HONEY BEE VENOM MELITTIN

Correlation of Nonspecific Inflammatory Activities with Amino Acid Sequences

B. F. MACKLER\(^1\) and G. KREIL\(^2\)

\(^1\)Dental Science Institute, The University of Texas Health Science Center, Houston, Texas 77025, and \(^2\)Department of Chemistry, Institute for Molecular Biology of the Austrian Academy of Sciences, Vienna, Austria

Abstract—The nonspecific (nonallergic) inflammatory activity of melittin, a protein toxin from honeybees, was correlated with specific amino acid sequences. Two different amino acid sequences of melittin were found to contribute to nonspecific inflammatory activities in guinea pig skin. Carboxy terminal peptide sequences of 7–10 amino acids induced immediate inflammatory reactions that reached maximum intensities by 15–30 min, then rapidly dissipated. The amino (N) terminal hydrophobic sequence, although not directly inflammatory, intensified the immediate reaction, causing a severe lesion evident by 2 h and characterized by massive polymorphonuclear leukocyte infiltration. A conceptual model of bee venom–induced inflammation in nonallergic individuals is suggested.

INTRODUCTION

The sting of a honey bee, *Apis mellifica*, usually induces a localized lesion that becomes swollen and quite painful even in nonallergic individuals. In allergic patients, this localized lesion is more severe, with occasional anaphylactoid reactions. Although considerable work has been reported concerning clinical allergenic hypersensitivities (1–5), relatively little is known about the mechanism of nonspecific bee venom-induced inflammation. The presence of nonspecific inflammation during skin testing has made the recognition and diagnosis of bee sting (Hymenoptera) allergy quite difficult (4); frequently, patients lacking clinical sensitivity give positive skin tests to whole honey bee extracts (6, 7). Atopic patients were found to give specific skin reactions to major bee venom allergens that could be distinguishable from the nonspecific inflammatory reactions (8).

Honey bee venom contains several pharmacologically active components
which upon subcutaneous injection induce nonspecific swelling and erythema (9). Melittin, a 26-amino acid peptide that accounts for about 50% of dry venom (10), is presumed to mediate the nonspecific inflammatory reactions caused by bee venom (11). The most prominent nonspecific effect of melittin is reported to be mast cell rupture with histamine release (12, 13). Recent studies confirmed these observations and demonstrated that carboxy terminal peptide fragments of melittin released histamine at levels comparable to that released by a whole molecule, but without cell disruption (14).

As the amino acid structure of melittin is known, it seemed appropriate to correlate the nonspecific skin inflammatory properties of melittin with specific regions of its amino acid sequence. In particular, the inflammatory activity of nonlytic histamine-releasing melittin peptides were compared with those of the whole molecule. Inflammation was assessed by quantifying changes in vascular permeability in normal guinea pig skin. Evidence was found indicating that melittin induced multiple nonspecific inflammatory responses: an early immediate response reaching maximum intensity in 15–30 min, then rapidly dissipating; and a later, more severe inflammatory response evident at 30 min but reaching maximum intensity after 120 min.

### MATERIALS AND METHODS

**Preparation of Melittin and Its Peptide Fragments.** Melittin was purified from lyophilized bee venom collected from European (Mack, Illerissen, West Germany) and American (Sigma Chemical Company, St. Louis, Mo.) honey bees. Details of the purification method have been fully described elsewhere (15).

Hydrolysis of melittin with chymotrypsin under controlled conditions (23) yielded fragments Mel₁₋₁₉ and Mel₁₀₋₂₆, as well as small amounts of Mel₁₀₋₂₆ (-NH₃), which has a smaller net positive charge due to deamidation of one of the three amide bonds present in the C-terminal gln-gln-NH₂ sequence. The C-terminal Mel₁₀₋₂₆ heptapeptides were also produced by hydrolysis with N-bromosuccinimide and thermolysin (14). The N-terminal heptapeptide Mel₁₋₇ was isolated from a trypsin hydrolysate of melittin (30 mg melittin in 5 ml H₂O, 1.5 mg trypsin, pH 8.5, 37°C, 4 h). The nonapeptide Mel₁₈₋₂₆ was obtained after digesting melittin with elastase (40 mg melittin in 5 ml H₂O, 60 µg elastase, 37°C, 1 h). The decapptide Mel₁₇₋₂₆ could be isolated from a peptic digest of melittin as described previously (16). All fragments except Mel₁₋₁₉ were purified by high-voltage paper electrophoresis at pH 4.8 on Whatman 3MM paper (1% pyridine acetate buffer, 50 V/cm, 60–120 min). The hydrophobic fragment Mel₁₋₁₉ was extracted from an aqueous suspension with n-butanol (butanol–acetic acid–water ratio 4:1:2) and purified by descending paper chromatography.

The amino acid sequences of these fragments as well as intact melittin are shown in Figure 1. Synthetic melittin and α-N-formyl-melittin were generously supplied by Dr. K. Lübke (Schering AG, West Berlin).

**Guinea Pig Skin Inflammation.** The inflammatory activities of melittin and its fragments were assayed by intradermal injection into the back of normal Hartley strain guinea pigs (250–300 g). At least 24 h prior to testing, guinea pigs were shaved and depilated. The test