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Spasmogenic activity of chemotactic *N*-formylated oligopeptides: Identity of structure–function relationships for chemotactic and spasmogenic activities

(formyl peptide receptor/prokaryotic signal peptides/smooth muscle contraction/chemotactic peptides)

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ABSTRACT **The chemotactic *N*-formylated oligopeptides are potent spasmogenic agents for guinea pig ileum. Structure–activity studies with various *N*-formylated peptides suggest the presence of a specific receptor that resembles in specificity the formyl peptide receptor on leukocytes. A competitive antagonist of the formyl peptide receptor on leukocytes also inhibits formyl peptide-induced ileum contraction, whereas the antihistamine diphenhydramine is without effect. The contractile response caused by the synthetic *N*-formylated peptides differs from those induced by acetylcholine, histamine, and substance P. In particular, a latent period after treatment with the *N*-formyl peptides is seen before the onset of the response, and a sustained contractile response is not maintained. In addition, tachyphylaxis does occur, but complete recovery of activity is seen after a 20- to 30-min rest period. These observations suggest broad biological roles of prokaryotic signal peptides from bacteria as acute inflammatory mediators.**

N-formylmethionyl peptides are potent chemotactic agents for both neutrophils (PMN) and macrophages (1) and are believed to be the analogues of the naturally occurring NH_2-terminal signal peptides produced by bacteria (2, 3). These products may be responsible for the accumulation of the acute inflammatory cells in tissues containing bacteria. The binding of these bacterial peptides to specific receptors on inflammatory cells triggers a number of biological responses such as aggregation, superoxide production, enzyme secretion, and chemotaxis (4–7). Histamine release from the basophil also can occur (8).

The broad range of biological responses induced by these bacterial products is similar to the effects of C5a, a complement-derived anaphylatoxin, which appears to play a major role in mediating acute inflammatory reactions. In addition to having a variety of biological effects on the neutrophil (e.g., chemotaxis, aggregation, enzyme secretion, and superoxide production), C5a also possesses potent spasmogenic activity for smooth muscle derived either from the small intestine or from the lung (9–12).

The current studies were designed to determine if chemotactic formyl oligopeptides have spasmogenic activity for smooth muscle. As defined by guinea pig ileum contraction (13, 14), we will show that the formyl peptides are potent spasmogenic agents. Furthermore, structure–activity studies with various *N*-formylated peptides support the possibility that the contractile response results from the binding of the formyl peptides to a specific receptor.

MATERIALS AND METHODS

The *N*-formylated peptides used were: fMet-Leu-Phe from Sigma; fNle-Leu-Phe (Nle, norleucine), Met-Leu-Phe, fMet-Leu-Phe-Phe, fMet-Leu-Glu, and fMet-Abu-Phe (Abu, α-aminobutyric acid) from R. Freer (Dept. of Pharmacology, Medical College of Virginia, Richmond, VA); and Boc-Phe-Leu-Phe-Leu-Phe (Boc, *tert*-butoxycarbonyl) from Peninsula Laboratories (Belmont, CA). Substance P, acetylcholine, histamine, and diphenhydramine were purchased from Sigma.

The terminal guinea pig ileum was used in all experiments. The isolated ileum (1.5 cm in length) was suspended in a 50-ml organ bath containing Krebs solution (130 mM NaCl/4.7 mM KCl/1.18 mM KH_2PO_4/1.17 mM $MgSO_4 \cdot 7H_2O$/1.6 mM $CaCl_2$/14.9 mM $NaHCO_3$/5.5 mM dextrose at 37°C bubbled with 95% O_2/5% CO_2. The resting tension was 1.0 g, and the isometric contractions were recorded by means of a force displacement transducer (Grass) and displayed on an oscillograph (Grass). The ileum was allowed to equilibrate for at least 2 hr with frequent washes prior to initiation of the experiments. Triplicate ileum strips from one animal or single ileum strips from two animals were used in all experiments.

Concentration–response curves were determined for each of the agents tested. Concentration–response curves for histamine, acetylcholine, and substance P were obtained by adding the drug cumulatively, so that the concentration in the bath was increased by a factor of 2 whenever a steady response to the previous concentration had been reached. For the formyl peptides, a steady-state response did not occur, and concentration–response curves reflect the maximal contraction that occurred at each peptide concentration tested. A period of 20–30 min was allowed to elapse before starting a new concentration–response curve. Repeated washings were made during this time.

In some experiments the competitive antagonist, Boc-Phe-Leu-Phe-Leu-Phe was added to the organ bath 30 sec to 1 min before addition of the agonists. Concentration–response curves were then generated as described. Boc-Phe-Leu-Phe-Leu-Phe alone at 1 μM gave no contractile response. Ileum strips in the absence of the antagonist served as a control.

RESULTS

To test whether the formyl peptides were able to cause ileum contraction, a concentration–response curve for fMet-Leu-Phe was generated (Fig. 1*A*). fMet-Leu-Phe caused contraction of ileum in a concentration-dependent manner. The sustained contractile response was not seen with fMet-Leu-Phe, and the ileum returned to the baseline resting tension within 2 min after addition of fMet-Leu-Phe. Rechallenging with an increasing concentration of fMet-Leu-Phe generated a second contraction that was greater in magnitude than the response was to the lower dose of peptide. Maximum contractile response equaled 2.29 ± 0.11 g. At high concentrations of fMet-Leu-Phe, tachyphylaxis did occur; however, the smooth muscle regained reactivity after a 20- to 30-min rest period between challenges.

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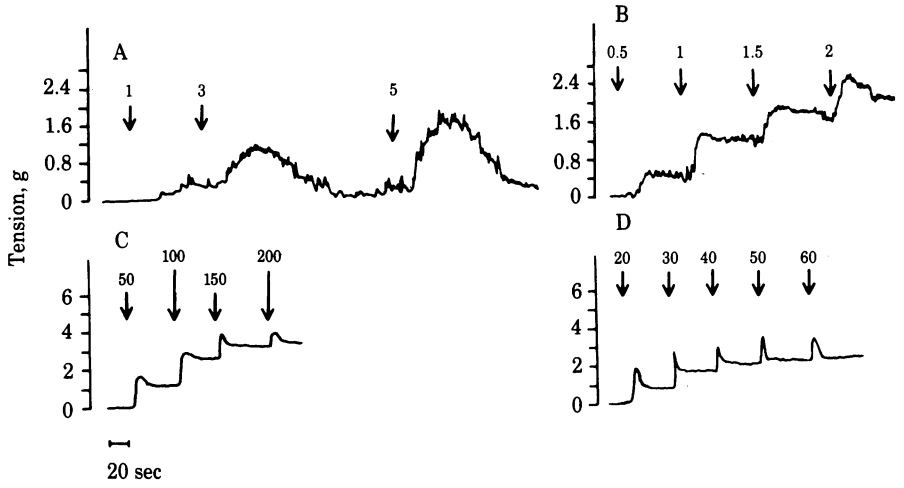

FIG. 1. Concentration–response curves for ileum contraction after treatment with increasing concentrations of various drugs. Arrows mark sequential addition of drug to the tissue bath, and the accompanying number indicates the nanomolar concentration. (*A*) f Met-Leu-Phe. Final cumulative concentrations are 1, 3, and 5 nM. Maximal contraction resulted in tension of 1.70 ± 0.05 g. (*B*) Substance P. Final cumulative concentrations are 0.5, 1, 1.5, and 2 nM. Maximal contraction resulted in tension of 2.16 ± 0.03 g. (*C*) Histamine. Final cumulative concentrations are 50, 100, 150, and 200 nM. Maximal contraction resulted in tension of 3.2 ± 0.01 g. (*D*) Acetylcholine. Final cumulative concentrations are 20, 30, 40, 50, and 60 nM. Maximal contraction resulted in tension of 2.6 ± 0.02 g. (Values for tension are expressed as the mean ± SEM of triplicate determinations.)

To define further the formyl peptide spasmogenic activity, dose–response curves for histamine, acetylcholine, and substance P [which binds to the formyl peptide receptor on rabbit neutrophils (15)] were compared to fMet-Leu-Phe. The nature of the response seen with fMet-Leu-Phe was different from the responses seen with substance P, histamine, and acetylcholine, respectively (Fig. 1 *B–D*). Furthermore the ileum contracted abruptly on exposure to substance P, histamine, and acetylcholine, whereas a 25- to 40-sec latent period was seen after addition of fMet-Leu-Phe (Fig. 2*A*). Both the latency and duration of contraction were independent of the concentration of fMet-Leu-Phe used and occurred with all formyl peptides tested (see below).

Tachyphylaxis was not observed with substance P, histamine, or acetylcholine. Prior treatment with substance P did not affect the subsequent response to fMet-Leu-Phe (Fig. 2*B*). Exposure to high concentrations of substance P (0.1 μM) did not cause cross-desensitization or tachyphylaxis to fMet-Leu-Phe when added to the organ bath prior to washing (data not shown). Addition of fMet-Leu-Phe in the presence of either acetylcholine or histamine resulted in a biphasic response (Fig. 2 *C* and *D*) that showed the same profile obtained when each agent was added separately. The presence of the antihistamine diphenhydramine (2 μM) had little effect (7.6% inhibition) on 0.1 μM fMet-Leu-Phe-induced contraction while greatly inhibiting contraction caused by histamine (by 75.3%). The data presented above suggest that the spasmogenic activity of fMet-Leu-Phe is different from that associated with histamine, acetylcholine, and substance P.

Structure–activity studies with various *N*-formylated peptides suggest the presence of a specific receptor for the formyl peptides (Fig. 3*A*). A greater than 24,000-fold range of activity was seen for the six peptides tested. The intensity of the contractile response was identical for all of the peptides. In addition, the rank order of peptide reactivity, fMet-Leu-Phe-Phe > fMet-Leu-Phe > fNle-Leu-Phe > fMet-Abu-Phe > Met-Leu-Phe > fMet-Leu-Glu, resembled the specificity of the formyl peptide receptors on neutrophils and macrophages seen in guinea pig and other species (4–6, 16–18). In particular, the mandatory requirement of the NH_2-terminal formyl group was evident. Methionine in position 1 and phenylalanine in position 3 conferred maximum activity. The addition of the nonpolar amino acid phenylalanine in position 4 also increased biological responsiveness.

Specific competitive antagonists of the formyl peptide receptor inhibit both the *in vivo* and *in vitro* responses of leukocytes (19). The antagonist Boc-Phe-Leu-Phe-Leu-Phe at 1 μM also antagonized, by approximately 5-fold, the contractile response caused by fMet-Leu-Phe (Fig. 3*B*). No antagonism of

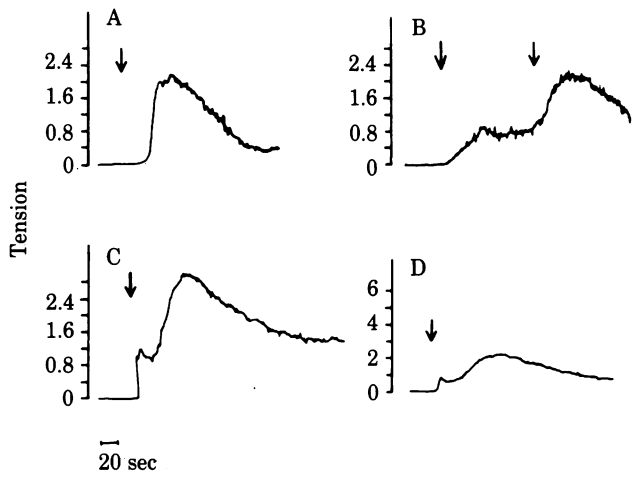

FIG. 2. (*A*) Time course of contraction after addition (arrow) of 0.5 μM f Met-Leu-Phe. Maximal contraction resulted in tension of 2.29 ± 0.11 g. (*B*) Contraction of ileum after treatment with 0.4 nM substance P (left arrow) (tension, 0.84 ± 0.05 g) followed by 600 nM f Met-Leu-Phe (right arrow) (tension, 2.28 ± 0.13). No significant decrease in contraction by f Met-Leu-Phe was seen after pretreatment with substance P. (*C*) Biphasic response after simultaneous addition of 30 nM acetylcholine and 600 nM f Met-Leu-Phe. Steady-state level and maximal contraction resulted in tensions of 1.2 ± 0.01 and 2.92 ± 0.04 for acetylcholine and f Met-Leu-Phe, respectively. (*D*) Biphasic response after simultaneous addition of 50 nM histamine and 5 nM f Met-Leu-Phe. Steady-state level and maximal contraction resulted in tensions of 0.46 ± 0.05 and 2.43 ± 0.07 g for histamine and f Met-Leu-Phe, respectively. (Values for tension are expressed as the mean ± SEM of triplicate determinations.)

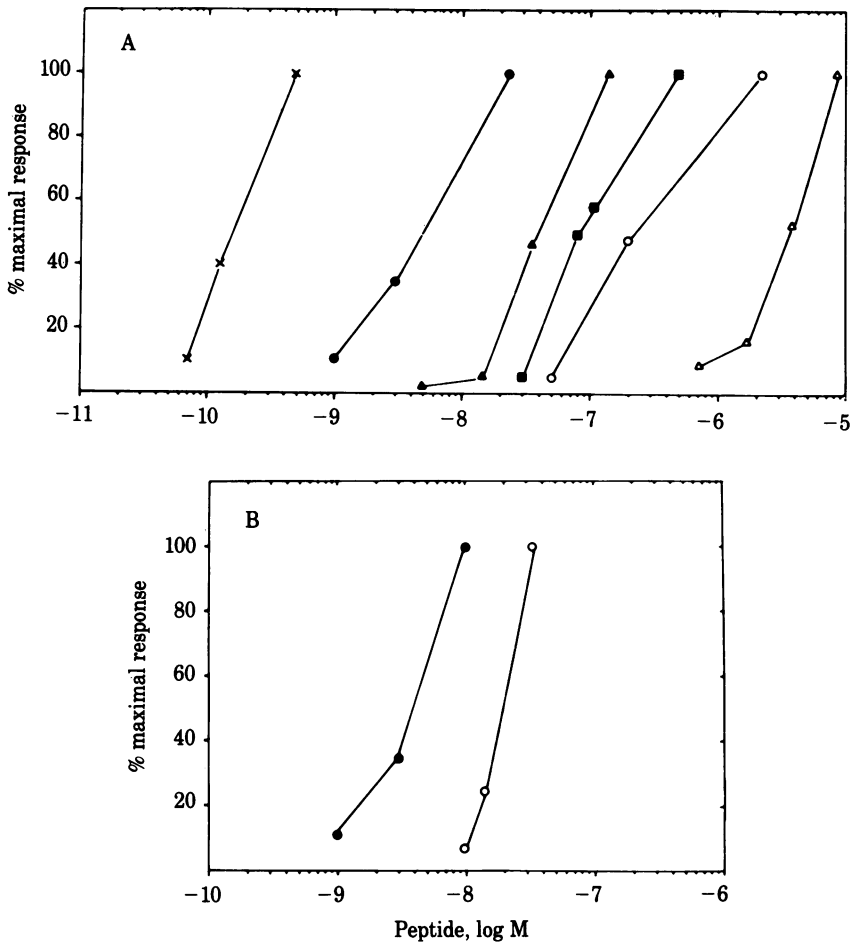

FIG. 3. (*A*) Ability of *N*-formylated peptides to cause guinea pig ileum contraction. x, fMet-Leu-Phe-Phe; ●, fMet-Leu-Phe; ▲, fNle-Leu-Phe; ■, fMet-Abu-Phe; ○, Met-Leu-Phe; and △, fMet-Leu-Glu. Dose–response curves were generated by adding the peptide cumulatively to the organ bath after relaxation to a previous exposure of lesser peptide had occurred. Dose–response curves reflect the maximal contraction that occurred at each peptide concentration tested. Each point reflects the mean of duplicate determinations. (*B*) Ability of the competitive antagonist Boc-Phe-Leu-Phe-Leu-Phe to antagonize the contractile response caused by fMet-Leu-Phe. ●, Control; ○, response in the presence of 1 μM Boc-Phe-Leu-Phe-Leu-Phe. Each point represents the mean of duplicate determinations.

histamine- or acetylcholine-induced contraction was seen (data not shown).

DISCUSSION

It can be concluded that formyl peptides initiate a contractile response in guinea pig ileum in a manner that is distinct from the response to histamine, substance P, and acetylcholine. In addition, the ileum strips remained responsive to multiple challenges with the various formyl peptides during the entire 6-hr range of some of these experiments.

Structure–activity studies with various *N*-formylated peptides led to the hypothesis of the presence of a specific receptor for the formyl peptides on the neutrophil (17). By this criterion, our studies also support the presence of a specific receptor for the formyl peptides within guinea pig ileum strips; however, the cellular distribution of the receptor remains unknown. The observed contractile response may be the result of a direct effect caused by binding of the formyl peptides to a receptor on the smooth muscle cell. However, because a variety of inflammatory cells including lymphocytes, macrophages, and other leukocytes are present in the intestinal lamina propria (20), the possibility that these findings represent an indirect effect mediated through the release of soluble mediators cannot be excluded. A direct effect on the nerve cells causing release of an excitatory transmitter is also possible.

Recent studies have demonstrated histamine release from human leukocytes after treatment with the formyl peptides (8). In addition, the ileum contraction caused by the anaphylatoxin C5a is at least in part mediated by histamine (21). However, the fMet-Leu-Phe-induced contraction is insensitive to inhibition by the antihistamine diphenhydramine in concentrations that inhibit maximum histamine-induced contraction of smooth muscle. Moreover, a sustained contractile response does not occur with fMet-Leu-Phe treatment (Figs. 1*A* and 2*A*), in contrast to the effects of histamine, acetylcholine, and substance P (Fig. 1 *B–D*).

Several substances possessing smooth muscle-contracting activity have been recovered from leukocytes and tissues treated with chemotactic factors. Among these are products of arachidonic acid metabolism from the cyclooxygenase and lipoxygenase pathways (11–13, 22). The spasmogenic activity produced by fMet-Leu-Phe appears distinct from the effects seen with slow reacting substance of anaphylaxis because a sustained contraction is observed with the latter, and complete relaxation of the ileum occurs only after repeated washings. In addition, tachyphylaxis is not observed with slow reacting substance of anaphylaxis (23, 24). With fMet-Leu-Phe-induced contraction, complete relaxation occurs after each contractile peak (Fig. 1*A*), and tachyphylaxis is observed. Whether any of the leukotrienes, prostaglandins, thromboxanes, or mono- and di-hydroxyicosatetraenoic acids are involved in the contractile response produced by the formyl peptides remains a possibility. If the contractile response is the result of secondary mediator release, the cell(s) producing these agents would appear capable of resynthesis because complete recovery of activity is seen after 20–30 min. However, regardless of the mechanism or mediator involved, the generation of smooth muscle-contracting activity within the ileum strips after treatment with the formyl peptides is clearly significant.

Our present knowledge of the biological role of the formyl peptides is incomplete. The effects of these chemolocomotory peptides in mobilization and subsequent activation of the neu-

trophils and macrophages are well established. Furthermore, because it is now established that the synthetic chemotactic oligopeptides are immunochemically identical to chemotactic peptides produced by bacteria (16), these studies suggest that the formyl peptides produced by bacteria may play an important role in the triggering of an acute inflammatory response in tissues containing bacteria. That the same peptides possess potent smooth muscle contracting activity represents a new biological dimension of these bacterial products. The combination of chemotactic and spasmogenic activity suggests that these peptides may play major roles in the acute inflammatory responses to bacteria. As pointed out above, the structure–activity data suggest that the receptor for the formyl peptides on leukocytes and macrophages may be similar if not identical to the receptor we describe in the guinea pig ileum.

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