or vehicle (subcutaneously) 30 min before MD, from postnatal days 5 to 13, and then sacrificed. The control (CT) pups were injected with vehicle (subcutaneously), and then returned to their dam. (A) Goblet, (B) Paneth and (C) enteroendocrine cells were stained. Values represent the means±SEM (n=6). a, $p<0.05$ vs vehicle (MD rats); b, $p<0.05$ vs CT.

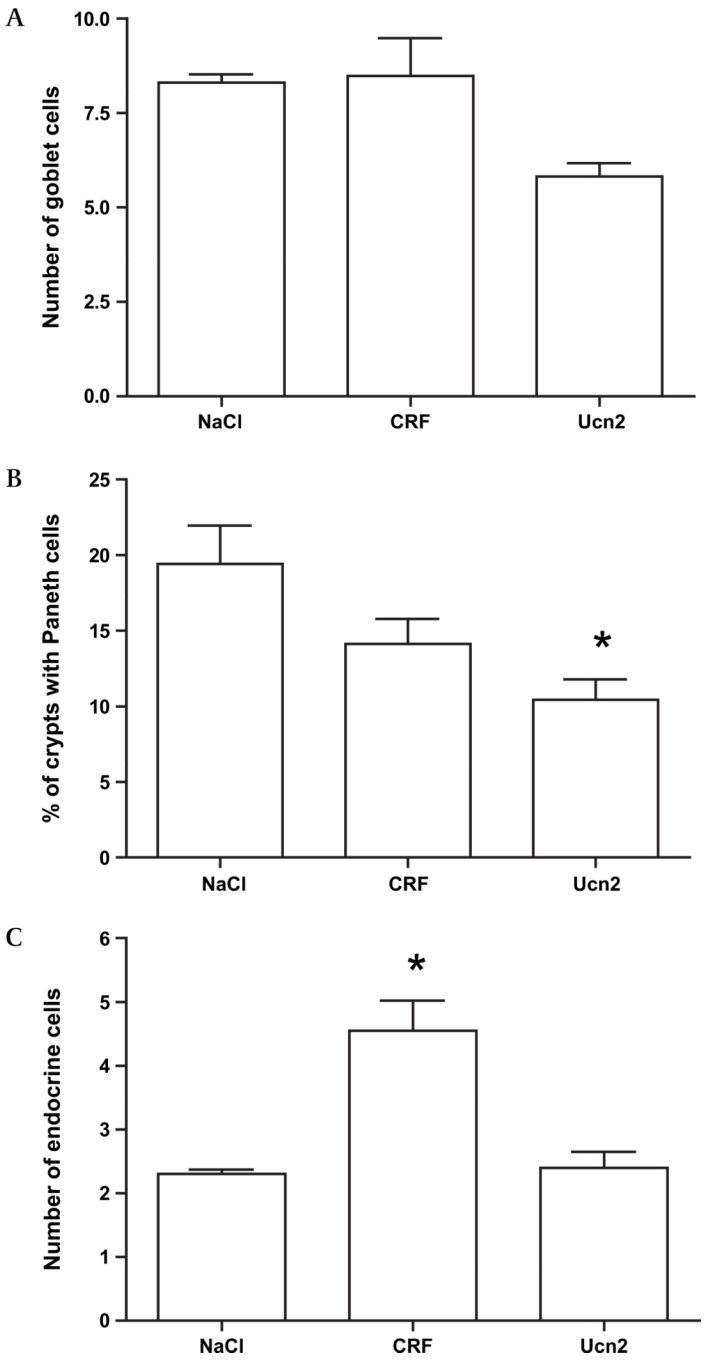

Figure 6.

Effects of corticotropin-releasing factor (CRF) (30 μg/kg, intraperitonally) and urocortin 2 (Ucn 2; 20 μg/kg, intraperitonally) versus saline on the epithelial secretory cell lineage in the rat duodenum. Rats were injected daily from postnatal days 5 to 13. Effects on (A) goblet cells, (B) Paneth cells and (C) enteroendocrine cells. Values represent the means ±SEM (n=6). *p<0.05.

Table 1

Antibodies

Antigen	Species	Source	Dilution
MUC2	Rabbit	Santa Cruz (H300)	1:250
Lysozyme	Rabbit	Zymed Laboratories	1:100
Chromogranin A	Rabbit	Immunostar	1:500
Serotonin	Rabbit	Sigma-Aldrich	1:500
Proliferating cell nuclear antigen	Mouse	Santa Cruz (PC10)	1:1000

Immunostar, Santa Cruz Biotechnology, Santa Cruz, California, USA; Sigma-Aldrich, St Louis, Missouri, USA; Zymed Laboratories, San Francisco, California, USA.