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Anti-allergic constituents in the culture medium of *Ganoderma lucidum.* (II) The inhibitory effect of cyclooctasulfur on histamine release

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Abstract

For centuries, *Ganoderma lucidum* has been used in Oriental medicine for the treatment of chronic bronchitis. Sequential fractions of the culture medium of this plant revealed that one of the active constituents was cyclooctasulfur. The latter effectively inhibited histamine release from rat peritoneal mast cells and impeded ^{45}Ca uptake into these cells without affecting the cyclic AMP content. SDS-PAGE analysis indicated that cyclooctasulfur induced some changes in protein bands obtained from the membrane fraction of mast cells, suggesting that this compound interacts with membrane proteins so as to inhibit ^{45}Ca uptake, and that this may be the main cause of histamine release inhibition.

Introduction

"Ganoderma" has been used in Oriental medicine from ancient times as a tonic and sedative, and for the treatment of chronic bronchitis. It has been shown that the culture medium of *Ganoderma lucidum* (Fr.) Karst. contains active constituents which inhibit histamine release from mast cells. In the present study, the inhibitory mechanism of the active components in *G. lucidum* which affect the histamine release from rat peritoneal mast cells was studied.

Materials and methods

Extraction of G. lucidum culture medium

The *G. lucidum* culture medium was extracted according to the method previously described [1]. After centrifugation of the chloroform extract of the medium, the soluble fraction was dried to make a light brown powder. The powder was extracted with chloroform and evaporated to obtain a dark brown oily material. The extract was chromatographed on silica gel with benzene, chloroform, and chloroform/methanol in ratios of 95:5, 90:10, and 50:50, respectively (Fig. 1a). Active fractions were further extracted with hexane, yielding pale yellow crystals. The latter were analyzed with an electron impact ionization mass (EI-mass) spectrometer (Hitachi N80B).

Histamine release from rat peritoneal mast cells

Peritoneal mast cells isolated from the abdominal cavity of male Wistar rats were suspended in physiological buffered solution (PBS) containing (m*M*) NaCl 154, KCl 2.7, $CaCl_2$ 0.9, glucose 5.6, HEPES 5; pH 7.4. The cells were preincubated with test compounds suspended in 0.25% carboxymethylcellulose sodium at 37 °C for 15 min, and subsequently exposed to compound 48/80 (48/80) at 0.5 μg/ml for 1 min. The amounts of histamine in the supernatant and residual hista-

mine in the cells were determined separately using a fluorometric assay [2].

^{45}Ca uptake into mast cells

^{45}Ca uptake into mast cells was measured as described previosly [2]. Rat mast cells, purified to more than 95% by means of Percoll centrifugation, were incubated with ^{45}Ca for 1 min at 37 °C. Thereafter, pretreatment with cyclooctasulfur was carried out for 15 min and the cells were incubated with 48/80 (0.5 μg/ml) for 5 min. The mast cells were washed twice with fresh PBS, then solubilized with Triton X-100 (10%). Radioactivity was measured by liquid scintillation counting.

Determination of cyclic-3',5'-AMP content

After incubation with cyclooctasulfur at 37 °C for 15 min, the cells were stimulated with 0.5 μg/ml of 48/80 for 5 min. Cells were disrupted using the freeze-thawing method and the cAMP content was measured with a radioimmunoassay kit (Yamasa) [3].

SDS-PAGE analysis

After incubation with cyclooctasulfur for 15 min at 37 °C, the cells were disrupted by 2 sec of sonication in 0.25 *M* of sucrose. The disrupted cells were centrifuged at 50,000 × g to obtain the membrane fraction. SDS-PAGE analysis was carried out according to the method of Laemmli [4] in the presence or absence of 2-mercaptoethanol (2-ME).

Differential scanning calorimetry (DSC)

Test compounds were sonicated with 2 m*M* of dipalmitoylphosphatidylcholine (DPPC) for 1 min at 45 °C, and calorimetric scans were carried out with a BSC-50 apparatus (Sinku-Riko) at a rate of 0.8 °C/min [5].

Results

The chloroform extract of the culture medium of *G. lucidum* was sequentially fractionated with benzene, chloroform and the mixtures of chloroform/ methanol. As indicated in Fig. 1a, histamine release from rat peritoneal mast cells was strongly inhibited by fractions A-4, C-1 and D-1. TLC analysis revealed that the active constituent of A-4 was oleic acid. Since, after TLC analysis, C-1 and D-1 were evaluated as containing the same active constituent, the two fractions were

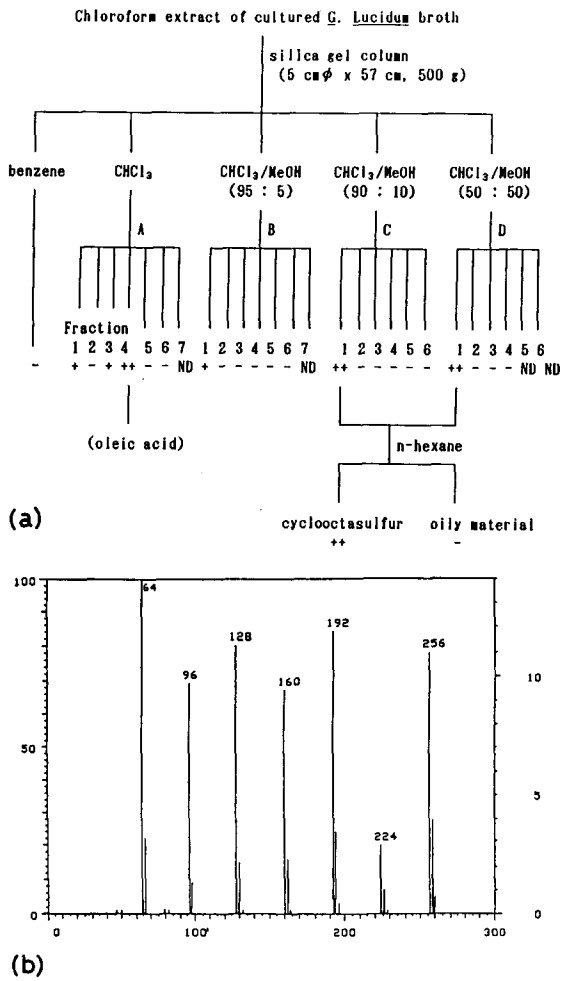

Figure 1
Fractionation of chloroform extract of *G. lucidum* by means of silica gel chromatography and mass spectrum (EI-mass) of the final active material. (a) Extraction procedure: + +: strongly active in histamine release inhibition from rat peritoneal mast cells induced by compound 48/80 (0.35 μg/ml, with 35.5 ± 0.4% of histamine release; almost complete inhibition). +: active in histamine release inhibition. –: not effective in inhibiting histamine release. ND: not done (due to low solubility). (b) Mass spectrum (EI-mass).

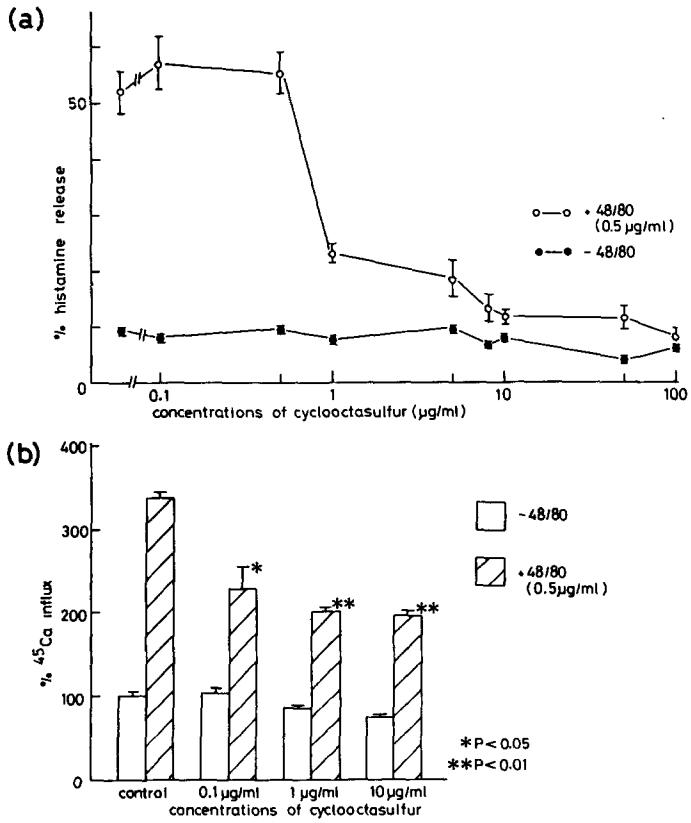

Figure 2
Inhibitory effects of cyclooctasulfur on histamine release and ^{45}Ca uptake in rat peritoneal mast cells induced by compound 48/80 (0.5 μg/ml). (a) Histamine release. 52.2 ± 4.8% of histamine release was induced by 48/80 alone. (b) ^{45}Ca uptake. ^{45}Ca uptake determined in control cells was 2407.3 ± 13.34 cpm/10^6 cells.

extracted simultaneously with hexane, yielding pale yellow crystals. From the EI-mass spectrum (Fig. 1b), the material was judged to be cyclooctasulfur. The melting point was 116.0–116.5 °C, which was close to the authentic value. An authentic sample of cyclooctasulfur strongly inhibited the histamine release from rat peritoneal mast cells induced by 48/80, and the potency was almost the same as that of the extracted material (Fig. 2a).

In order to study the inhibitory mechanism of cyclooctasulfur on mast cell histamine release, its effect on ^{45}Ca uptake was measured. While 48/80 increased ^{45}Ca uptake into mast cells at a rate 3.5 times the control, cyclooctasulfur strongly inhibited the increment of ^{45}Ca uptake (Fig. 2b). However, changes in the cAMP content of rat mast cells induced by 48/80 were not affected by pretreatment with cyclooctasulfur. DSC analysis revealed that cyclooctasulfur did not affect the phase transition profile of DPPC. Since 2-ME disrupts disulfide bonds, the effect of cyclooctasulfur on the membrane protein of rat mast cells was analyzed by means of SDS-PAGE in the presence or absence of 2-ME. In the presence of 2-ME, the profiles of mast cell membrane proteins treated with cyclooctasulfur was quite similar to that of the control cells. In the absence of 2-ME, however, dense protein bands were observed at molecular weights of 26, 29.3 and 30 kdaltons, after treatment with cyclooctasulfur, suggesting that the latter elicited disulfide coupling of membrane proteins. In the presence of 2-ME, 48/80 decreased the density of protein bands of 29.3 and 68

kdaltons, and increased that of 44- and 25.6-kdalton bands. In the absence of 2-ME, it decreased the density of protein bands of 33, 29.3 and 26 kdaltons, and increased that of 44- and 25.6-kdalton bands. Cyclooctasulfur pretreatment inhibited almost completely those changes induced by 48/80.

Discussion

From the present study, it became apparent that one of the active constituents of *G. lucidum* was cyclooctasulfur. Cyclooctasulfur strongly inhibited the histamine release from rat peritoneal mast cells induced by 48/80. Although cyclooctasulfur did not affect the decrease in cAMP content of the rat mast cell caused by 48/80, this compound strongly inhibited 48/80-induced ^{45}Ca uptake into these cells. This may be one of the reasons for the histamine release inhibition. DSC analysis indicated that cyclooctasulfur did not interact with DPPC liposomes, suggesting that this compound may not interact with the lipid bilayer region of the cell membrane. By means of SDS-PAGE analysis, it became evident that a disulfide exchange reaction probably takes place in the cell membrane. Thus, it is reasonable to conclude that cyclooctasulfur may interact with membrane proteins so as to decrease the Ca uptake from the extracellular medium, causing inhibition of histamine release.

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