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Suppression of L-Histidine Decarboxylase mRNA Expression by Methyleugenol

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We investigated the effect of methyleugenol on anaphylaxis. Methyleugenol completely inhibited systemic anaphylaxis induced by compound 48/80 in mice. Methyleugenol also inhibited local anaphylaxis activated by anti-dinitrophenyl (DNP) IgE. Moreover, methyleugenol dose-dependently inhibited histamine release in mast cells activated by compound 48/80 or anti-DNP IgE. Northern-blot analysis demonstrated that significantly reduced level of the mRNA of L-histidine decarboxylase (HDC) was expressed in mast cells treated with methyleugenol, compared to that without methyleugenol. We conclude that methyleugenol directly affect histamine release and HDC gene over-expression in mast cells. © 1997 Academic Press

Methyleugenol (1-allyl-3, 4-dimethoxybenzene) may be ingested in very small amounts by humans as natural components of certain spices, essential oils, or vegetables. Mast cell has long been thought to play a crucial role in the development of many physiologic changes during anaphylactic and allergic responses (1). Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine remaines the best characterized and most potent vasoactive mediator implicated in the acute phase of type I allergic reactions (2). Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (3). The most potent secretagogues include the synthetic compound 48/80, polymers of basic amino acids (4). Substance P and others appear to induce histamine release by interacting with the same site as compound 48/80 (5). L-histidine decarboxylase (HDC) catalyzes the formation of histamine from its precursor, histidine, in a single step. Mouse mastocytoma P-815 cells are the proper cell type for elucidating the mechanism underlying histamine formation in mast cells, because they synthesize HDC in response to various stimuli. Mast cells also release inflammatory mediators through the reaction of cell-bound antibodies with multivalent (6,7). We have evaluated the effect of methyleugenol on both compound 48/80-induced systemic anaphylactic shock and anti-DNP IgE-induced local anaphylactic reaction. Here we show that methyleugenol suppresses HDC gene expression increased by substance P.

MATERIALS AND METHODS

Materials. Methyleugenol, compound 48/80, substance P, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO). The α-minimal essential medium (α-MEM) was purchased from Flow Laboratories (Irvine). Fetal calf serum (FCS) was purchased from Gibco Laboratories (Grand Island, NY). The original stock of BALB/c mice and Wistar rats were purchased from The Korean Research Institute of Chemical Technology (Taejeon, Chungnam), and the animals were maintained in the College of Pharmacy, Wonkwang University. The animals were housed five to ten per cage in a laminar air flow room maintained under the temperature of 22±1°C and relative humidity of 55±10% throughout the study.

Systemic anaphylaxis. Mice were given an intraperitoneal injection of 8 μg/g body weight (BW) of the mast cell degranulator compound 48/80. Methyleugenol was dissolved in saline and administered by intraperitoneal injection with 0.01 to 100.0 μg/g BW at 1 h before the injection of compound 48/80. Mortality was determined for 1 h after induction of anaphylactic shock. After mortality test the blood was obtained from each mouse's heart.

Passive cutaneous anaphylaxis (PCA). An IgE-dependent cutaneous reaction was generated by sensitizing skin with an intradermal injection of anti-DNP IgE followed 48 h after with an injection of DNP-HSA into the rat tail vein. The DNP-HSA was diluted in PBS. Rats were injected intradermally with 100 μg of anti-DNP IgE into each of 4 dorsal skin sites that had been shaved 48 h previously. Sites were outlined with a water-insoluble red marker. Forty-eight hours later each rat received an injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:4) via tail vein. Methyleugenol (100 μ g/g BW) was orally administered 1 h before the challenge. Thirty min after the challenge, the rats were sacrificed, the dorsal skin was removed for measurement of pigment area. The amount of

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The abbreviations used are: DNP, dinitrophenyl; HDC, L-histidine decarboxylase; HSA, human serum albumin; PCA, passive cutaneous anaphylaxis; RPMC, rat peritoneal mast cells.

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dye was then determined colorimetrically after extraction with 1 ml of 1.0 N KOH and 9 ml of mixture of aceton and phosphoric acid (5:13) based on the method of Katayama et al. (8). The absorbant intensity of extraction was measured at 620 nm in a spectrofluorometer, and the amount of dye was calculated with the measuring-line Evans blue.

Preparation of serum and histamine determination. The blood was allowed to 400 × g for 10 min. The serum was withdrawn and histamine content was measured by the o-phthalaldehyde spectroflurometric procedure of Shore et al. (9). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer.

Preparation of rat peritoneal mast cells (RPMC). RPMC were isolated as previously described (10). In brief, rats were anesthetized by ether, and 20 ml of Tyrode buffer B (NaCl, glucose, $NaHCO_3$, KCl, NaH_2PO_4) containing 0.1% gelatin (Sigma Chemical Co.) was injected into the peritoneal cavity, and the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150 × g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from major components of rat peritoneal cells, *i.e.* macrophages and small lymphocytes, according to the method described by Yurt et al. (11). In brief, peritoneal cells suspended in 1 ml Tyrode buffer B were layered on 2 ml of 22.5% w/v metrizamide (density, 1.120 g/ml, Sigma Chemical Co.) and centrifuged at room temperature for 15 min at 400 × g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1ml Tyrode buffer A containing calcium. Mast cell preparations were about 95% pure as assessed by Toluidine Blue staining. More than 97% of cells were viable as judged by trypan blue uptake.

Inhibition of histamine release. Purified mast cells were resuspended in Tyrode buffer A containing calcium for the treatment of compound 48/80. Mast cell suspensions (1×10^6 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80. The cells were preincubated with the methyleugenol preparations, and then incubated (10 min) with the compound 48/80. The reaction was stopped by cooling tubes in ice. The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400 × g for 5 min at 4°C.

Assay of histamine release. The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Histamine release without methyleugenol} - \text{Histamine release with methyleugenol}}{\text{Histamine release without methyleugenol}} \times 100$$

Molecular probes. The HDC probe used a ^{32}P-labeled *PstI*-digested cDNA insert from a mouse mastocytoma P-815 cells cDNA clone kindly provided Dr. A. Ichikawa, Kyoto University, Kyoto, Japan (12).

RNA extraction and Northern blot analysis. Mastocytoma P-815 cells were maintained as a suspension culture in α-MEM with 10% FCS. Total RNA was prepared by using the modified LiCl-urea method (13), electrophoresis in 1.2% agarose-formaldehyde gels, and transferred to nylon membranes by capillary action in 20× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.2). After prehybridization, the filters were hybridized with random [α-^{32}P]-dCTP-labeled probes having specific activity of 1 to × 10^8 cpm/μg in 10% dextran sulfate, 50% formamide, 4× SSC, 1× Denhardt's solution, and 10 μg/ml salmon sperm DNA for 24 h at 42°C. Then the filters were washed, dried, and examined by autoradiography.

TABLE I

Effects of Methyleugenol on Compound 48/80-Induced Systemic Anaphylaxis

Treatment	Dose in μg/g BW	Compound 48/80 (8 μg/g BW)	Mortality (%)
Saline	—	+	100
Methyleugenol	0.01	+	100
Methyleugenol	0.1	+	100
Methyleugenol	1.0	+	80
Methyleugenol	10.0	+	0
Methyleugenol	100.0	+	0

Groups of mice were intraperitoneally pretreated with 200 μl saline or methyleugenol was given at various doses 1 h before (n = 10/group) compound 48/80 injection. Compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injection was represented as No. of dead mice × 100/total No. of experimental mice.

Statistical analysis. The results obtained were expressed as means ± S.E. for the number of animals. The Student's *t*-test was used to make a statistical comparison between the groups. Results with $P<0.05$ were considered statistically significant.

RESULTS

To assess the contribution of methyleugenol in anaphylactic reactions, we first used the *in vivo* model of systemic anaphylaxis. We used compound 48/80 (8 μg/g BW) as systemic fatal anaphylaxis inducer. The mortality of mice was determined for 1 h after injection of compound 48/80. As shown in Table I, intraperitoneal injection of 200 μl saline as control induced fatal shock in 100% of the each group. When methyleugenol was pretreated at concentration ranging from 0.01 to 100 μg/g BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. Especially, methyleugenol inhibited compound 48/80-induced anaphylaxis 100% with the dose of 10 to 100 μg/g BW (Table I).

The ability of methyleugenol to influence compound 48/80-induced serum histamine release was investigated. Methyleugenol was given with 0.01 to 100 μg/g BW 1 h before (n=5/group) compound 48/80 injection. While serum levels of histamine were markedly elevated after compound 48/80 injection in all groups of mice, mice injected methyleugenol showed significant reduction in serum histamine levels (Fig. 1).

Another way to test anaphylactic reactions is to induce PCA. As described in the experimental procedures, local extravasation is induced by local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Oral administration of methyleugenol (100 μg/g BW) showed the marked inhibition rate (81.6%) in PCA reaction (Table II).

The inhibitory effects of methyleugenol on compound 48/80-induced or IgE-mediated histamine release from RPMC following 10 min preincubation are shown in

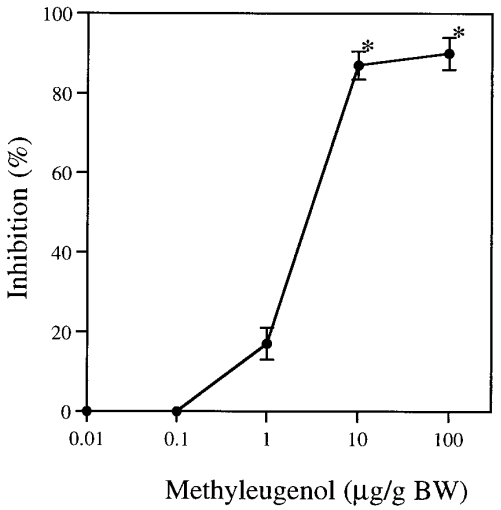

FIG. 1. Effect of methyleugenol on compound 48/80-induced serum histamine release. Groups of mouse were intraperitoneally pretreated with 200 μl saline or methyleugenol. Each drug was given with various doses at 1 h before (n=7/group) compound 48/80 injection. Compound 48/80 solution was intraperitoneally given to the group of mice. *P<0.05; significantly different from the saline value.

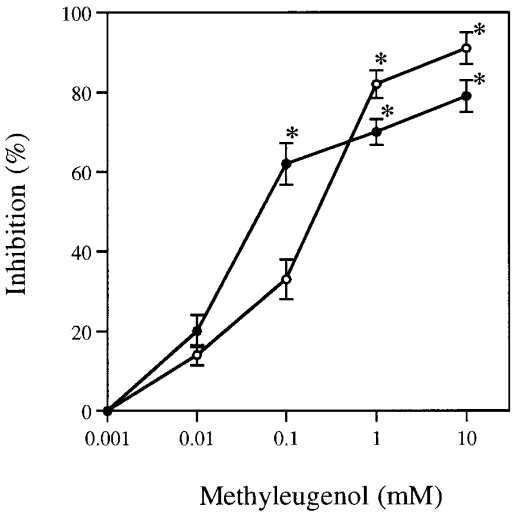

FIG. 2. Effect of methyleugenol on compound 48/80-induced (●) or IgE-mediated (○) histamine release from RPMC. RPMC (2×10^5 cells/ml) were preincubated with drug at 37°C for 10 min prior to incubation with each stimulator for 10 min. *P<0.05; significantly different from the saline value.

Fig. 2. Methyleugenol dose-dependently inhibited compound 48/80-induced or IgE-mediated histamine release at concentrations of 0.001 to 10 mM.

Data in Fig. 3 show the analysis of Northern blot. As shown in a previous study (12), the mRNA encoding the amino acid sequence of HDC in mastocytoma P-815 cells is 2.7 kb in size. Methyleugenol suppressed the increase in substance P-induced accumulation of HDC mRNA on incubation for 6 h. No significant cytotoxicity of these compounds on culture was observed in the concentration used in the experiments as assessed by trypan blue uptake.

DISCUSSION

The present study showed that methyleugenol pretreatment profoundly affected compound 48/80-induced systemic anaphylaxis and anti-DNP IgE-induced PCA reactions. Methyleugenol inhibited the release of histamine induced by specific antigens as well as non-specific mechanisms from mast cells. We simply speculate that these results indicate that anaphylactic degranulation of mast cells is inhibited by methyleugenol. There is absolutely no doubt that stimulation of mast cells with compound 48/80 or anti-DNP IgE initiates the activation of signal-transduction pathway which lead to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (14,15). The evidence indicates that the protein is Gi-like and that the activation is inhibited by benzalkonium chloride (16). Tasaka et al. reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane (17). This result indicates that the membrane permeability increase may be essential trigger for the release of the mediator from mast cells.

TABLE II

Effect of Methyleugenol on the 48-h PCA in Rats

Treatment	Dose (μg/g BW)	Amount of dye (8 μg/g BW)	Inhibition (%)
Saline	—	15.630 ± 0.025	—
Methyleugenol	100	2.869 ± 0.005*	81.6

Drug was administered orally 1 h prior to challenge with antigen. Each amount of dye represents the mean ± S.E. of 4 experiments. *P < 0.05; significantly different from the saline value.

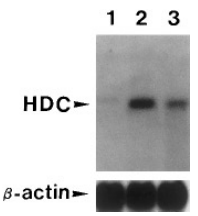

FIG. 3. Effect of methyeugenol on HDC mRNA level in mastocytoma P-815 cells. Cells were incubated in the absence (lane 1) or presence of 100 nM substance P (lane 2), substance P plus 100 nM methyeugenol for 6 h (lane 3) at 37°C. The cells were harvested and washed, and then total RNA was prepared, and HDC mRNA was analysed, by Northern hybridization as described in the Materials and Methods. The β-actin probe was used to verify that an equal amount of total RNA (20 μg) was loaded in each lane.

We have demonstrated that methyleugenol suppressed in substance P-induced accumulation of HDC mRNA. The mechanism by which the methyleugenol suppression result in increased HDC gene transcription is of interest. In previous studies, it was reported that substance P can specifically bind to murine mast cells to trigger release of histamine (18, 19). Methyleugenol may work as an antagonist in receptor interaction. This is supported by a previous reports that benzalkonium chloride and another selective antagonists inhibit the histamine release incuced by compound 48/80 and basic neuropeptides such as substance P (5, 20). The methyleugenol-administered rats are protected from IgE-mediated anaphylaxis. The possible mechanism of protection against anti-DNP IgE, while not clear at present, may be evidenced only in particular conditions. In conclusion, the results obtained in the present study provide evidence that methyleugenol importantly contributes to the prevention or tratment of anaphylactic diseases.

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