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Short communication

The carboxamide feG(NH2) inhibits endotoxin perturbation of intestinal motility

Daimen Tan [b], Catherine Rougeot [c], Joseph S. Davison [a], Ronald Mathison [a,*]

[a] *Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1*
[b] *Department of Pediatrics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1*
[c] *Unité de Génétique et Biochimie du Développement, Département d'Immunologie, Institut Pasteur, 25–28 rue du Docteur Roux, 75724, Paris cedex 15, France*

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Abstract

The submandibular gland rat-1 (SMR1) salivary gland prohormone contains several peptides, submandibular gland peptide-T (SGP-T) and the tripeptide, FEG, which possess anti-inflammatory activities. The D-isomeric form of FEG, feG, also is a potent anti-inflammatory peptide. In this study, we compared the inhibitory activity of feG and its carboxamide derivative, feG(NH2), on the perturbations of intestinal motility induced by intravenous lipopolysaccharide. feG(NH2) was 20–30 times more potent than feG in reducing the motility disturbances induced by lipopolysaccharide. feG may undergo □-amidation to yield a hormone that strongly down-regulates intestinal responsiveness to endotoxin. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Salivary glands contain polypeptide hormones such as renin and epidermal growth factor (EGF), but the discovery of a variable coding sequence-1 (VCS-1) gene encoding a 146 amino acid protein named SMR1 (submandibular gland rat-1; Rosinski-Chupin et al., 1988) resulted in the identification of a new prohormone that is synthesized by submandibular glands. A heptapeptide (SGP-T; sequence = TDIFEGG) and a tripeptide (FEG), which are found at the carboxyl-terminal of the SMR1 prohormone, were isolated from rat submandibular glands and found to possess anti-inflammatory activity (Mathison et al., 1997a, 1998). The tripeptide FEG and its D-isomeric form, feG, are potent inhibitors of intestinal, cardiovascular and pulmonary anaphylaxis (Mathison et al., 1998; Befus et al., 2000).

Many mammalian bioactive hormones, neurotransmitters and growth factors have a carboxyl terminal carboxamide (Prigge et al., 1997), and since the C-terminal sequence of SMR1 of FEGGGK (Rosinski-Chupin et al., 1988) is indicative of C-terminal amidation, it is possible that carboxamide derivatives of FEG or its glycine extended analogues may possess biological activity. In this study, we compared the modulation of LPS induced perturbations of intestinal motility by feG and feG(NH2). The D-isomeric forms of the peptides were used to provide metabolically stable peptides which could be administered orally (Mathison et al., 1998).

2. Material and methods

2.1. Animals

All experiments were carried out in accordance with the Canadian Council on Animal Care guidelines, and received prior approval from the University of Calgary Animal Care Committee. Male Sprague Dawley rats, weighing 200–250 g were raised at the Life and Environmental Sciences Animal Resource Centre, The University of Calgary. The rats were maintained with lights on from 7:00 to 19:00 h, and were provided food and water ad libitum. With the

* Corresponding author. Tel.: +1-403-220-6986; fax: +1-403-283-8225.
E-mail address: rmathiso@ucalgary.ca (R. Mathison).

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intestinal motility experiments lipopolysaccharide (LPS; *Salmonella typhosa*; Sigma, St. Louis, MO) was injected intravenously at a dose of 20 μg/kg transit (Hellström et al., 1997).

2.2. Intestinal motility

The methods for recording intestinal motility in rats have been described (Scott and Tan, 1996). Under halothane anesthesia, following an 18-h fast, three pairs of Teflon-coated stainless steel bipolar electrodes were sown onto the longitudinal muscle of the jejunum at 2.5-cm intervals, with the first pair placed 2.5 cm from the ligament of Treitz. The electrodes of each pair were sutured 3 mm apart for bipolar recording. The wires exited the peritoneal cavity through a stab incision and subcutaneously tunneled to the anterior abdominal wall and exited through a plastic cannula positioned in an intrascapular region. Indwelling jugular catheters were installed for subsequent administration of peptides. On the 7th day after surgery, the rats were fasted overnight and the following day, after stabilization in the recording chamber, the three pairs of electrodes were connected to bioelectric amplifiers (Hewlett–Packard, model 8811A) and the electric signals generated by the migrating myoelectric complexes recorded on a chart recorder (Hewlett–Packard, model 7858A) for three cycles before, and 120 min after intravenous challenge with 20 μg/kg of LPS. The peptides were given intravenously 20 min before injecting LPS.

Changes in intestinal motility were detected by measuring the cycle period of migrating myoelectric complexes between the ends of phase III activity (the marked increase in regular phasic myoelectric and motor activity) of successive migrating myoelectric complexes (Scott and Tan, 1996). Since endotoxin totally disrupts the standard migrating myoelectric complexes that occur in fasting animals and produces a pattern of intense, irregular myoelectric electricity, the activity of the peptides can be determined by measuring the time required to reestablish normal MMC activity.

2.3. Peptide synthesis and preparation

The peptides feG and feG(NH2) were synthesized and purified at Core Laboratories, Queen's University, Kingston, Ontario, using standard solid phase techniques. The peptides are water-soluble, and were dissolved in 0.9% saline immediately before administration to the animals.

2.4. Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) and differences between groups were determined using the Student's *t*-test for unpaired samples.

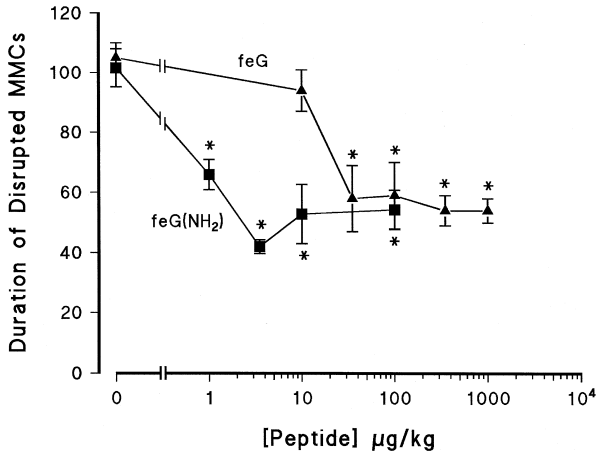

Fig. 1. Rats receiving intravenous lipopolysaccharide (LPS) showed a disruption of their migrating myoelectric complexes that lasted 102 ± 6 min. Intravenous injection of feG (▲) or feG(NH2) (■) 20 min before injection of 20 μg/kg of lipopolysaccharide, reduced the length of time the migrating myoelectric complexes were disrupted by the LPS. Control rats received an intravenous injection of saline instead of peptide. Number of animals = 5–22. Significance: $^{*}P < 0.05$; when compared to saline-treated control.

All data were expressed as mean ± S.E.M. and significance was accepted at $P < 0.05$.

3. Results

The migrating myoelectric complexes in fasted rats exhibit an intrinsic rhythmicity with a cycle period of 11.6 ± 1.8 min, which was not affected by treatment with the peptides. Within 16 ± 2 min of the intravenous injection of 20 μg/kg of LPS, the normal fasting pattern of intestinal migrating myoelectric complexes changed into the fed pattern of activity and this disruption persisted for 102 ± 6 min ($n = 22$).

When feG was administered intravenously, 20 min before LPS injection, a 50% inhibition of the disrupted intestinal migrating myoelectric complexes was observed (Fig. 1) in doses ranging from 35 to 100 μg/kg. Doses of feG lower than 35 μg/kg were ineffective. The amidated derivative of feG (feG(NH_2)) was effective at a much lower dose, with a maximally effective dose of 3.5 μg/kg. By comparing comparable minimally effective doses feG(NH_2) was approximately 20–30 times more potent than feG.

4. Discussion

The LPS released from cell walls of gram-negative bacteria contributes to diarrheal disease and with more severe intoxication, gastrointestinal dysmotility or ileus frequently develops. Small doses of LPS (20 μg/kg) in the rat have pronounced effects by disturbing intrinsic

resting motility and substantially increasing intestinal transit (Hellström et al., 1997). Identification of strategies for the effective management of LPS-induced intestinal toxicity remains an important priority in gastrointestinal research (Chieveley-Williams and Hamilton-Davies, 1999).

The tripeptide FEG, an active fragment (Mathison et al., 1998) of the heptapeptide SGP-T (Mathison et al., 1997a), and its D-isomeric form, feG, are potent inhibitors of cardiovascular and intestinal (Mathison et al., 1997b) as well as pulmonary (Befus et al., 2000) anaphylaxis. Although these peptides have similar potency in reducing cardiovascular anaphylactic reactions, feG is more effective than SGP-T in preventing the disruption of migrating myoelectric complexes elicited by LPS (unpublished), and anaphylaxis-induced pulmonary inflammation (Befus et al., 2000). The present study shows that the carboxamide of feG, feG(NH_2), is an even more potent inhibitor of endotoxin-induced perturbation of intestinal motility. The apparent absence of a significant dose response effect of feG cannot be readily explained, but may reflect a substrate availability limiting effect if feG must be converted to feG(NH_2) before it can exert its effects on LPS-induced motility changes.

The disruption of migrating myoelectric complexes by LPS is effected through the nitric oxide and arachidonate pathways, with both nitric oxide synthase (NωL-arginine methyl ester) and cycloxygenase (indomethacin) inhibitors providing almost complete protection against the LPS-induced disruptions in intestinal motility (Hellström et al., 1997). Dexamethasone pretreatment 6 h before, also prevented LPS effects on motility. Although the mechanism by which the tripeptides, feG and feG(NH_2), reduced LPS perturbations of intestinal motility is not known, these peptides only reduced the effects of LPS by approximately 50%, even at the highest doses used. However, the peptides were effective at μg/kg doses, whereas, mg/kg doses of nitric oxide synthase and cycloxygenase inhibitors are required to achieve inhibition (Hellström et al., 1997).

Bioactive peptides resulting from the conversion of a prohormone are derived through a sequence of processing events, including cleavage at specific sites and modifications such as carboxy-terminal amidation. It has been observed that C-terminal amides are essential for full biological activity of α-melanocyte-stimulating hormone, gastrin and cholecystokinin (Bradbury et al., 1982). Even though the present study was performed with D-isomeric peptides, it is postulated that a similar processing step for FEGGGK C-terminal sequence of the SMR1 precursor after removal of the basic residue by carboxypeptidase B-like enzyme. The amidation enzymes operating in tandem (peptidyl-glycine α-amidating monooxygenase (PAM) and peptidylamidoglycolate lyase (PGL)), could convert biologically modestly active glycine-extended peptide to active carboxamide product. Thus, future studies should establish whether FEG is generated in the salivary glands before being released into the circulation or whether the peptide is released into the blood with a free COOH-terminus and, blood and tissue peptidyl-glycine α-amidating monooxygenase and peptidylamidoglycolate lyase generate the more biologically active FEG-carboxamide.

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