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Stimulation of Lipoxygenase Product Synthesis in Human Leukocytes and Platelets by Melittin

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SUMMARY

The effects of melittin on the synthesis of lipoxygenase metabolites of arachidonic acid in human leukocytes and platelets were studied using high performance liquid chromatography. Melittin was found to stimulate strongly the formation of leukotrienes and hydroxy-eicosatetraenoic acids (HETEs) in a concentration-dependent fashion. The metabolites detected were LTB_4, ω-OH-LTB_4, ω-COOH-LTB_4, LTC_4, 5-HETE, 12-HETE, 15-HETE, 5*S*,12*S*-DiHETE, and 5*S*,15*S*-DiHETE. These results suggest that the action of melittin on the formation of arachidonic acid metabolites might be involved in its ability to release endogenous substrates required for the synthesis of 5-, 15-, and 12-lipoxygenase products in leukocytes and platelets, respectively.

INTRODUCTION

Melittin is a 26-amino acid peptide which constitutes more than 50% of bee venom proteins. It is an amphipatic peptide in which amino acids 1–20 are largely hydrophobic and 21–26 are hydrophilic (1). The peptide strongly interacts with phospholipids, lipid bilayers, and cell membranes (2–4) and induces a variety of biochemical reactions in cells including the release of AA[1] and formation of prostaglandins through activation of membrane-bound enzymes (5–7). Although previous studies indicated that melittin might induce the formation of leukotriene B_4 in PMNLs (8), its action on the synthesis of lipoxygenase products of AA has not yet been fully investigated. Thus we investigated the effect of melittin on the formation of 5-, 15-, and 12-lipoxygenase metabolites of AA in human leukocytes and platelets, respectively.

EXPERIMENTAL PROCEDURES

Materials. Melittin and bee venom PLase A_2 (Activity 4,000–10,000 units/g) and Ficoll-Paque were obtained from Sigma Chemical Co. (St. Louis, MO). The ionophore A23187 was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Radiochemicals were obtained from Amersham (Oakville, Ontario, Canada).

Preparation of human leukocytes and platelets. Blood from healthy subjects was collected using citrate/phosphate/dextrose/adenine solution as anticoagulant and centrifuged at 160 × *g* for 15 min. Platelet-rich plasma was discarded, and the red cells were removed by dextran sedimentation and NH_4Cl lysis (9, 10). This cell suspension was either used directly or further purified on Ficoll-Paque to obtain a pure PMNL population. The leukocyte/platelet ratio of the final cell suspension was approximately 1:3. Leukocytes were incubated at a concentration of 10^7 cells/ml (incubation volume 1 ml) with different concentrations of melittin for 5 and 10 min in Dulbecco's phosphate-buffered saline. Reactions were stopped by addition of 1 volume of methanol:acetonitrile (1:1, v/v). The samples were centrifuged at 3000 × *g* for 30 min. The supernatants were acidified to pH 3 by PO_4H_3, and the entire sample (2 ml) was injected into the HPLC apparatus without any further purification.

Analysis of lipoxygenase products by RP-HPLC. RP-HPLC was carried out using a C_{18} Radial Pak column (10-μm particles, Waters Associates) as reported (11). The AA metabolites were detected by UV detection at 280 nm and 229 nm (Waters 440 detectors). This HPLC system permitted a lower limit of detection of approximately 5 pmol. The metabolites of AA were then quantified by comparison of their peak areas with that of the internal standard prostaglandin B_2 and after correction for differences in molar absorption coefficient and attenuation settings (12). Compounds were identified on the basis of their comigration with standards and UV absorbance.

RESULTS

Human leukocytes and platelets were incubated with 5 μg/ml of melittin for 10 min. Analysis of the incubation media by RP-HPLC revealed a number of lipoxygenase metabolites of AA, i.e., ω-COOH-LTB_4, ω-OH-LTB_4, LTB_4, 5S,12S-DiHETE, 5S,15S-DiHETE, 15-HETE, 12-HETE, 5-HETE, and LTC_4 (Fig. 1). These results demonstrated that melittin stimulated the formation of metabolites from the three lipoxygenase pathways (i.e., 15-, 12-, and 5-lipoxygenase) in human leukocytes and platelets. Concentration-response studies with melittin

[1] The abbreviations used are: AA, arachidonic acid; LT, leukotriene; LTB_4, 5*S*,12*R*-dihydroxy-6,8,10,14-(*Z*,*E*,*E*,*Z*)-eicosatetraenoic acid; LTC_4, 5*S*-hydroxy-6*R*-*S*-glutathionyl-7,9,11,14-(*E*,*E*,*Z*,*Z*)-eicosatetraenoic acid; HETE, hydroxy-eicosatetraenoic acid; 12-HETE, 12*S*-hydroxy-5,8,10,14-(*Z*,*Z*,*E*,*Z*)-eicosatetraenoic acid; 15-HETE, 15*S*-hydroxy-5,8,11,13-(*Z*,*Z*,*Z*,*E*)-eicosatetraenoic acid; 5-HETE, 5*S*-hydroxy-6,8,11,14-(*E*,*Z*,*Z*,*Z*)-eicosatetraenoic acid; RP-HPLC, reversed phase-high performance liquid chromatography; PLase, phospholipase; PMNL, polymorphonuclear leukocyte.

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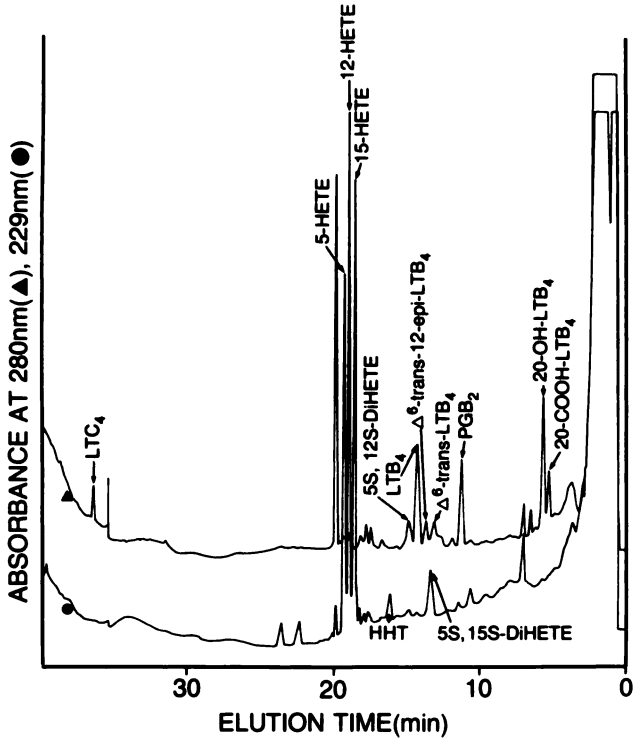

FIG. 1. *RP-HPLC chromatogram of the metabolites of AA formed in 10^7 human leukocytes and 3 × 10^7 platelets incubated 10 min at 37° with 5 μg/ml of melittin*

PGB_2 (prostaglandin B_2), 200 ng. Full-scale deflection at 229 nm and 280 nm corresponded to 1 and 0.5 optical density unit, respectively.

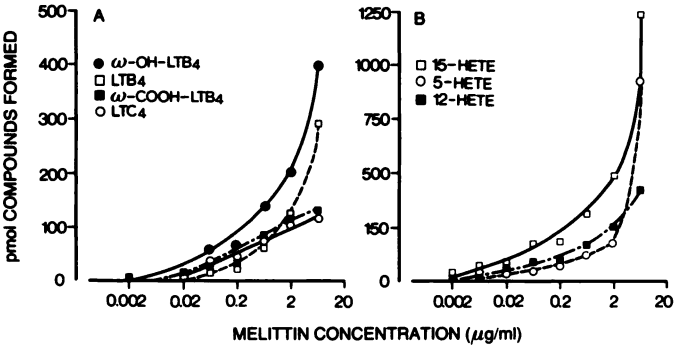

FIG. 2. *Effect of increasing concentrations of melittin on the formation of LTs (A) and HETEs (B) in human leukocytes and platelets (1:3) after 10 min of incubation*

Each *point* is the mean of 4 experiments. Standard errors ranged from 15–30% of the mean.

(Fig. 2) indicated that the peptide stimulated these transformations in a concentration-dependent manner. Melittin also stimulated the formation of 5- and 15-lipoxygenase products in a purified PMNL preparation (Fig. 3). However, melittin at concentrations above 5 μg/ml induced cellular cytotoxicity, as demonstrated by the trypan blue exclusion test (data not shown).

The effects of ionophore A23187 and melittin were compared in time course experiments. Both stimuli induced a rapid synthesis of lipoxygenase products (Fig. 4). At 10 μg/ml, melittin was at least as potent as the ionophore A23187 at 2 μg/ml for the formation of 5- and 12-lipoxygenase products: melittin, but not the ionophore, also stimulated the formation of 15-HETE (Fig.

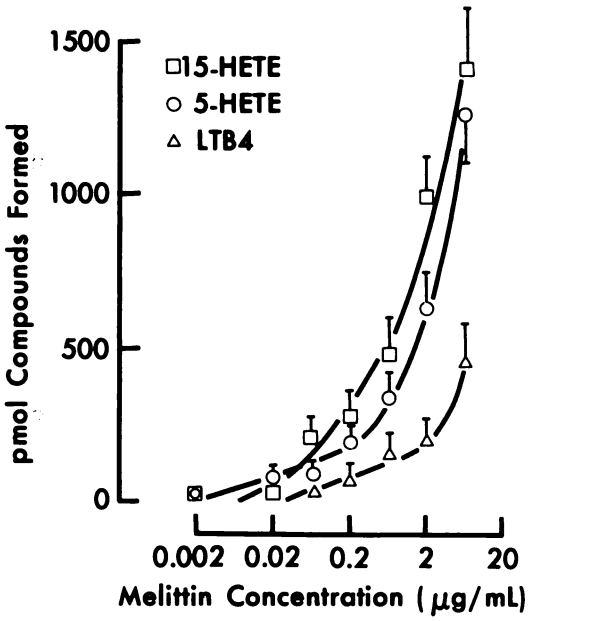

FIG. 3. *Effect of increasing concentrations of melittin on the formation of 15-HETE, 5-HETE, and LTB_4 in human polymorphonuclear leukocytes (10^7 cells/ml) after 10 min incubation*

Values are means ± SE; $n = 4$.

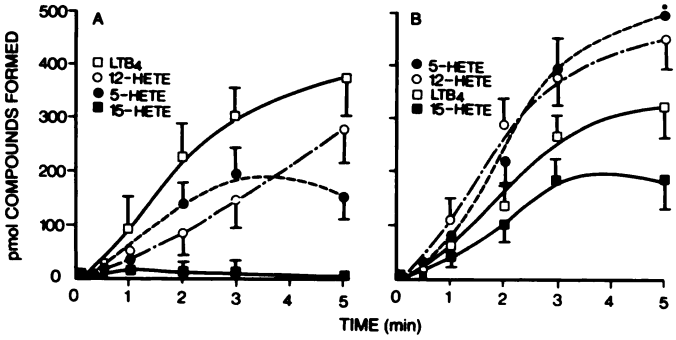

FIG. 4. *Time course of the effect of 2 μg/ml of ionophore A23187 (A) and 10 μg/ml of melittin (B) on the formation of LTs and HETEs by 10^7 human leukocytes and 3 × 10^7 platelets*

Values are means ± SE; $n = 4$ (SE = 60).

4). Further studies were carried out to determine the action of melittin in the presence of exogenous AA. Our data showed that the addition of exogenous AA to the milittin-stimulated cells did not increase the formation of LTs, whereas it increased the formation of HETEs by 2–3-fold (data not shown).

The presence of PLase A_2 in the melittin preparation (6 μg/mg of melittin, as measured by radiometric assay; Ref. 13) made it necessary to investigate the effect of PLase A_2 on AA metabolite formation in platelets and leukocytes. Leukocytes were incubated with different concentrations of PLase A_2 for 10 min. Analysis of the incubation mixture showed that the PLase A_2 stimulates the formation of lipoxygenase products at concentrations above 10 μg/ml (Fig. 5), indicating that the stimulatory action of melittin may not be due to the presence of contaminating PLase A_2 in melittin preparations.

DISCUSSION

These data demonstrate that melittin stimulates the formation of lipoxygenase metabolites of AA in leuko-

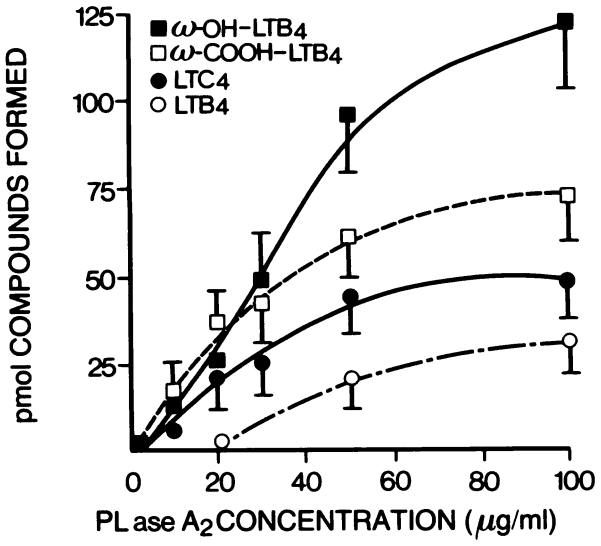

FIG. 5. *Effect of increasing concentrations of PLase A_2 on the formation of LTs by 10^7 human leukocytes and 3×10^7 platelets in 10-min incubations*

Values are means ± SE; $n = 4$.

cytes and platelets within the dose range of 0.1–10 μg/ml. The response is concentration related, and at concentrations below 5 μg/ml the peptide stimulated product formation without decreasing cell viability as assessed by trypan blue exclusion. The mechanism by which melittin causes lipoxygenase product formation certainly involves its ability to stimulate PLase A_2 and AA release, since the synthesis of metabolities was observed both in the absence and presence of exogenous AA. In addition, it has previously been shown that melittin activates PLase A_2 in a variety of intact cell systems (1, 5, 14). The activation of PLase A_2 and the release of AA by melittin may account for the formation of 15- and 12-lipoxygenase products in leukocytes and platelets, respectively. However, it does not fully explain the mechanism of 5-lipoxygenase metabolite formation, since it is well known that addition of AA alone to leukocytes does not induce the formation of LTs (15). Thus, our results suggest that melittin might stimulate both PLase A_2 and 5-lipoxygenase activities. These effects of melittin on AA metabolism in leukocytes and platelets may be related to the property of the peptide to alter membrane permeability to calcium, since both PLase A_2 and 5-lipoxygenase are known to be calcium-dependent enzymes (16). Accordingly, the ability of melittin to stimulate the formation of LTs was reduced by more than 95% in the absence of calcium (data not shown).

Another significant observation of this study was that melittin stimulated the formation of metabolites from the three lipoxygenase pathways, whereas the ionophore A23187 stimulated the synthesis of 5- and 12-lipoxygenase products only in the absence of exogenous AA. Although this may be an indication that melittin can specifically stimulate the formation of 15-HETE and 5S,15S-DiHETE, the explanation for it is that the peptide might cause the release of larger amounts of endogenous AA than the ionophore, which could be used as a substrate for 15-lipoxygenase, since, in a study on the effect of exogenous AA on lipoxygenase product synthesis in human leukocytes, it was shown that the synthesis of 15-HETE occurs only at a relatively high concentration of AA (15). Alternatively, melittin might stimulate the release of linoleic acid, which has recently been shown to be a better substrate for 15-HETE formation than AA (17).

In conclusion, the present data indicate that melittin is a potent stimulator of 5- and 15-lipoxygenase product synthesis in human leukocytes and of 12-lipoxygenase product in human platelets.

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