JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Food-Derived Peptides Stimulate Mucin Secretion and Gene Expression in Intestinal Cells

Daniel Martínez-Maqueda,[†] Beatriz Miralles,[†] Sonia De Pascual-Teresa,[§] Inés Reverón,[⊗] Rosario Muñoz,[⊗] and Isidra Recio*[,†]

[†]Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), Nicolás Cabrera 9, 28049 Madrid, Spain

[§]Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), CSIC, José Antonio Novais 10, 28040 Madrid, Spain

[⊗]Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

ABSTRACT: In this study, the hypothesis that food-derived opioid peptides besides β-casomorphin 7 might modulate the production of mucin via a direct action on epithelial goblet cells was investigated in HT29-MTX cells used as a model of human colonic epithelium. Seven milk whey or casein peptides, a human milk peptide, and a wheat gluten-derived peptide with proved or probable ability to bind μ- or δ-opioid receptors were tested on the cell culture. Significantly increased secretion of mucins was found after exposure to six of the assayed peptides, besides the previously described β-casomorphin 7, as measured by an enzyme-linked lectin assay (ELLA). Human β-casomorphin 5 and α-lactorphin were selected to study the expression of mucin 5AC gene (MUC5AC), the HT29-MTX major secreted mucin gene. α-Lactorphin showed increased expression of MUC5AC from 4 to 24 h (up to 1.6-fold over basal level expression), although differences were statistically different only after 24 h of exposure. However, this increased expression of MUC5AC did not reach significance after cell treatment with human β-casomorphin 5. In conclusion, six food-derived peptides have been identifed with described or probable opioid activity that induce mucin secretion in HT29-MTX cells. Concretely, α-lactorphin is able to up-regulate the expression of the major secreted mucin gene encoded by these cells.

KEYWORDS: *opioid peptides, milk, gene expression, intestinal goblet cells, mucins*

INTRODUCTION

The gastrointestinal lumen is covered by a viscous solution, known as mucus, which lubricates the epithelia, helping in the passage of substances and particles through the digestive tract, and forms a protective layer against noxious chemicals, microbial infections, dehydration, and changing luminal conditions.[1] The intestinal mucus gel owes its properties to secreted mucins, which are high molecular weight glycoproteins produced by goblet cells of the epithelium. Not surprisingly, mucin gene expression, biosynthesis, and secretion are highly regulated. Disruption of the barrier integrity and invasion of microbes with subsequent chronic inflammation and further disturbance of the mucosal architecture are hallmarks of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.[2] Even colon cancer has been linked to a faulty mucin expression in rat model experiments.[3]

Certain dietary components such as fiber, short-chain fatty acids, and probiotics can positively influence the characteristics of the intestinal mucus, although the mode of action of each compound may differ. Oat bran, rye bran, and soybean hull were shown to increase globlet cell volume density in the proximal and distal small intestine of hamsters.[4] Among the three main short-chain fatty acids produced in the human colon (i.e., acetate, propionate, and butyrate), butyrate appears to be the most effective in stimulating mucus release.[5] The modulation of mucin gene (MUC) expression in intestinal epithelial goblet cells has been subsequently demonstrated.[6] Besides, the mechanisms that regulate butyrate-mediated effects on MUC2 synthesis have been studied.[7] Recently, it has been reported that selected probiotics can induce MUC3 expression of mucosal intestinal epithelial cells in a reproducible, although time-limited, manner.[8]

With regard to dietary proteins, no information about their impact was available until two milk protein hydrolysates (casein and lactalbumin hydrolysates) and the peptide β-casomorphin 7, specifically, were shown to induce a strong release of mucin in the jejunum of the rat through the activation of the enteric nervous system and opioid receptors.[9] Trompette et al.[10] provided evidence that peptides that had shown this effect need to be absorbed and participate in the regulation of intestinal mucus discharge through activation of μ-opioid receptors on intestinal cells. The presence of opioid receptors on these cells suggests the possibility that food-derived peptides with opioid agonist structure, which can be produced in the intestinal lumen during gastrointestinal digestion, might modulate the production of mucin via a direct action on epithelial goblet cells. Rat and human mucus-secreting cell lines can be used as models to avoid interactions with the nervous system. In rat DHE cells, β-casomorphin 7 has been shown to increase mucin secretion and the expression of rat mucin rMuc2 and rMuc3. In human HT29-MTX cells, this peptide increased as well mucin secretion and MUC5AC mRNA levels.[11] The aim of this work was to evaluate if other food peptides with proved or probable

Received: March 27, 2012
Revised: July 9, 2012
Accepted: August 6, 2012
Published: August 23, 2012

Table 1. Primers for Real-Time PCR

gene	base pairs	primers		ref
MUC5AC	240	5′-CGACCTGTGCTGTGTACCAT-3′	(2870−2889)	11
		5′-CCACCTCGGTGTAGCTGAA-3′	(3109−3091)	
human cyclophillin	165	5′-TCCTAAAGCATACGGGTCCTGGCAT-3′	(280−304)	11
		5′-CGCTCCATGGCCTCCACAATATTCA-3′	(445−421)	
human β-actin	197	5′-CTTCCTGGGCATGGAGTC-3′	(879−896)	31
		5′-GCAATGATCTTGATCTTCATTGTG-3′	(1076−1053)	

ability to bind μ- or δ-opioid receptors can induce mucin secretion and MUC5AC expression on human HT29-MTX intestinal cells.

MATERIALS AND METHODS

Peptides. Bovine β-casomorphin 7 (YPFPGPI) and (D-Ala2,*N*-Me-Phe4,glycinol5) enkephalin (DAMGO) were purchased from Bachem (Bubendorf, Switzerland). Bovine neocasomorphin (YPVEPF), human β-casomorphin 5 (YPFVE), bovine α-casein exorphin (YLGYLE), bovine β-lactorphin (YLLF-NH_2), human and bovine α-lactorphin (YGLF-NH_2), gluten exorphin A5 (GYYPT), and bovine α-casein fragments 90−94 (RYLGY) and 143−149 (AYFYPEL) were synthesized using conventional solid-phase FMOC synthesis with a 433A peptide synthesizer (Applied Biosystems, Warrington, UK). Their purity (>90%) was verified in our laboratory by reverse phase high-performance liquid chromatography and tandem mass spectrometry.

Cell Culture. HT29-MTX, a human colon adenocarcinoma-derived mucin-secreting goblet cell line, was provided by Dr. Thécla Lesuffleur (INSERM UMR S 938, Paris, France).[12] The cell line was grown in plastic 75 cm^2 culture flasks in DMEM supplemented with 10% FBS and 10 mL/L penicillin−streptomycin solution (all from Gibco, Paisley, UK) at 37 °C in a 5% CO_2 atmosphere in a humidified incubator. Cells were passaged weekly using trypsin/EDTA 0.05% (Gibco). The culture medium was changed every 2 days. To study the effect of peptides or DAMGO, cells were seeded at a density of 5×10^5 cells per well in 12-well culture plates (Nunc, Roskilde, Denmark). The cell line was used between passages 12 and 19. Experiments were performed 21 days after confluency. Twenty-four hours before the studies, the culture medium was replaced by serum- and antibiotic-free medium to starve the cells and to eliminate any interference from extraneous proteins or hormones. After serum-free medium removal, the monolayer was washed twice with PBS. Serum-free medium with or without peptide (0.05−0.5 mM) or DAMGO (0.001 mM) was added to the cells and incubated at 37 °C for 10 min−24 h in a 5% CO_2 humidified atmosphere. The supernatants were collected, frozen, and stored at −70 °C. The total RNA was isolated with Nucleospin RNA II (Macherey-Nagel, Düren, Germany).

Enzyme-Linked Lectin Assay (ELLA). To measure mucin-like glycoprotein secretion, an ELLA reported by Trompette et al.,[13] slightly modified, was used. Briefly, wells of a microtiter plate were coated with sample diluted in sodium carbonate buffer (0.5 M, pH 9.6) and incubated overnight at 4 °C. The plates were then washed with PBS containing 0.1% Tween 20 (PBS−Tween) and blocked with 2% BSA in PBS-Tween (PBS−Tween−BSA) for 1 h at 37 °C. After samples were washed six times, biotinylated wheat germ agglutinin (Vector Laboratories, Peterborough, UK) in PBS−Tween−BSA (1:1000) was added, and the samples were incubated for 1 h at 37 °C. Subsequently, avidin−peroxidase conjugate (Vector Laboratories) (1:50000) was added for signal amplification. Colorimetric determination using *o*-phenylenediamine dihydrochloride solution (Dako, Glostrup, Denmark) was performed at 492 nm.

The mucin-like glycoprotein content of samples was determined from standard curves prepared from gastric porcin mucin (Sigma, Steinheim, Germany). All experiments were performed three times for at least three biological replicates. Data were analyzed by a two-way ANOVA, using GraphPad Prism 4 software, followed by the Bonferroni test for single comparisons. Differences between means and controls were considered to be significant with $P < 0.05$ (∗), $P < 0.01$ (∗∗), or $P < 0.001$ (∗∗∗).

Real-Time Quantitative RT-PCR Assays (qRT-PCR). Quantitative RT-PCR amplification was carried out with the real-time fluorescence method using a 7500 Fast System (Applied Biosystems). RNA (375 ng) was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instruction. The specific primers (target and reference genes) used for the RT-PCR assays are listed in Table 1. The SYBR Green method was used, and each assay was performed with cDNA samples in triplicate. Each reaction tube contained 12.5 μL of 2× SYBR Green real-time PCR Master Mix (Applied Biosystems), 5 μL of 1 μM gene-specific forward and reverse primers, and 2.5 μL of a 1:10 dilution of cDNA. Amplification was initiated at 50 °C for 2 min and at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Control PCRs were included to confirm the absence of primer−dimer formation (no-template control) and to verify that there was no DNA contamination (without RT enzyme negative control). All real-time PCR assays amplified a single product as determined by melting curve analysis and by electrophoresis in 2% agarose gels. A standard curve was plotted with cycle threshold (Ct) values obtained from amplification of known quantities of cDNAs and used to determine the efficiency (E) as $E = 10^{-1/\text{slope}}$.

The relative expression levels of the target gene were calculated using the comparative critical threshold method (ΔΔCt). Human cyclophilin and β-actin were tested as reference genes. The cyclophilin gene was chosen to calculate the threshold cycles because it had previously been shown to be constant under all conditions used. All experiments were performed at least three times in triplicate ($n = 9$). Data were analyzed by a two-way ANOVA, using GraphPad Prism 4 software. For a better comparison of the concentration versus control data for each time, data were analyzed by a one-way ANOVA, followed by the Newman−Keuls test. Differences between means and controls were considered to be significant with $P < 0.05$ (∗), $P < 0.01$ (∗∗), or $P < 0.001$(∗∗∗).

RESULTS

Determination of Mucin Secretion of HT29-MTX Cell Culture over 24 h. HT29-MTX cells form a homogeneous monolayer of polarized goblet cells that exhibit a discrete apical brush border.[14] Previous studies have shown that the morphological differentiation of the cells, as well as the secretion of mucins when it occurs, is a growth-related phenomenon, starting after the cells have reached confluency.[12] To get quantitative information about the mucin production by HT29-MTX cells, mucins were quantified by ELLA during 24 h, when the proportion of cells that express mucus reaches 100% and remains stable (21 days after confluency).[12] Figure 1 shows the concentration of mucin-like glycoproteins found in the culture medium at increasing times between 30 min and 24 h. The values exhibited a steep increased secretion of mucin between 4 and 8 h (10 times higher) that was followed by a further increase reaching 6 times the 8 h value at 24 h. Close

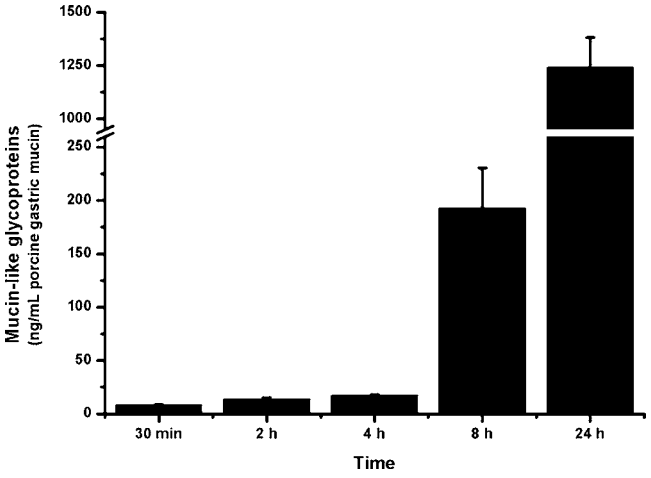

Figure 1. Time course secretion of mucin by HT29-MTX cells determined by enzyme-linked lectin assay. Data are expressed as mucin-like glycoprotein secretion. Each bar represents the mean ± SE of six biological replicates in triplicate.

values were measured in two independent experiments at each time. Therefore, the cell culture proved to be a reliable tool for the study of gastrointestinal mucin secretion.

Mucin Secretion of HT29-MTX Cells under the Effect of Different Food Peptides. Various synthetic milk- or wheat-derived gluten peptides with proved ability to bind μ- or δ-opioid receptors and two casein-derived peptides that had shown a potent antihypertensive effect and sequences that may anticipate opioid activity were added to the cell culture.[15] The specific μ-receptor agonist DAMGO was used as a positive control.

Table 2 summarizes the maximum mucin secretion by HT29-MTX cells after addition of the assayed peptides at 0.1 mM. Six of the eight newly studied food peptides showed significant activity on mucin secretion by HT29-MTX cells. From the casein-derived peptides, human β-casomorphin 5 showed the highest secretion value. Both whey-derived peptides, α-lactorphin and β-lactorphin, showed significantly higher values than the control. Among the studied peptides, gluten exorphin showed the lowest activity with an increase of 157% of control. The specific μ-receptor agonist DAMGO behaved as a potent mucus secretagogue in HT29-MTX cells, as it was used at a 100 times lower concentration than the food-derived peptides. The activity of this agonist had been previously reported in rat ex vivo experiments[10] and DHE cells[11] but not in human cells. From this first screening, the whey-derived peptide, α-lactorphin, and the human β-casomorphin 5 were selected for further experiments.

Figure 2 shows time course experiments of addition of different doses (0.05, 0.1, and 0.5 mM) of α-lactorphin (A) and human β-casomorphin 5 (B) and subsequent determination of secreted mucin by ELLA. Both peptides stimulated the release of mucin-like glycoprotein at 0.5 and 2 h after exposure, which denotes the enhanced mucus discharge in this time range (Figure 2). Although the secretion values did not allow clear evidence of a dose−response effect, in general, higher releases were found with the highest dose (0.5 mM) at 0.5 and 2 h for α-lactorphin and at 0.5 h for human β-casomorphin 5.

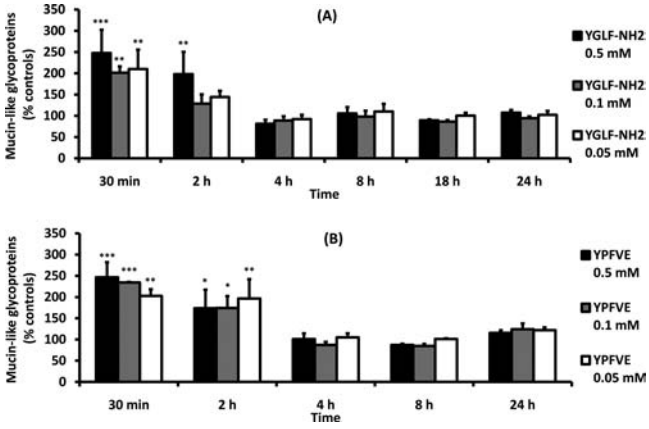

Figure 2. Time course effect at three different concentrations (0.05, 0.1, and 0.5 mM) of α-lactorphin, YGLF-NH2 (A), and human β-casomorphin 5, YPFVE (B), on mucin secretion in HT29-MTX cells determined by enzyme-linked lectin assay. Data are expressed as mucin-like glycoprotein secretion as a percentage of control (untreated cells). Each point represents the mean ± SE of three biological replicates in triplicate. Significant differences of each concentration versus control were determined by two-way ANOVA applying the Bonferroni test: (***)$P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$.

MUC5AC Expression in HT29-MTX Cells under the Effect of Different Food Peptides. Quantitative PCR was used to determine the level of transcripts of MUC5AC in HT29-MTX cells treated with α-lactorphin and human β-

Table 2. Maximum Mucin Secretion Respect to Control (Untreated HT29-MTX cells) upon Addition of 0.1 mM Different Food Peptides and 0.001 mM DAMGO Determined by Enzyme-Linked Lectin Assay[a]

peptide				
sequence	denomination	food protein	% control	*P*
YPFPGPI	bovine β-casomorphin 7	β-casein A2 f(60−66)	282	<0.001
YPVEPF	bovine neocasomorphin	β-casein f(114−119)	-	
YPFVE	human β-casomorphin 5	β-casein f(51−55)	234	<0.001
YLGYLE	bovine α-casein exorphin 2−7	α-casein f(91−96)	-	
RYLGY		α-casein f(90−94)	191	<0.001
AYFYPEL		α-casein f(143−149)	221	<0.001
YLLF-NH_2	bovine β-lactorphin	β-lactoglobulin f(102−105)	453	<0.001
YGLF-NH_2	bovine and human α-lactorphin	α-lactalbumin f(50−53)	201	<0.001
GYYPT	gluten exorphin A5	wheat glutenin	157	<0.05
	DAMGO		232	<0.01

[a]Data were obtained by three biological replicates in triplicate. Significant differences between average values and control by two-way ANOVA (Bonferroni test).

casomorphin 5. MUC5AC was selected because it is the gene that codifies an abundant secreted mucin and presents high levels of mRNA in HT29-MTX cells.[12] β-Casomorphin 7 was used as positive control, and it showed increased expression levels of MUC5AC after 24 h of exposure (1.7-fold basal level), according to the previously reported results (data not shown).[11] Different concentrations of peptides between 0.05 and 0.5 mM were added to the incubation medium, and cells were incubated during a time range of 10 min−24 h (Figure 3).

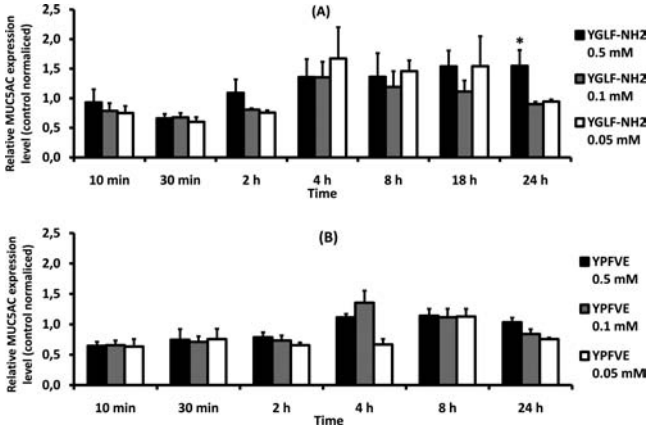

Figure 3. Time course effect at three different concentrations (0.05, 0.1, and 0.5 mM) of α-lactorphin, YGLF-NH2 (A), and human β-casomorphin 5, YPFVE (B), on MUC5AC mRNA level in HT29-MTX cells determined by quantitative RT-PCR. Data are expressed as relative MUC5AC expression level of control (untreated cells), using cyclophilin as reference gene. Each point represents the mean ± SE of three biological replicates in triplicate. Significant differences of each concentration versus control were determined by one-way ANOVA applying the Newman−Keuls test: (*) $P < 0.05$.

α-Lactorphin showed a trend of increased MUC5AC expression from 4 to 24 h, but, due to the high variability, differences of expression reached significance ($P < 0.05$) only at 24 h (1.6-fold basal level expression at 0.5 mM). On the contrary, time course experiments for human β-casomorphin 5 did not induce a significant increase in MUC5AC mRNA levels compared to untreated cells at the assayed times.

DISCUSSION

Mucin secretion by nontreated HT29-MTX goblet cells increased noticeably throughout the 24 h period studied. The slow rise between 0.5 and 4 h may be related to cell adaptation when starvation medium was added. The observed trend is in accordance with information provided in the literature about the high mucin-producing capacity by intestinal goblet cells, based on the important role that mucins play in the epithelium protection and lubrication, as well as its constant renewal.[16]

β-Casomorphin 7 was the first food peptide reported with opioid activity.[17] It is the most studied food-derived opioid peptide, and its influence on the mucin secretion has been evaluated in vitro (human and rat) and ex vivo (rat).[10,11] Its activity on the mucin secretion and MUC5AC expression by goblet cells was confirmed. The present study shows that a whey protein-derived peptide, α-lactorphin, with proved opioid activity, although with lower affinity toward μ-receptors than β-casomorphin 7,[18] can induce mucin secretion and MUC5AC expression. Our results have not found a relationship of the effect of α-lactorphin to dose, because significance in levels of transcripts of MUC5AC was found only at the highest dose assayed (0.5 mM). With regard to the time, α-lactorphin showed increased MUC5AC expression from 4 to 24 h after exposure, although significance was reached at 24 h. The mucin discharge coupled with the corresponding increase of MUC expression to replenish the intracellular mucin pool is a behavior that can be found in other secreting cells such as pancreatic β cells, which respond to changes in blood glucose by first secreting insulin and next increasing insulin biosynthesis.[19] The time range at which mucin exocytosis and stimulation of glycoprotein synthesis reach their maximum under the effect of external agents is still unknown. A study on the treatment of rat cells with butyrate showed that the significant increase in rat mucin gene (rMuc) expression was observed after 24 h but not at earlier time points (1, 3, and 8 h).[6]

Human β-casomorphin 5 showed a significant mucin-secreting activity but no significant overexpression of MUC5AC at the assayed times. Human β-casomorphin 5 displays opioid activity with affinity for μ- and δ-receptors, although it is 2.6 times less potent than β-casomorphin 7.[20]

Two peptides from bovine α-casein, RYLGY and AYFYPEL, showed significant mucin-secretor values. These sequences have not been reported as opioid but have been described in a hydrolysate prepared by our research group for which antihypertensive activity has been demonstrated.[15] Peptide RYLGY is included in the sequence of a casein exorphin (RYLGYL) with moderate opioid activity and μ- and δ-receptor affinity.[21] Peptide AYFYPEL had not been previously described as an opioid peptide but shows an aromatic residue, Tyr, in the second position and Phe together with Tyr in the third and fourth positions, respectively, which forms a favorable structure to bind opioid receptors.[22] Gluten exorphin A5, a peptide having demonstrated opioid activity[23] presented mucin secretion activity. The low value encountered is in accordance with the lower μ-receptor affinity of this peptide compared to δ-receptor.[23] Even so, this is the first report of the mucin-secretory activity of this opioid peptide of vegetal origin on human HT29-MTX goblet cells.

Finally, peptides showing no stimulatory activity, neocasomorphin and α-casein exorphin 2−7, although having been previously reported as opioid peptides, have shown lower activity affinity for μ- and δ-receptor than β-casomorphin 7.[24] However, this lower affinity cannot explain the lack of activity found for these peptides, because the affinity of neocasomorphin for μ-receptors is higher than that of α-lactorphin. Although it has been described that enzymatic activity and expression of intestinal peptidases are lower in HT29 compared with Caco-2 cells,[25] it is possible that some of these sequences are susceptible to the action of cell peptidases and, therefore, peptides could be hydrolyzed to an inactive form. This point will be considered in future studies because it can explain the lack of activity of previously described opioid sequences.

The fact that not only casein-derived but also whey-derived peptides provoke stimulation of mucin secretion and modulation of mucin expression in goblet cells opens a new perspective, with regard to previous works, wherein β-casomorphins were solely considered to play an important physiological role in this cell type. In fact, casein has demonstrated in vivo an effect on intestinal mucin expression in the rat, whereby a significant increase of rMuc3 mRNA in the small intestinal tissue and rMuc4 mRNA in the colon has been observed, when a diet containing hydrolyzed casein compared

to a synthetic amino acid mixture or a protein-free diet was orally administered.[26] There have been studies related to the administration of bovine α-lactalbumin and the stimulation of mucus metabolism in gastric mucosa,[27,28] and some reports had evidenced the activity of α-lactalbumin and hydrolysates of this protein on gastric ulcer on rat models in vivo.[29] Furthermore, a hydrolysate of this protein induced a strong release of mucin in the jejunum of the rat ex vivo.[9] However, some researchers support the view that the protection by whey protein on induced colitis in rats has to be attributed to its high content in threonine and cysteine and to a reduced gene expression of inflammation markers such as interleukin 1β, calprotectin, and inducible nitric oxide synthase.[30] Indubitably, the mechanisms involved in the protective effect of dietary peptides on gastrointestinal mucosa need to be ascertained.

In conclusion, six food-derived peptides have been shown to induce mucin secretion in HT29-MTX human colonic goblet-like cells for the first time. Some of them had been previously described as opioid peptides but two sequences had not, although their structure may be favorable to bind opioid receptors. Concretely, α-lactorphin increased the expression of MUC5AC. This is a first step in finding new food peptides that can be included in the wide variety of stimuli that provoke mucin secretion in goblet cells and therefore play a role in the modulation of this protective function. These findings may assist in the development of dietary strategies to augment mucus layer formation as protection against inflammatory bowel disease effects.

AUTHOR INFORMATION

Corresponding Author

*E-mail: i.recio@csic.es.

Funding

This work was supported by Projects AGL2008-01713, AGL2011-26643, and Consolider-Ingenio FUN-C-Food CSD 2007-063 from Ministerio de Economía y Competitividad. We are participants in the FA1005COST Action INFOGEST on food digestion. D.M.-M. acknowledges CSIC for a JAE Program fellowship.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are deeply grateful to T. Lessuffleur for generously providing HT29-MTX cells.

REFERENCES

(1) Pérez-Vilar, J. Gastrointestinal mucus gel barrier. In *Oral Delivery of Macromolecular Drugs*, 1st ed.; Bernkop-Schnürch, A., Ed.; Springer: New York, 2009; pp 21−47.

(2) Otte, J.-M.; Ilka, W.; Stephan, B.; Ansgar, M. C.; Frank, S.; Michael, K.; Wolfgang, E. S. Human β defensin 2 promotes intestinal wound healing in vitro. *J. Cell. Biochem.* **2008**, *104*, 2286−2297.

(3) Velcich, A.; Yang, W.; Heyer, J.; Fragale, A.; Nicholas, C.; Viani, S.; Kucherlapati, R.; Lipkin, M.; Yang, K.; Augenlicht, L. Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science* **2002**, *295*, 1726−1729.

(4) Lundin, E.; Zhang, J. X.; Huang, C. B.; Reuterving, C. O.; Hallmans, G.; Nygren, C.; Stenling, R. Oat bran, rye bran, and soybean hull increase goblet cell-volume density in the small-intestine of the golden-hamster. A histochemical and stereologic light-microscopy study. *Scand. J. Gastroenterol.* **1993**, *28*, 15−22.

(5) Shimotoyodome, A.; Meguro, S.; Hase, T.; Tokimitsu, I.; Sakata, T. Short chain fatty acids but not lactate or succinate stimulate mucus release in the rat colon. *Comp. Biochem. Phys. A* **2000**, *125*, 525−531.

(6) Gaudier, E.; Jarry, A.; Blottiere, H. M.; De Coppet, P.; Buisine, M. P.; Aubert, J. P.; Laboisse, C.; Cherbut, C.; Hoebler, C. Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2004**, *287*, 1168−1174.

(7) Burger van Paassen, N.; Vincent, A.; Puiman, P. J.; Van der Sluis, M.; Bouma, J.; Boehm, G.; Van Goudoever, J. B.; Van Seuningen, I.; Renes, I. B. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem. J.* **2009**, *420*, 211−219.

(8) Dykstra, N. S.; Hyde, L.; Adawi, D.; Kulik, D.; Ahrne, S. I. V.; Molin, G.; Jeppsson, B.; Mackenzie, A.; Mack, D. R. Pulse probiotic administration induces repeated small intestinal muc3 expression in rats. *Pediatr. Res.* **2011**, *69*, 206−211.

(9) Claustre, J.; Toumi, F.; Trompette, A.; Jourdan, G.; Guignard, H.; Chayvialle, J. A.; Plaisancié, P. Effects of peptides derived from dietary proteins on mucus secretion in rat jejunum. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2002**, *283*, 521−528.

(10) Trompette, A.; Claustre, J.; Caillon, F.; Jourdan, G.; Chayvialle, J. A.; Plaisancié, P. Milk bioactive peptides and β-casomorphins induce mucus release in rat jejunum. *J. Nutr.* **2003**, *133*, 3499−3503.

(11) Zoghbi, S.; Trompette, A.; Claustre, J.; Homsi, M. E.; Garzon, J.; Jourdan, G.; Scoazec, J.-Y.; Plaisancié, P. β-Casomorphin-7 regulates the secretion and expression of gastrointestinal mucins through a μ-opioid pathway. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2006**, *290*, 1105−1113.

(12) Lesuffleur, T.; Porchet, N.; Aubert, J. P.; Swallow, D.; Gum, J. R.; Kim, Y. S.; Real, F. X.; Zweibaum, A. Differential expression of the human mucin genes MUC1 to MUC5 in relation to growth and differentiation of different mucus-secreting HT-29 cell subpopulations. *J. Cell Sci.* **1993**, *106*, 771−783.

(13) Trompette, A.; Blanchard, C.; Zoghbi, S.; Bara, J.; Claustre, J.; Jourdan, G.; Chayvialle, J. A.; Plaisancié, P. The DHE cell line as a model for studying rat gastro-intestinal mucin expression: effects of dexamethasone. *Eur. J. Cell Biol.* **2004**, *83*, 347−358.

(14) Lesuffleur, T.; Barbat, A.; Dussaulx, E.; Zweibaum, A. Growth adaptation to methotrexate of HT-29 human colon carcinoma cells is associated with their ability to differentiate into columnar absorptive and mucus-secreting cells. *Cancer Res.* **1990**, *50*, 6334−6343.

(15) Contreras, M. d. M.; Carrón, R.; Montero, M. J.; Ramos, M.; Recio, I. Novel casein-derived peptides with antihypertensive activity. *Int. Dairy J.* **2009**, *19*, 566−573.

(16) Linden, S. K.; Sutton, P.; Karlsson, N. G.; Korolik, V.; McGuckin, M. A. Mucins in the mucosal barrier to infection. *Mucosal Immunol.* **2008**, *1*, 183−197.

(17) Brantl, V.; Teschemacher, H.; Henschen, A.; Lottspeich, F. Novel opioid peptides derived from casein (β-casomorphins). I. Isolation from bovine casein peptone. *Hoppe-Seyler's Z. Physiol. Chem.* **1979**, *360*, 1211−1216.

(18) Yoshikawa, M.; Tani, F.; Yoshimura, T.; Chiba, H. Opioid peptides from milk proteins. *Agric. Biol. Chem.* **1986**, *50*, 2419−2421.

(19) Webb, G. C.; Akbar, M. S.; Zhao, C.; Steiner, D. F. Expression profiling of pancreatic β cells: glucose regulation of secretory and metabolic pathway genes. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5773−5778.

(20) Koch, G.; Wiedemann, K.; Teschemacher, H. Opioid activities of human β-casomorphins. *Naunyn Schmiedebergs Arch. Pharmacol.* **1985**, *331*, 351−354.

(21) Loukas, S.; Varoucha, D.; Zioudrou, C.; Streaty, R. A.; Klee, W. A. Opioid activities and structures of α-casein-derived exorphins. *Biochemistry* **1983**, *22*, 4567−4573.

(22) Meisel, H. Overview on milk protein-derived peptides. *Int. Dairy J.* **1998**, *8*, 363−373.

(23) Fukudome, S.; Jinsmaa, Y.; Matsukawa, T.; Sasaki, R.; Yoshikawa, M. Release of opioid peptides, gluten exorphins by the action of pancreatic elastase. *FEBS Lett.* **1997**, *412*, 475−479.

(24) Jinsmaa, Y.; Yoshikawa, M. Enzymatic release of neocasomorphin and β-casomorphin from bovine β-casein. *Peptides* **1999**, *20*, 957−962.
(25) Howell, S.; Kenny, A. J.; Turner, A. J. A survey of membrane peptidases in two human colonic cell lines, Caco-2 and HT-29. *Biochem. J.* **1992**, *284*, 595−601.
(26) Han, K.-S.; Deglaire, A.; Sengupta, R.; Moughan, P. J. Hydrolyzed casein influences intestinal mucin gene expression in the rat. *J. Agric. Food Chem.* **2008**, *56*, 5572−5576.
(27) Ushida, Y.; Shimokawa, Y.; Matsumoto, H.; Toida, T.; Hayasawa, H. Effects of bovine α-lactalbumin on gastric defense mechanisms in naive rats. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 577−583.
(28) Ushida, Y.; Shimokawa, Y.; Toida, T.; Matsui, H.; Takase, M. Bovine α-lactalbumin stimulates mucus metabolism in gastric mucosa. *J. Dairy Sci.* **2007**, *90*, 541−546.
(29) Mezzaroba, L. F. H.; Carvalho, J. E.; Ponezi, A. N.; Antônio, M. A.; Monteiro, K. M.; Possenti, A.; Sgarbieri, V. C. Antiulcerative properties of bovine α-lactalbumin. *Int. Dairy J.* **2006**, *16*, 1005−1012.
(30) Sprong, R. C.; Schonewille, A. J.; Van der Meer, R. Dietary cheese whey protein protects rats against mild dextran sulfate sodium-induced colitis: role of mucin and microbiota. *J. Dairy Sci.* **2010**, *93*, 1364−1371.
(31) Tai, E. K.; Wong, H. P.; Lam, E. K.; Wu, W. K.; Yu, L.; Koo, M. W.; Cho, C. H. Cathelicidin stimulates colonic mucus synthesis by up-regulating MUC1 and MUC2 expression through a mitogen-activated protein kinase pathway. *J. Cell. Biochem.* **2008**, *104*, 251−258.