Br. J. Pharmacol. (1991). **102**, 730–734

Peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) activates capsaicin-sensitive primary afferent nerves in guinea-pig atria and urinary bladder

[1]Sandro Giuliani, Paolo Santicioli, *Manuela Tramontana, *Pierangelo Geppetti & Carlo Alberto Maggi

Pharmacology Department, A. Menarini Pharmaceuticals, Via Sette Santi 3, 50131 Florence, Italy and *Department of Internal Medicine, University of Florence, Italy

1 We have investigated the ability of the N-formyl-methionyl-leucyl-phenylalanine (FMLP) a synthetic analogue of a chemotactic peptide derived from a variety of bacteria, to activate capsaicin-sensitive primary afferents in the guinea-pig atria and urinary bladder.

2 In the isolated, electrically-driven left atria from reserpine-pretreated guinea-pigs (atropine in the bath), FMLP (3 nM–1 μM) produced a biphasic positive inotropic response. The late component of this response was selectively abolished by *in vitro* capsaicin pretreatment while both the early and late responses were abolished by indomethacin.

3 The inotropic response to FMLP in the guinea-pig atria was unaffected by ruthenium red. The late but not the early response was strongly inhibited or abolished by tetrodotoxin (TTX), ω-conotoxin (CTX) or by the C-terminal fragment (8–37) of human α-calcitonin gene-related peptide (hCGRP). hCGRP-(8-37) acts as competitive antagonist at CGRP receptors.

4 In the guinea-pig isolated bladder, FMLP (10 nM–10 μM) produced a concentration-dependent contraction which was unchanged by previous *in vitro* capsaicin, TTX or CTX pretreatment. The response to low concentrations of FMLP was suppressed by indomethacin, irrespective of the capsaicin pretreatment.

5 FMLP (10 μM) produced a significant increase in the outflow of CGRP-like immunoreactivity (CGRP-LI) from superfused guinea-pig atria or urinary bladder. CGRP-LI outflow induced by FMLP was blocked by indomethacin or *in vitro* capsaicin pretreatment.

6 These findings indicate that FMLP activates the 'efferent' function of capsaicin-sensitive primary afferents via prostanoid generation. This action could provide a neurogenic contribution to the overall inflammatory response produced by bacteria-derived peptides in inflamed tissues. In addition the present data indicate that endogenous prostanoids generated during exposure to FMLP produce peptide secretion from sensory nerves via a TTX- and CTX-sensitive but ruthenium red-resistant mechanism.

Introduction

N-formyl-methionyl-leucyl-phenylalanine (FMLP) is a powerful synthetic analogue of a chemotactic peptide derived from a variety of bacteria (Schiffmann *et al.*, 1975; Showell *et al.*, 1976). FMLP is known to produce a variety of biological responses including airway smooth muscle contraction (Hamel *et al.*, 1984; Smith *et al.*, 1985), vascular contraction and relaxation (Crowell *et al.*, 1989; Laplante *et al.*, 1989), leucocyte and macrophage chemotaxis and granule enzyme secretion (Smith & Iden, 1980). The action of FMLP on granulocytes is mediated by specific receptors (Aswanikumar *et al.*, 1977) and some of its biological actions are indirectly mediated by production of prostanoids (Laplante *et al.*, 1989; Crowell *et al.*, 1989).

In recent years much attention has been directed to investigation of the mechanisms leading to activation of the 'efferent' function played by capsaicin-sensitive afferents (Szolcsányi 1984; Maggi & Meli, 1988). Several chemical stimuli including bradykinin (Geppetti *et al.*, 1988; Saria *et al.*, 1988) or arachidonic acid metabolites (Manzini *et al.*, 1990) have been shown to induce secretion of neuropeptides from peripheral endings of capsaicin-sensitive afferents. This process is thought to be of relevance for the pathophysiology of various organs and systems and particularly to play a pivotal role in the genesis of neurogenic inflammation.

In this study we present functional and neurochemical evidence indicating that FMLP activates, via prostanoid generation, capsaicin-sensitive afferents in the guinea-pig atria and urinary bladder. Additionally, we investigated the effects of various pharmacological tools, such as ω-conotoxin and ruthenium red on the response to FMLP in the guinea-pig atria. These pharmacological tools have been recently used (Maggi *et al.*, 1988; 1989a) to distinguish between different modes of peptide secretion from peripheral endings of capsaicin-sensitive afferents.

[1] Author for correspondence.

Methods

General

Male albino guinea-pigs weighing 250–300 g were stunned and bled. The urinary bladder and left atria were excised and placed in oxygenated (96% O_2 and 4% CO_2, pH 7.4 at 37°C) Krebs solution (urinary bladder) or Tyrode solution (atria), as described previously (Maggi *et al.*, 1988; 1989a; Giuliani *et al.*, 1989).

Functional experiments

The left atria or longitudinal strips of the urinary bladder (mucosa-free, 1 cm long 3 mm wide) were placed in 5 ml baths for isolated organs, maintained at 37°C. Tension was recorded isometrically (atria, load 5 mN) or isotonically (urinary bladder 10 mN) by means of Basile 7050 Unirecord. The atria were obtained from reserpine-pretreated (5 mg kg^{-1}, i.p., 72–96 h beforehand) animals and placed in Tyrode solution containing atropine (1 μM), and driven at a frequency of 3 Hz (0.5 ms pulse width, maximal voltage) by means of a Grass S88 stimulator, as described previously (Maggi *et al.*, 1988; 1989a; Giuliani *et al.*, 1989).

All experiments started after a 90 min equilibration period. In the atria, the positive inotropic response produced by trains of electrical field stimulation (10 Hz for 2.5 s, 1 ms pulse width, 60 V) was determined at 15–20 min intervals by means of the second output channel of the stimulator until reproducible inotropic responses, shown previously to depend on antidromic activation of sensory nerves (Maggi *et al.*, 1988; 1989a).

Concentration-response curves to FMLP were obtained for the atria or urinary bladder with 30 min intervals between doses. In both cases the effect of FMLP was allowed to develop for 10 min before washing. No significant desensitization was found with this protocol for the response to 10 μM FMLP checked at the end of the equilibration period or at the end of the concentration-response curve.

In some experiments the effect of FMLP was studied in preparations which were desensitized to capsaicin *in vitro*. This was done by applying 10 μM capsaicin for 15 min at the end of the equilibration period followed by washing.

The effects of indomethacin (10 μM) on responses to FMLP was investigated by adding the drug to the Krebs or Tyrode solution from the beginning of the experiment.

In the atria the response to 1 μM FMLP was also investigated in the presence of 0.1 μM ω-conotoxin fraction GVIA (CTX, 20 min before), tetrodotoxin (TTX, 0.3 μM, 10 min before), ruthenium red (10 μM, 10 min before) or the CGRP antagonist, hCGRP-(8-37) (1 μM, 10 min before). The concentrations of CTX, TTX and ruthenium red were selected from previous studies in which these drugs have been shown to inhibit responses produced by activation of sensory nerves without exerting postjunctional effects (Maggi *et al.*, 1988; 1989a). The C-terminal fragment of hCGRP, hCGRP-(8-37) is a competitive antagonist at CGRP receptors, as shown by Chiba *et al.* (1989). The concentration of the CGRP antagonist used here was selected from a previous study in which it was shown to antagonize competitively the response to hCGRP in guinea-pig left atria without affecting the inotropic response to isoprenaline (Maggi *et al.*, 1991).

In the atria the response to FMLP was expressed as % variation of resting inotropism. In the urinary bladder it was expressed as % of the response to KCl (20 mM, added to the bath for 60 s) chosen as an appropriate internal standard. The response to KCl was not modified by previous exposure to capsaicin or indomethacin.

Release experiments

Release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) was determined by radioimmunoassay in superfusates from guinea-pig atria and mucosa-free urinary bladder, using the same technique as described previously (Maggi *et al.*, 1989a,b). Tissue slices (100–150 mg) were placed in a 2 ml bath maintained at 37°C and superfused at a rate of 0.3 ml min^{-1} with oxygenated (96% O_2 and 4% CO_2) Krebs solution containing 1 μM thiorphan. Five-min (1.5 ml) fractions were collected before (2 fractions), during (4 fractions) and after (1 fraction) the stimulus. After a 90 min equilibration period the tissues were exposed for 20 min to the stimulus. Samples were added with acetic acid to give 2 N final concentration and freeze-dried. At the end of the experiments the tissues were blotted two–three times with filter paper and weighed.

Statistical analysis

Each value in the text and figures is mean ± standard error of the mean (s.e.mean). Statistical analysis was performed by means of the Student's *t* test for paired or unpaired data or by means of analysis of variance, as indicated in the legends. A P value <0.05 was considered statistically significant.

Drugs

Drugs used were: N-formyl-Met-Leu-Phe (FMLP), human calcitonin gene-related peptide (hCGRP), hCGRP-(8-37), ω-conotoxin GVIA (CTX) (Peninsula), thiorphan, indomethacin, tetrodotoxin (TTX) (Sigma), ruthenium red (Aldrich), capsaicin, reserpine (Serva).

Results

Functional experiments: guinea-pig atria

FMLP (3 nM–1 μM) produced, after a latency of 5–10 s, a concentration-dependent slowly developing positive inotropic effect (Figures 1 and 2) that was fully reproducible at 30 min interval. At 0.1–1 μM the response to FMLP was clearly biphasic, as shown in a typical tracing in Figures 1 and 2. During

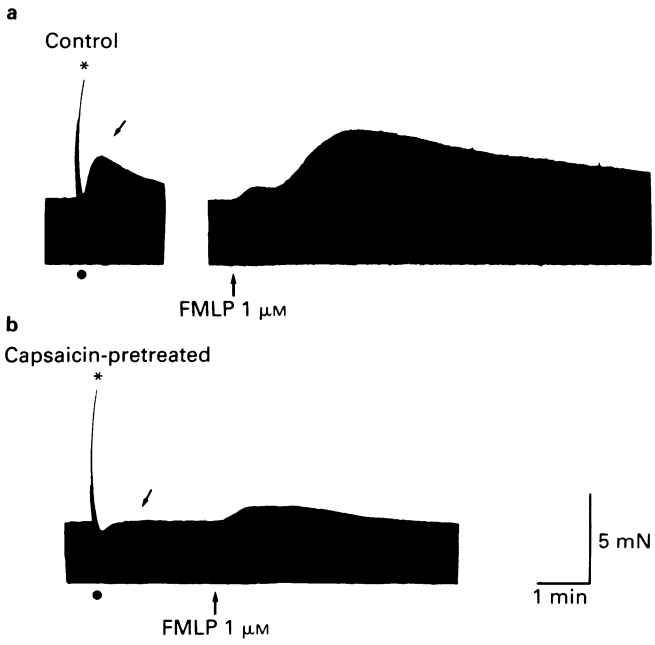

Figure 1 Typical tracings showing the positive inotropic response to electrical field stimulation (EFS 10 Hz, 1 ms, 60 V for 2.5 s) and to N-formyl-Met-Leu-Phe (FMLP, 1 μM) administration in control (a) and in capsaicin-pretreated (10 μM for 15 min) electrically-driven guinea-pig left atria (b). EFS, applied at the dots, produced a post stimulus potentiation (indicated by asterisks) followed by a delayed neurogenic positive inotropic response (indicated by arrows) which is due to antidromic activation of capsaicin-sensitive sensory nerves. FMLP produced a biphasic positive inotropic effect. *In vitro* capsaicin desensitization abolished both the delayed positive inotropic response to EFS and the late inotropic response to FMLP.

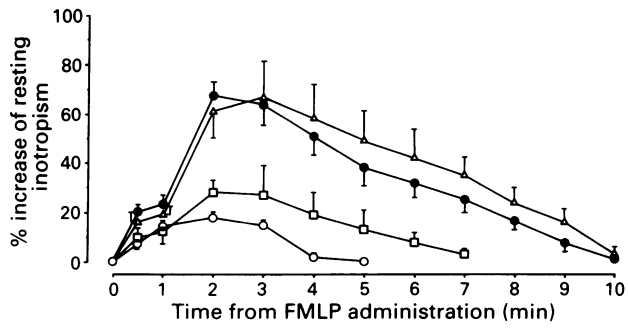

Figure 2 Time-course of the inotropic effect of N-formyl-Met-Leu-Phe (FMLP) on guinea-pig isolated left atria at various concentrations (○ 3 nM; □ 10 nM; △ 0.1 μM; ● 1 μM). Maximal effect was reached at 0.1–1 μM concentration with a clearly biphasic response. Each value is the mean of 5–6 experiments with s.e. shown by vertical bars.

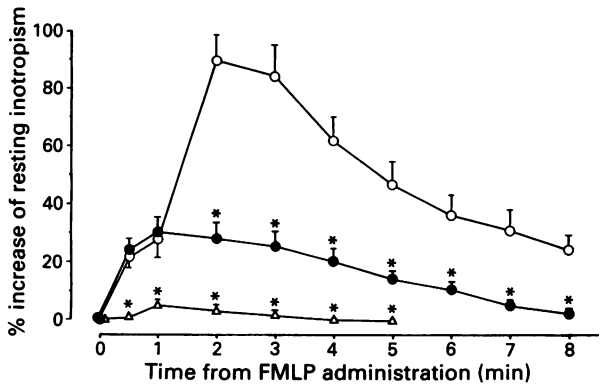

Figure 3 Time-course of the inotropic response to N-formyl-Met-Leu-Phe (FMLP) at 1 μM concentration (○ control response) on isolated electrically-driven left atria of guinea-pig. Pre-exposure to capsaicin (● 10 μM for 15 min) significantly reduced the late inotropic response to 1 μM FMLP leaving the early one unchanged. In the presence of indomethacin (△ 10 μM) the inotropic response to FMLP was abolished.
* $P < 0.05$ significantly different from control response (Student's t test for paired data). Each value is the mean of 5–7 experiments with s.e.mean shown by vertical bars.

the first min after FMLP application inotropism slowly increased and reached a plateau at about 20–30% over resting tension. At this stage a further, marked increase in atrial inotropism was observed which peaked at about 2–3 min after FMLP administration. Data in Figures 3 and 4 show that pre-exposure of the preparations to a desensitizing concentration of capsaicin (10 μM for 15 min) failed to affect the early inotropic response to FMLP while the late component was significantly reduced and the overall response to FMLP appeared as a monophasic inotropic response with a peak at 2 min after FMLP administration. In the presence of indomethacin (10 μM) the inotropic response to FMLP was abolished (Figures 3 and 4).

In subsequent experiments we investigated the action of various substances on the biphasic inotropic response to 1 μM FMLP on the assumption that the biphasic behaviour observed in control conditions reflected activation of two distinct mechanisms, the latter involving peptide release from capsaicin-sensitive afferents. Tension developed in the presence of FMLP was therefore calculated at 1 and 3 min after FMLP administration and the effect of test substances evaluated at these two times after FMLP administration.

Data in Figure 4 show that the early response to FMLP was unaffected by either TTX (0.3 μM), CTX (0.1 μM), ruthenium red (10 μM) or the CGRP antagonist, h-CGRP(8-37) (1 μM). Ruthenium red was also without effect on the delayed response to FMLP which was, by contrast, significantly and markedly reduced by TTX, CTX or the CGRP antagonist (Figure 4). Data in Figure 4 also show, for comparison, the effect of various pretreatments on the inotropic response produced in the same preparation by electrical field stimulation which, in the present experimental conditions, has been proposed to depend upon CGRP release from capsaicin-sensitive afferents (Saito *et al.*, 1987; Maggi *et al.*, 1988; 1989a): the response to electrical field stimulation was abolished or markedly inhibited by *in vitro* capsaicin desensitization, TTX, CGRP antagonist, CTX while it was unaffected by indomethacin or ruthenium red (Figure 4).

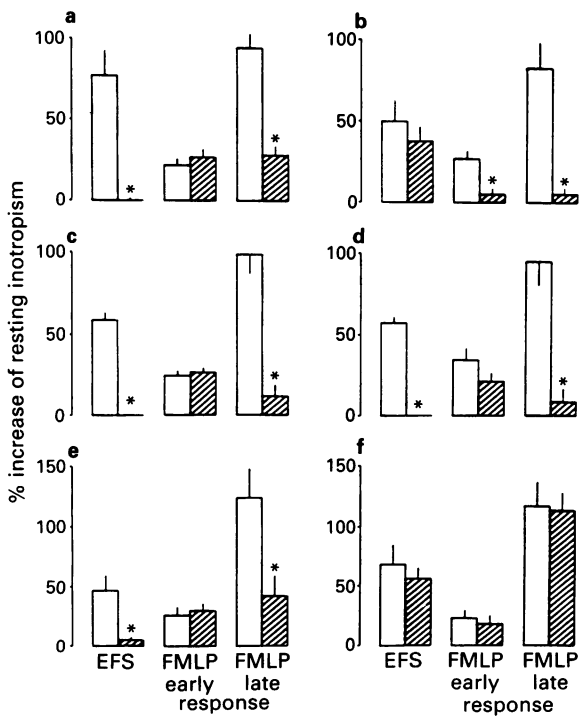

Figure 4 Effect of capsaicin, indomethacin, tetrodotoxin (TTX), human calcitonin gene-related peptide-(8-37) (hCGRP-(8-37)), conotoxin (CTX) and ruthenium red (RR) on the inotropic effect (early and late responses calculated at 1 and 3 min respectively) produced by N-formyl-Met-Leu-Phe (FMLP, 1 μM) and for comparison by electrical field stimulation (EFS 10 Hz, 1 ms, 60 V for 2.5 s). In all panels (a–f), open columns are controls; hatched columns: in (a) capsaicin 10 μM; (b) indomethacin 10 μM; (c) TTX 0.3 μM; (d) hCGRP-(8-37) 1 μM; (e) CTX 0.1 μM; (f) RR 10 μM.
* $P < 0.05$ significantly different from the respective control (Student's t test for paired data). Each column is the mean of 4–6 experiments with s.e.mean shown by vertical lines.

Functional experiments: guinea-pig urinary bladder

FMLP (10 nM–10 μM) produced a concentration-dependent contraction of mucosa-free strips from the guinea-pig urinary bladder. At maximal concentrations (1–10 μM) the response approached 40–50% of that to KCl (20 mM) and was not significantly affected by indomethacin (10 μM), capsaicin-pretreatment (10 μM for 15 min followed by repeated washings) or a combination of these two treatments (Figure 5, $n = 5$ for each group). At low FMLP concentrations (10–100 nM) no response was observed in the presence of indomethacin (Figure 5). The response to FMLP in capsaicin-pretreated strips was not different from controls (Figure 5). Likewise, the response to FMLP was not significantly modified by pretreatment with either TTX (0.3 μM) or CTX (0.1 μM) ($n = 4$ each, Figure 5).

Release experiments

Data in Figure 6, show that 10 μM FMLP evoked a significant increase in CGRP-LI outflow from both guinea-pig atria and urinary bladder. The FMLP-evoked peptide release was abolished, in both tissues, in the presence of indomethacin (10 μM) or in tissues pre-exposed to capsaicin (10 μM for 15 min) in order to inactivate capsaicin-sensitive primary afferents (Figure 6).

Discussion

The present findings provide functional and neurochemical evidence indicating that the bacterial chemotactic peptide FMLP activates indirectly, via prostanoid generation, the 'efferent' function of capsaicin-sensitive primary afferents and determines the release of CGRP-LI. In both the guinea-pig atria and urinary bladder, CGRP-LI is entirely stored in peripheral endings of capsaicin-sensitive primary afferents (Su *et al.*, 1986; Franco-Cereceda *et al.*, 1987) from which it has been shown to be released in response to a variety of stimuli including capsaicin, high potassium depolarizing media or bradykinin (Maggi & Meli, 1988 for review).

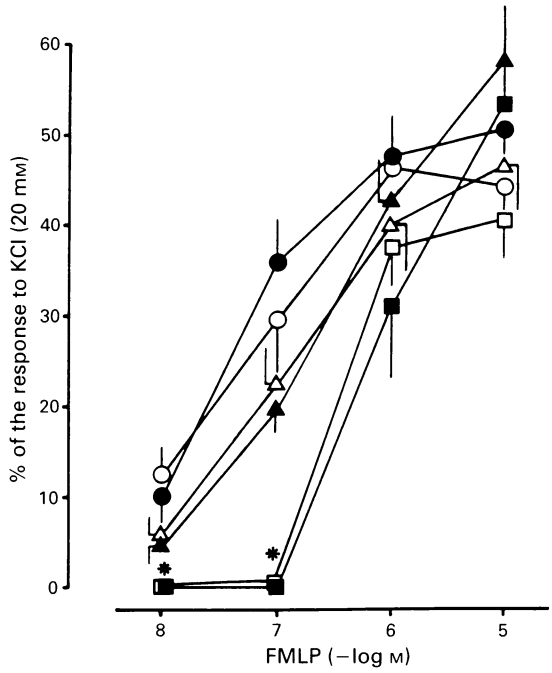

Figure 5 Concentration-response curves showing the contractile effect of N-formyl-Met-Leu-Phe (FMLP, 0.01–10 μM) on guinea-pig isolated urinary bladder (○ control response) alone and in the presence of indomethacin (□ 10 μM), following *in vitro* capsaicin desensitization (● 10 μM for 15 min), a combination of these two treatments (■), tetrodotoxin (△) or conotoxin (▲).
* $P < 0.05$ significantly different from control (Student's t test for unpaired data). Each value is the mean of 4–5 experiments; vertical lines show s.e.mean.

In the atria, the functional response produced by FMLP was clearly biphasic with a late component ascribable to sensory nerve activation. The early response, which persisted after *in vitro* capsaicin desensitization, is most likely ascribable to the inotropic effect of prostanoids generated by FMLP being abolished by indomethacin. Release experiments indicate that FMLP activates the efferent function of capsaicin-sensitive afferents even in the guinea-pig urinary bladder. As in the case of the atria, this effect of FMLP can be ascribed to prostanoid generation, CGRP-LI release being abolished by indomethacin. However, in the case of the guinea-pig bladder, peptide release from sensory nerves by FMLP does not play a relevant role in the local motor response, which was unchanged by capsaicin, TTX or CTX pretreatment. In spite of this, the contraction of the bladder induced by FMLP is partially indomethacin-sensitive. It appears therefore that the motor effect produced by sensory nerve activation in the guinea-pig bladder is masked by the larger contraction produced by FMLP through capsaicin-insensitive mechanisms. However, we cannot exclude the possibility that the local release of sensory neuropeptides by FMLP in the bladder might have a prominent action on other smooth muscle cells, e.g. in the regulation of blood flow and vascular permeability, thus contributing to the overall symptoms of cystitis.

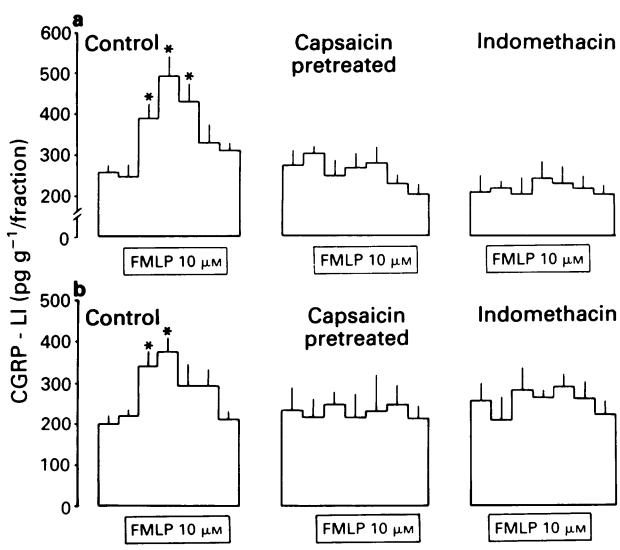

Figure 6 Effect of superfusion with N-formyl-Met-Leu-Phe (FMLP, 10 μM) on the calcitonin-gene related peptide like (CGRP-LI) outflow from slices of guinea-pig urinary bladder (a) or atria (b) in control preparations (left panels), preparations pre-exposed to capsaicin (10 μM for 15 min, middle panels) or in presence of 10 μM indomethacin (right panels).
* $P < 0.05$ significantly different from the basal values (by Analysis of Variance). Each bar indicates the mean of at least 5 experiments; vertical lines show s.e.mean.

The electrically driven left atria from reserpine-pretreated guinea-pigs (atropine in the bath) have been repeatedly shown to provide an excellent bioassay for functional responses produced by activation of capsaicin-sensitive primary afferents (Saito *et al.*, 1988; Maggi *et al.*, 1988; 1989a). In these particular experimental conditions, sensory nerve activation determines a positive inotropic response which is thought to be mediated by endogenous CGRP (Franco-Cereceda *et al.*, 1987; Saito *et al.*, 1988). This is further supported by the observation that hCGRP-(8-37), which acts as a competitive antagonist at certain CGRP receptors (Chiba *et al.*, 1989), selectively blocked the inotropic response to hCGRP (pA_2 6.8) without affecting that to isoprenaline (Maggi *et al.*, 1991). The CGRP antagonist blocked the positive inotropic response produced by electrical field stimulation and the late response to FMLP without affecting the inotropic response to isoprenaline (Maggi *et al.*, 1991) or the early response to FMLP. This result provides pharmacological evidence for an involvement of CGRP in the late component of the functional response to FMLP.

Recently, evidence has been presented indicating that two distinct mechanisms for activation of the 'efferent' function of capsaicin-sensitive primary afferents exist (Maggi *et al.*, 1988; 1989a). The first mechanism, activated by capsaicin itself, involves a TTX-resistant direct stimulation of sensory nerve endings that is selectively blocked by ruthenium red and does not involve the voltage-sensitive calcium channels. The second mechanism, activated by electrical field stimulation, involves the generation of a propagated action potential (TTX-sensitive) which invades antidromically sensory nerve endings and determines peptide secretion via CTX-sensitive calcium channels. This second mode of sensory nerve activation is unaffected by ruthenium red at concentrations which block the effect of capsaicin (Maggi *et al.*, 1989a).

Present data indicate that the indirect action of FMLP on sensory nerves, via prostanoid generation, fits this second mechanism of activation e.g. mimics an axon reflex arrangement.

As discussed in the Introduction, FMLP is a synthetic chemoattractant peptide derived from bacteria. It has been proposed that FMLP-like peptides generated during bacterial infections play a pathogenic role in inflammatory diseases by virtue of their powerful chemotactic activity. The present results indicate an additional mechanism through which bacteria-derived peptides could exert a local pro-inflammatory action i.e. sensory neuropeptide release from peripheral endings of capsaicin-sensitive primary afferents. This might be important in the case of the urinary bladder bacterial cystitis and, in the heart, for pericarditis/myocarditis. The action of FMLP on sensory nerves at this level could participate in the genesis of symptoms by stimulating reflexes (pain, cardiovascular reflexes) and, through peptide release from sensory nerves, contribute to the overall inflammatory process.

References

ASWANIKUMAR, S., CORCORAN, B., SCHIFFMANN, E., DAY, A.R., FREER, R.J., SHOWELL, H.J., BECKER, E.L. & PERT, C.B. (1977). Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. *Biochem. Biophys. Res. Commun.*, **74**, 810–817.

CHIBA, T., YAMAGUCHI, A., YAMATANI, T., NAKAMURA, A., MORISHITA, T., INUI, T., FUKASE, M., NODA, T. & FUJITA, T. (1989). Calcitonin gene-related peptide receptor antagonist human CGRP (8-37). *Am. J. Physiol.*, **256**, E331–E335.

CROWELL, R.E., VAN EPPS, D.E. & REED, W.P. (1989). Responses of isolated pulmonary arteries to synthetic peptide F-Met-Leu-Phe. *Am. J. Physiol.*, **257**, H107–H112.

FRANCO-CERECEDA, A., HENKE, H., LUNDBERG, J.M., PETERMANN, J.B., HÖKFELT, T. & FISHER, J.A. (1987). Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. *Peptides*, **8**, 399–410.

GEPPETTI, P., MAGGI, C.A., PERRETTI, F., FRILLI, S. & MANZINI, S. (1988). Simultaneous release by bradykinin of substance P and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart. *Br. J. Pharmacol.*, **94**, 288–290.

GIULIANI, S., MAGGI, C.A. & MELI, A. (1989). Prejunctional modulatory action of neuropeptide Y on peripheral terminals of capsaicin-sensitive sensory nerves. *Br. J. Pharmacol.*, **98**, 407–412.

HAMEL, R., FORD-HUTCHINSON, A.W., LORD, A. & CIRINO, M. (1984). Bronchoconstriction induced by N-formyl-methionyl-leucyl-phenylalanine in the guinea pig; involvement of arachidonic acid metabolites. *Prostaglandins*, **28**, 43–56.

LAPLANTE, C., TREMBLAY, B. & MARCEAU, F. (1989). Relaxant effect of N-Formyl-Methionyl-Leucyl-Phenylalanine on rabbit vascular strips. *J. Pharmacol. Exp. Ther.*, **248**, 774–780.

MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmacol.*, **19**, 1–43.

MAGGI, C.A., PATACCHINI, R., GIULIANI, S., SANTICIOLI, P. & MELI, A. (1988). Evidence for two independent modes of activation of the efferent function of capsaicin-sensitive nerves. *Eur. J. Pharmacol.*, **156**, 367–373.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., DEL BIANCO, E., GEPPETTI, P. & MELI, A. (1989a). The 'efferent' function of capsaicin-sensitive nerves: ruthenium red discriminates between mechanisms of activation. *Eur. J. Pharmacol.*, **170**, 167–177.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GEPPETTI, P., CECCONI, R., GIULIANI, S. & MELI, A. (1989b). Multiple mechanisms in the motor responses of the guinea-pig isolated urinary bladder to bradykinin. *Br. J. Pharmacol.*, **98**, 619–629.

MAGGI, C.A., CHIBA, T. & GIULIANI, S. (1991). Human CGRP-(8-37) as an antagonist of exogenous and endogenous calcitonin gene-related peptide. *Eur. J. Pharmacol.*, (in press).

MANZINI, S., BALLATI, L., GEPPETTI, P., RUBINI, I., MEINI, S. & PERRETTI, F. (1990). Arachidonic acid-induced bronchomotor responses are partially mediated by release of sensory neuropeptides from capsaicin-sensitive structures. *Br. J. Pharmacol.*, **98**, 1077–1079.

SAITO, A., ISHIKAWA, T., KIMURA, S. & GOTO, K. (1987). Role of calcitonin gene-related peptide as cardiotonic neurotransmitter in guinea-pig left atria. *J. Pharmacol. Exp. Ther.*, **243**, 731–740.

SARIA, A., MARTLING, C.R., YAN, Z., THEODORSSON-NORHEIM, E., GAMSE, R. & LUNDBERG, J.M. (1988). Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenylpiperazinium and vagal nerve stimulation. *Am. Rev. Resp. Dis.*, **137**, 1330–1335.

SCHIFFMANN, E., CORCORAN, B. & WAHL, S.M. (1975). N-formylmethionyl peptides as chemoattractants for leucocytes. *Proc. Natl. Acad. Sci., U.S.A.*, **72**, 1059–1062.

SHOWELL, H.J., FREER, R.J., ZIGMOND, S.H., SCHIFFMANN, E., ASWANIKUMAR, S., CORCORAN, B. & BECKER, E. (1976). The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J. Exp. Med.*, **143**, 1154–1169.

SMITH, J.K., McCORMICK, J.R. & ELGUINDI, A.S. (1985). Contraction of human and guinea pig airway by a chemotactic peptide. *Chest*, **88**, 25S.

SMITH, R.J. & IDEN, S.S. (1980). Pharmacological modulation of chemotactic factor-elicited release of granule-associated enzymes from human neutrophils. Effects of prostaglandins, nonsteroid anti-inflammatory agents and corticosteroids. *Biochem. Pharmacol.*, **79**, 2389–2395.

SU, H.C., WHARTON, J., POLAK, J.M., MULDERRY, P.K., GHATEI, M.A., GIBSON, S.J., TERENGHI, G., MORRISON, J.F.B., BALLESTA, J. & BLOOM, S.R. (1986). Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunohistochemistry. *Neuroscience*, **18**, 727–747.

SZOLCSÁNYI, J. (1984). Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In *Antidromic Vasodilation and Neurogenic Inflammation* ed. Chahl, L.A., Szolcsányi, J. & Lembeck, F. pp. 26–52. Budapest: Akademiai Kiado.

(Received July 27, 1990
Revised October 25, 1990
Accepted October 31, 1990)