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The Effect of Acteoside on Histamine Release and Arachidonic Acid Release in RBL-2H3 Mast Cells

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The effect of acteoside, a phenylpropanoid glycoside isolated from *Clerodendron trichotomum Thunberg*, on histamine and arachidonic acid release was investigated in RBL 2H3 cells. Histamine was dose-dependently released from RBL 2H3 cells by melittin, arachidonic acid and thapsigargin. In extracellular Ca^{2+}-free solution, basal secretion of histamine increased by two fold. The response of histamine release to melittin and thapsigargin in Ca^{2+}-free solution was significantly decreased, whereas the response to arachidonic acid was significantly increased as compared with those in normal solution. Acteoside inhibited histamine release induced by melittin, arachidonic acid and thapsigargin in a dose-dependent manner in the presence or absence of extracellular Ca^{2+}. However, the inhibitory activity of acteoside was more potent in normal solution than that in Ca^{2+}-free solution. These data suggest that inhibitory mechanism of acteoside on histamine release may be related to extracellular Ca^{2+}. On the other hand, acteoside significantly inhibited arachidonic acid release and prostaglandin E_2 production induced by 0.5 μM melittin. It is possible that acteoside may be developed as an anti-inflammatory agent.

Key words: Acteoside, Histamine, Arachidonic acid, Prostaglandin E_2

INTRODUCTION

Inflammatory responses are related to phospholipase A_2 (PLA_2) activation, histamine release, reactive oxygen species (ROS) generation and nitric oxide (NO) production in neutrophils, macrophages and mast cells (Attur *et al.*, 2000; Petrone *et al.*, 1980). PLA_2 activation plays a key role in inflammatory responses. In particular, PLA_2 activation may have influence on histamine release and ROS production as well as eicosanoid complex production. Both melittin, an endogenous PLA_2 activator, and arachidonic acid significantly increased histamine release from mast cells (Gueck *et al.*, 2004; Battistella *et al.*, 1986).

Acteoside and other phenylpropanoid glycosides are contained in many plants that are widely used in traditional herbal medicine. Some of these phenylpropanoid glycosides appear to have various biological activities, such as antibacterial, analgesic, anti-inflammatory and antioxidant activity (Xiong *et al.*, 2000; Wong *et al.*, 2001; Sahpaz *et al.*, 2002). It has been reported that anti-inflammatory action of acteoside is related to the inhibition of lipopolysaccharide (LPS)-induced prostaglandin E_2, NO, and TNF-α production in mouse peritoneal macrophage (Diaz *et al.*, 2004). Despite of various biological activities of acteoside, there is no evidence of the effect on histamine release from mast cells. Therefore, to investigate the underlying mechanism of acteoside on histamine release, we measured the inhibitory effect of acteoside on histamine release induced by melittin, arachidonic acid and thapsigargin, and on arachidonic acid release in RBL-2H3 mast cells.

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MATERIALS AND METHODS

Materials

Acteoside, a phenylpropanoid glycoside, was isolated from *Clerodendron trichotomum Thunberg*. [^{3}H]arachidonic acid and enzyme-linked immunosorbent assay (ELISA) kit for the determination of prostaglandin E_2 (PGE_2) was provided by Amersham Pharmacia (Piscataway, NJ,

U.S.A.) and 1-palmytoyl-2-(10-pyrendecanoyl)-sn-glycero-3-phosphorylcholine (10-pyren PC) was from Molecular Probes (Eugene, OR, U.S.A.). Other reagents were purchased from Sigma Chemical Co. (U.S.A.).

Cell culture

The rat basophilic leukemia (RBL-2H3) cells were grown in Dulbecco's modified Eagle minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (Gibco) in 5% CO_2. The RBL-2H3 cells were harvested by incubating them in phosphate balanced saline (PBS) containing 1 mM EDTA and 0.25% trypsin for 5 min at 37°C and were used for measurements of histamine release and arachidonic acid release.

Cellular viability

Cellular viability was performed by using a 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based colorimetric assay (Woerdenbag *et al.,* 1994). Cells in 96-well plates (8×10^5 cells/well) were exposed to acteoside at 37°C for 3 h. The 20 μL MTT solution (5 mg/mL in phosphate buffered saline) was added and further incubated for 3 h. After aspirating the supernatant from the wells, 100 μL of DMSO were added for the dissolution of Formazan crystals. The absorbance of each well was then read at 520 nm using an ELISA plate reader.

Histamine assay

The harvested RBL-2H3 cells were washed with Krebs buffer (mM: NaCl 137, KCl 2.7, Na_2HPO_4 0.4, $MgCl_2$ 0.5, HEPES (pH 7.4) 10, $CaCl_2$ 1.8, glucose 5) and suspended in Krebs buffer at the density of 10^6 cells/mL. Ca^{2+}-free Krebs buffer was prepared by adding 2 mM EGTA instead of 1.8 mM $CaCl_2$.

The cells were treated with acteoside for 10 min, and then histamine release was induced by melittin, arachidonic acid or thapsigargin for 30 min at 37°C. The released histamine was assayed using the fluorometric method (Shore *et al.,* 1959). After centrifugation, histamine contents in both supernatant and pellet were measured with 0.1 mL of 1% *o*-phthaldialdehyde in methanol. After 4 minutes, the reaction was terminated by adding 0.2 mL of 3 N HCl. The fluorescence intensity was measured using excitation and emission wavelengths of 355 and 455 nm respectively, with fluorospectrophotometer (FL600, Microplate Fluorescence Reader, Bio-Tek). Data are expressed as % release (histamine contents in supernatant / histamine contents in supernatant and pellet × 100).

Measurement of [^{3}H]arachidonic acid release

RBL-2H3 cells were suspended in 20 ml of Krebs buffer and incubated with [^{3}H]arachidonic acid (0.2 μCi/mL) for 2 hr at 37°C. The cells were washed twice with Krebs buffer containing 0.5 mg/mL bovine serum albumin to trap the liberated [^{3}H]arachidonic acid. The cells were suspended in Krebs buffer at the density of 10^6 cells/mL and then [^{3}H]arachidonic acid release was induced by melittin for 60 min at 37°C. The radioactivity of [^{3}H]arachidonic acid released by melittin in the medium was measured by scintillation counting (Jesus *et al.,* 1994). Data are expressed as % release (radioactivity (cpm) in supernatant / radioactivity (cpm) in supernatant and pellet × 100).

Measurement of PGE_2 in melittin-stimulated mast cells

The RBL-2H3 cells were isolated and stimulated as described above. Total amount of PGE_2 was assayed using enzyme immunoassay protocol provided by Amersham Pharmacia Biotech and expressed as ng/mg protein. Protein measurement was made to correct the differences between preparations using the bincinchoninic acid (BCA) method (Smith *et al.,* 1985).

PLA_2 assay

The reaction solution of 50 mM Tris-HCl buffer, pH 7.5, containing 100 mM NaCl, 1 mM EDTA, 2 μM 10-pyren PC, 0.1% bovine serum albumin and 6 mM $CaCl_2$ was prepared (Radvanyi *et al.,* 1989). The reaction was initiated by the addition of PLA_2 and incubated for 20 min at 37°C. The fluorescence intensity was monitored using excitation and emission wavelengths of 345 nm and 398 nm, respectively.

Data analysis

The results are represented as means ± S.D. and analyzed statistically by analysis of variance (ANOVA), and differences between groups were determined with Newman-Keuls test. The level of significance was set at less than 5%.

RESULTS AND DISCUSSION

It has been reported that methanol extracts of *Clerodendron trichotomum Thunberg* inhibited carageenan-induced rat paw edema and LPS-induced PGE_2 production in Raw 264.7 cells (Choi *et al.,* 2004). In this experiment, the effect of acteoside isolated from *Clerodendron trichotomum Thunberg* on histamine release and arachidonic acid release was measured in RBL 2H3 cells. Acteoside did not show any cytotoxic activity in RBL 2H3 cells up to a concentration of 100 μM.

Effect of acteoside on histamine release induced by melittin

PLA_2 activation, especially cytosolic PLA_2, plays a

central role in histamine release from human basophils or mouse mast cells *via* generation of lysophosphatidylcholine or arachidonic acid (Morita *et al.*, 1983; Nakatani *et al.*, 2000).

Melittin, an endogenous PLA_2 activator (Da Silva *et al.*, 1995), increased histamine release dose-dependently from RBL-2H3 cells in the presence or absence of extracellular Ca^{2+} (Fig. 1A). It has been reported that melittin caused histamine release in rat peritoneal mast cells (Battistella *et al.*, 1986). Histamine release induced by melittin showed more prominent in the presence of extracellular Ca^{2+} than that in the presence of extracellular Ca^{2+}. However, basal histamine release increased by 2 fold from 14.9 ± 2.9% in normal Krebs solution to 28.4 ± 1.5% in Ca^{2+} free-Krebs buffer. Acteoside dose-dependently inhibited 0.5 μM melittin-induced histamine release in the presence or absence of extracellular Ca^{2+} (Fig. 1B) and inhibitory effect of acteoside was more prominent in the presence of extracellular Ca^{2+}. Although anti-inflammatory effect of acteoside is widely reported, this data may be the first evidence about the effect of acteoside on histamine release.

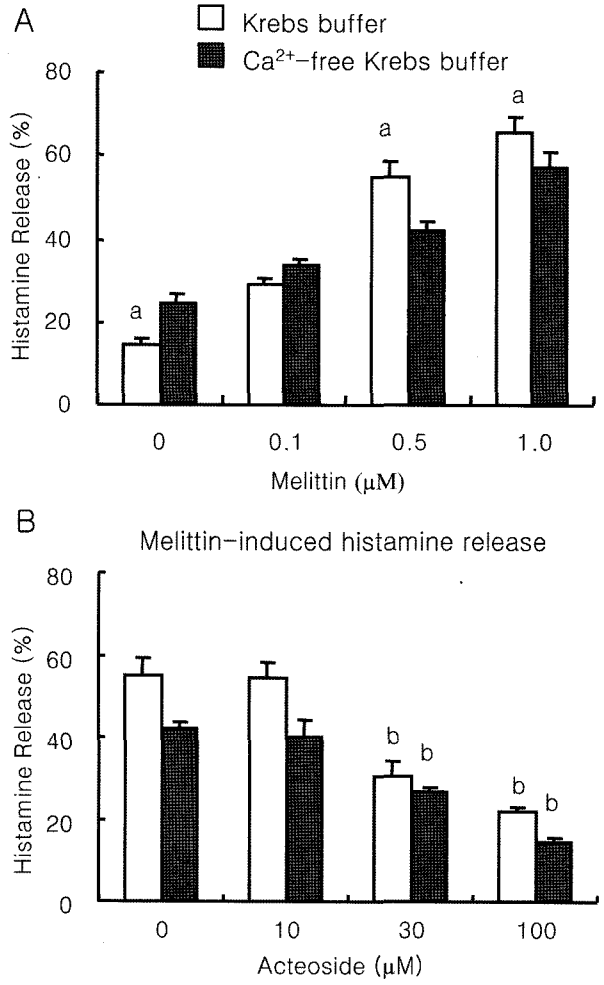

Fig. 1. Dose-response of histamine release to melittin (A) and inhibitory effect of acteoside on melittin-induced histamine release (B) in RBL-2H3 cells. Melittin stimulated dose-dependently histamine release in the presence or absence of extracellular Ca^{2+}. Acteoside significantly inhibited 0.5 μM melittin-induced histamine release. Results are mean ± SD of 6 separate experiments and expressed as % release (histamine contents in supernatant / histamine contents in supernatant and pellet × 100). a : Significantly different from histamine release in Ca^{2+}-free Krebs buffer at the same concentration ($P<0.05$). b : Significantly different from histamine release induced by melittin alone ($P<0.05$).

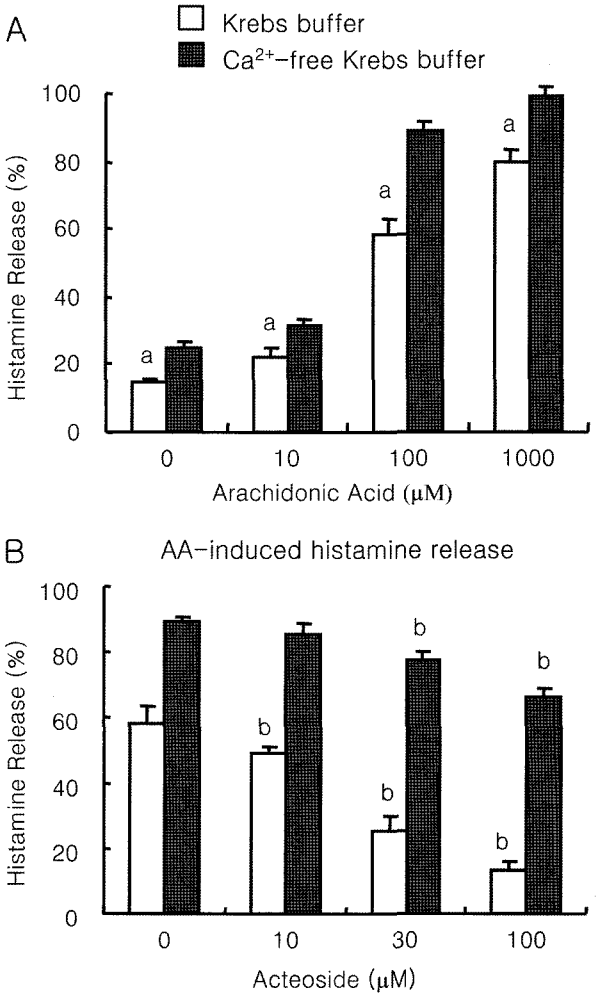

Fig. 2. Dose-response of histamine release to arachidonic acid (A) and inhibitory effect of acteoside on arachidonic acid-induced histamine release (B) in RBL-2H3 cells. Arachidonic acid (AA) stimulated dose-dependently histamine release in the presence or absence of extracellular Ca^{2+}. Acteoside significantly inhibited 100 μM arachidonic acid-induced histamine release. Results are mean ± SD of 6 separate experiments. a : Significantly different from histamine release in Ca^{2+}-free Krebs buffer at the same concentration ($P<0.05$). b : Significantly different from histamine release induced by arachidonic acid alone ($P<0.05$).

Effect of acteoside on histamine release induced by arachidonic acid

Arachidonic acid produced by PLA_2 activation dose-dependently increased histamine release from RBL-2H3 cells in the presence or absence of extracellular Ca^{2+} (Fig. 2A). It was previously reported that arachidonic acid stimulated histamine release significantly in C2 mast cells (Gueck *et al.*, 2004). Histamine release induced by arachidonic acid showed more prominent in the absence of extracellular Ca^{2+} than that in the presence of extracellular Ca^{2+}. These data suggest that arachidonic acid is involved in histamine release in RBL 2H3 cells. Acteoside inhibited dose-dependently 100 μM arachidonic acid-induced histamine release in the presence or absence of extracellular Ca^{2+} (Fig. 2B) and inhibitory effect of acteoside was more prominent in the presence of extracellular Ca^{2+}, which suggest that inhibitory action of acteoside may be related to the influx of extracellular Ca^{2+}. This suggestion is supported by the previous report that acteoside significantly suppressed bradykinin-induced increase in intracellular Ca^{2+} concentration in rat aortic endothelial cells (Lau *et al.*, 2004).

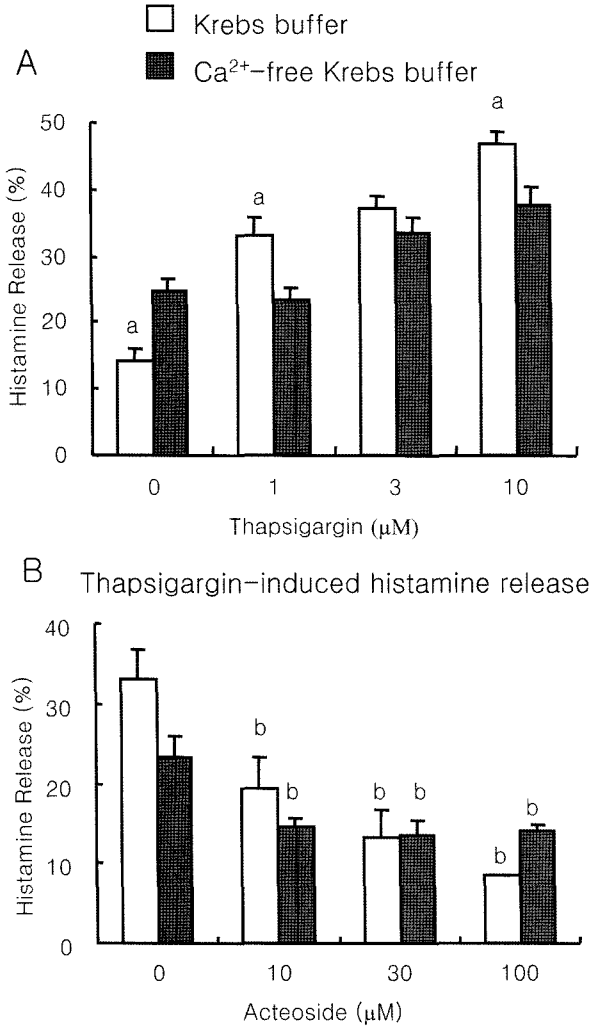

Fig. 3. Dose-response of histamine release to thapsigargin (A) and inhibitory effect of acteoside on thapsigargin-induced histamine release (B) in RBL-2H3 cells. Thapsigargin stimulated dose-dependently histamine release in the presence or absence of extracellular Ca^{2+}. Acteoside significantly inhibited 1 μM thapsigargin-induced histamine release. Results are mean ± SD of 6 separate experiments. a : Significantly different from histamine release in Ca^{2+}-free Krebs buffer at the same concentration ($P<0.05$). b : Significantly different from histamine release induced by thapsigargin alone ($P<0.05$).

Effect of acteoside on histamine release induced by thapsigargin

Thapsigargin, a Ca^{2+} pump inhibitor, increased intracellular Ca^{2+} level (Kitsukawa *et al.*, 1994). Thapsigargin increased dose-dependently histamine release from RBL-2H3 cells in the presence or absence of extracellular Ca^{2+} (Fig. 3A). Histamine release by thapsigargin was more prominent in the presence of extracellular Ca^{2+} because depletion of intracellular Ca^{2+} caused the influx of extracellular Ca^{2+}. Acteoside inhibited dose-dependently 1 μM thapsigargin-induced histamine release in the presence or absence of extracellular Ca^{2+} (Fig. 3B). Inhibitory effect of acteoside on histamine release induced by melittin, arachidonic acid or thapsigargin was more prominent in

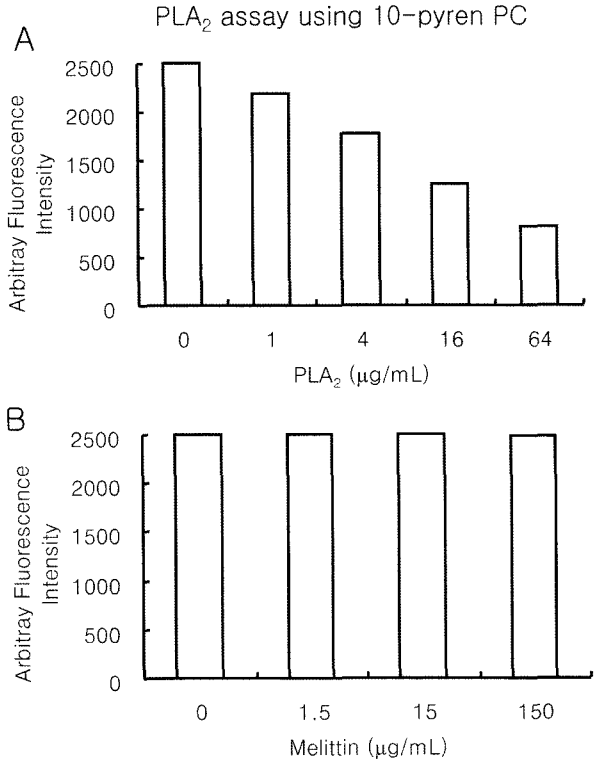

Fig. 4. Phospholipase A_2 (PLA_2) assay using 10-pyren PC. Results are mean of the representative experiment. The fluorescence intensity of 10-pyren PC hydrolyzed by phospholipase A_2 was decreased. The data show that melittin itself does not have phospholipase A_2 activity.

the presence of extracellular Ca^{2+}, suggesting that inhibitory action of acteoside is strongly related to extracellular Ca^{2+} influx.

Effect of acteoside on arachidonic acid release and PGE_2 production

To identify that melittin has PLA_2 activity, we measured PLA_2 assay using 10-pyren PC. Exogenous PLA_2 dose-dependently decreased fluorescence intensity of 10-pyren PC, whereas melittin did not affect (Fig. 4). This result suggests that melittin seems to activate an endogenous PLA_2. On the other hand, melittin increased dose-dependently arachidonic acid release in [^{3}H]arachidonic acid-labeled RBL 2H3 cells, but exogenous PLA_2 did not (Fig. 5). Exogenous PLA_2 (10 μg/mL) slightly increased histamine release from 14.9 ± 1.6% of basal secretion to 21.6 ± 1.0%. These data suggest that cytosolic PLA_2 rather than exogenous PLA_2 seems to be involved in histamine release and arachidonic acid release in RBL 2H3 cells. This suggestion is well in accord with the previous report that cytosolic PLA_2 plays a central role in histamine release from mouse mast cells (Nakatani *et al.*, 2000). Acteoside significantly inhibited arachidonic acid release and PGE_2 production stimulated by 0.5 mM melittin (Fig. 6). It has been reported that acteoside significantly inhibited LPS-induced PGE_2, NO and TNF-α production in mouse peritoneal macrophage (Diaz *et al.*, 2004). Considering on inhibitory effect of acteoside on histamine release and arachidonic acid release in RBL 2H3 cells, we suggest that acteoside has a potent anti-inflammatory activity.

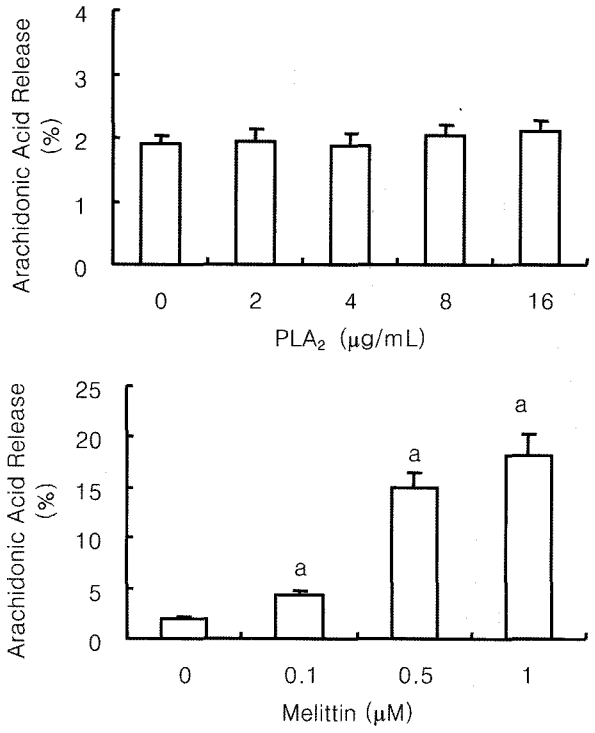

Fig. 5. Arachidonic acid release induced by exogenous phospholipase A_2 (PLA_2) and melittin, an endogenous phospholipase A_2 activator in [^{3}H]arachidonic acid-labeled RBL 2H3 cells. Results are mean ± SD of 3 separate experiments. a : Significantly different from control ($P<0.05$).

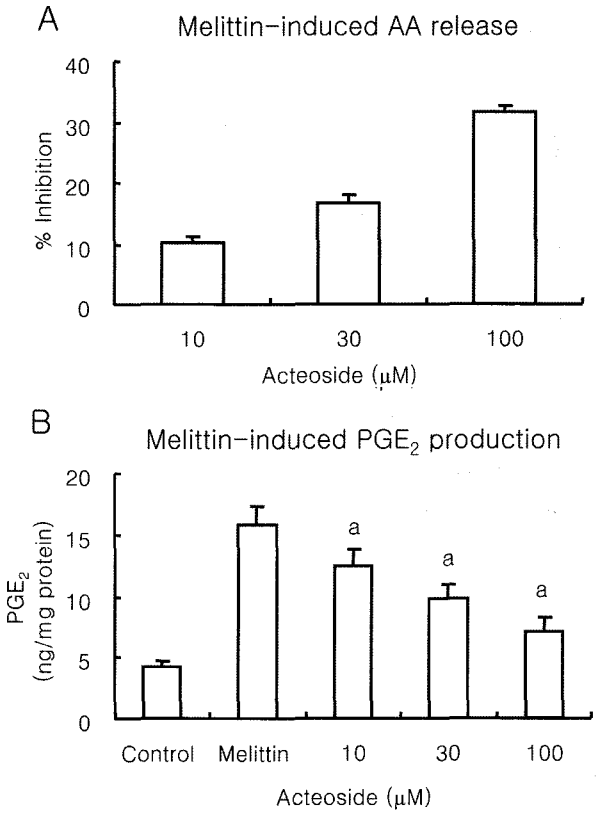

Fig. 6. Effect of acteoside on arachidonic acid (AA) release (A) and prostaglandin E_2 (PGE_2) production (B) in RBL 2H3 cells stimulated by 0.5 μM melittin. Acteoside inhibited both arachidonic acid release and prostaglandin E_2 production induced by melittin. Results are mean ± SD of 3 separate experiments. a : Significantly different from melittin ($P<0.05$).

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