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Effects of Extract and Ingredients Isolated from *Magnolia obovata* Thunberg on Catecholamine Secretion from Bovine Adrenal Chromaffin Cells

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ABSTRACT. The crude extract of magnolia bark, an herbal drug, inhibited the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine (ACh) in a concentration-dependent manner (200–900 μg/mL). The extract also diminished the secretion induced by high K^+, which is a stimulus directly depolarizing the plasma membranes, but its inhibition was weaker than that of ACh-evoked secretion. β-Eudesmol, honokiol, magnolol, and bornyl acetate, but not α- and β-pinenes, all of which are ingredients of magnolia bark, greatly reduced ACh-evoked secretion. β-Eudesmol and magnolol also inhibited high K^+-induced secretion to an extent similar to that of ACh-evoked secretion. However, honokiol and bornyl acetate inhibited the secretion induced by high K^+ much less than the secretion evoked by ACh. ACh-induced Na^+ influx and ACh- or high K^+-induced Ca^{2+} influx into the cells were diminished by β-eudesmol or honokiol. These results indicate that magnolia bark contains some effective components inhibiting the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by ACh due to the antagonism of Na^+ and Ca^{2+} influxes into the cells. However, inhibition by the extract of magnolia bark seems to be attributable to honokiol and bornyl acetate. Furthermore, the results indicate that the inhibitory effect of magnolia bark may be associated with its pharmacological effect on activities of the nervous system. BIOCHEM PHARMACOL **60**;3: 433–440, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. magnolia bark; catecholamine secretion; chromaffin cells; β-eudesmol; honokiol; bornyl acetate

The bark of *Magnolia obovata* Thunberg, a medicinal plant, has been well known as an important component of many Chinese traditional medicines for more than 2000 years. These Chinese medicines are used for the treatment not only of gastrointestinal disorders but also of anxiety, which led us to consider that magnolia bark may influence the central and/or autonomic nervous systems. The oldest Chinese traditional medical book, *Sheng-nong, Ben-cao Jing*, mentions that magnolia bark itself has a tranquilizing action. Actually, the extract of magnolia bark has been shown to have depressant actions on the central nervous system [1]. On the other hand, various ingredients have been isolated and identified from magnolia bark. They are β-eudesmol, α- and β-pinenes, and bornyl acetate, as essential oils; magnolol and honokiol, as diphenyl compounds; and magnocurarine and magnoflorine, as alkaloids. It also has been reported that some of these ingredients have pharmacological effects on nervous systems [2, 3]. However, until now, there has been no evidence showing the influence of magnolia bark and its components on autonomic nervous systems and clarifying the relationship between the pharmacological effects of magnolia bark and those of its ingredients.

Because the adrenal medulla is derived from neural crest tissue embryologically, it is widely used as a model of nervous systems, especially the sympathetic nervous system. The adrenal medulla can secrete catecholamines mainly via stimulation of the nicotinic ACh† receptors by a neurotransmitter, ACh, which is released from the terminals of the splanchnic nerve. Binding of ACh to the nicotinic receptors leads to depolarization of the cell membrane by an influx of Na^+ through receptor-operated cation channels, causing an influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels, and results in catecholamine secretion by exocytosis [4–6]. Therefore, adrenal chromaffin cells are widely used in studies of catecholamine secretion as a useful model of the sympathetic nervous system.

In this study, we investigated the effects of magnolia bark and its ingredients on the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by ACh. In addition, we also examined the ingredients responsible for the pharmacological effects of magnolia bark.

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† *Abbreviations*: ACh, acetylcholine; KRH, Krebs–Ringer–HEPES; $[Na^+]_i$, intracellular free sodium concentration; and SBFI, sodium-binding benzofuran isophthalate.

MATERIALS AND METHODS

Materials

The crude extract of magnolia bark was supplied by the Tsumura Co., Ltd. The extract was prepared by treatment of magnolia bark with hot water. β-Eudesmol, honokiol, magnolol, α- and β-pinenes, and bornyl acetate were purchased from Nacarai Tesque Inc. These ingredients and the extract were dissolved in dimethyl sulfoxide. The concentration of dimethyl sulfoxide in the incubation medium was 1%, which had no effect on the secretion of catecholamines from bovine adrenal chromaffin cells under the conditions employed in this study. Oxygenated KRH buffer (pH 7.4) was used as an incubation medium and was composed of 125 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM HEPES, 5.6 mM glucose, and 0.5% BSA. In 56 mM KCl–KRH buffer, the amount of NaCl was reduced to maintain the isotonicity of the medium. Tissue culture vessels were obtained from the Falcon Plastics Co. Eagle's minimum essential medium was from Nissui Seiyaku. SBFI tetraacetoxymethyl ester was from the Sigma Chemical Co. $^{45}CaCl_2$ (0.185 to 1.85 GBq/mg calcium) was from Amersham International, Ltd. All other chemicals were of the highest grade available from commercial sources.

Isolation and Primary Culture of Bovine Adrenal Chromaffin Cells

Bovine adrenal glands were provided by the Center of the Iwate Livestock Industry. Adrenal chromaffin cells were prepared by the method of collagenase digestion as described previously [7]. The isolated cells were suspended in Eagle's minimum essential medium containing 10% calf serum, 3.0 μM cytosine arabinoside, and antibiotics (100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.3 μg/mL of amphotericin B) and were maintained as a monolayer culture in 35-mm diameter dishes at a density of 2×10^6 cells. The cells were cultured at 37° in a CO_2 incubator (95% air/5% CO_2). A total of 2×10^6 cells contained 39.6 ± 1.6 μg catecholamines as epinephrine and norepinephrine.

Measurements of Catecholamine Secretion from the Chromaffin Cells and $^{45}Ca^{2+}$ Influx into the Cells

After 4 days in culture, the cells were washed twice with prewarmed KRH buffer and then preincubated with or without test agents in KRH buffer for 10 min at 37°. They were incubated with or without the test agents in the presence or absence of ACh or high K^+ (56 mM K^+) for 7 min. The reaction was terminated by transferring the incubation medium to tubes in an ice-cold bath. The catecholamines secreted into the medium were extracted with 0.4 M perchloric acid and adsorbed on aluminum hydroxide. Their amounts were estimated by the ethylenediamine condensation method [8], using a fluorescence spectrophotometer (650–10S; Hitachi) at an excitation wavelength of 420 nm and an emission wavelength of 540 nm. At these wavelengths, epinephrine and norepinephrine showed the same fluorescence intensity.

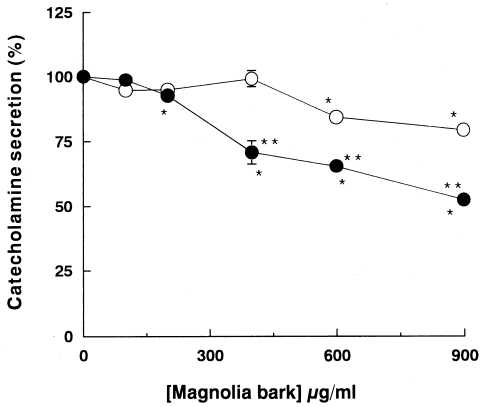

FIG. 1. Effect of magnolia bark extract on catecholamine secretion from bovine adrenal chromaffin cells. Cultured bovine adrenal chromaffin cells were washed twice with prewarmed KRH buffer and preincubated with different concentrations of magnolia bark extract (0–900 μg/mL) in KRH buffer for 10 min at 37°. Then the cells were incubated with different concentrations of the magnolia bark extract (0–900 μg/mL) mentioned above for 7 min in the presence or absence of 50 μM ACh (●) or high K^+ (○). Catecholamines secreted from the cells into the medium were determined as described in Materials and Methods. Basal values (basal secretions) were subtracted from the data, and the ACh- or the high K^+-induced response was assigned the value of 100%. The ACh-induced and the high K^+-induced secretions were 11.70 ± 0.42 and 8.12 ± 0.39 μg catecholamines/2×10^6 cells, respectively, and the basal secretion was 0.11 ± 0.03 μg catecholamines/2×10^6 cells. Values are means ± SD from four experiments. Key: (*) $P < 0.01$ compared with the corresponding control value; and (**) $P < 0.01$ compared with the corresponding high K^+-induced secretion.

After preincubation of the cells with or without test agents in KRH buffer for 10 min, the cells were incubated for 7 min with or without the test agents in the presence of $^{45}Ca^{2+}$ (37 kBq) in plain, ACh-containing, or high K^+-containing medium. The medium was removed, and the cells were cooled on ice immediately and washed three times with ice-cold Ca^{2+}-free KRH buffer. The cells were scraped and solubilized in 10% Triton X-100. Radioactivity was determined using a liquid scintillation counter (LSC-900) [7].

Measurement of $[Na^+]_i$

Loading of the chromaffin cells with SBFI was performed by a modification of the method of Harootunian *et al.* [9]. The isolated cells were cultured for 4 days on coverslips cut to fit into the spectrofluorometer cuvette. The cultured cells on the coverslip were incubated with 10 μM SBFI tetraacetoxymethyl ester and 0.02% Pluronic F-127 in KRH buffer for 3 hr at 37° and washed three times with KRH buffer. The coverslip was placed in the cuvette and preincubated with KRH buffer for 10 min at 37° in the fluorescence meter. The test agents then were added to the cuvette.

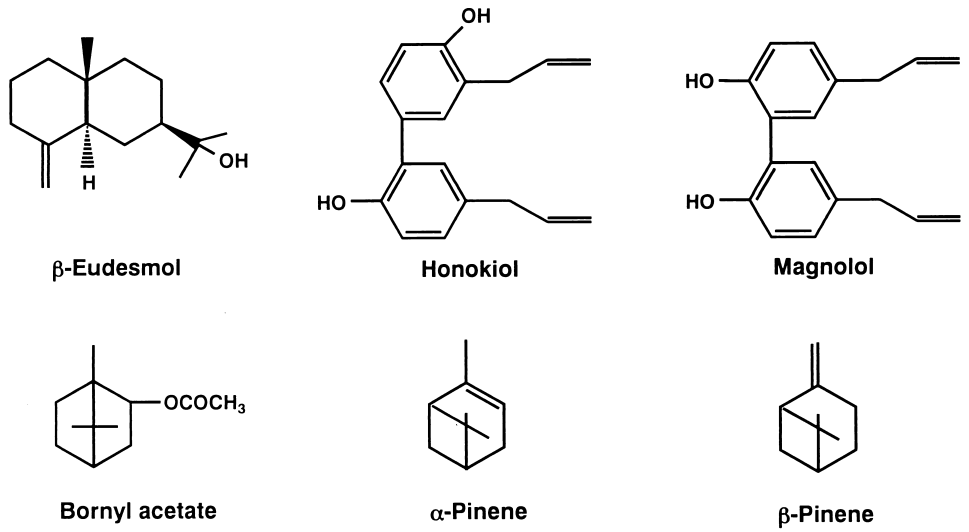

FIG. 2. Structures of β-eudesmol, honokiol, magnolol, bornyl acetate, α-pinene, and β-pinene.

Increases and decreases in the fluorescence induced from the SBFI–Na^+ complex were recorded simultaneously at excitation wavelengths of 340 and 380 nm, respectively, and at an emission wavelength of 500 nm. The change in $[Na^+]_i$ was expressed as the ratio of the fluorescence at excitation wavelengths of 340 and 380 nm.

Statistics

Statistical evaluation of data was performed by ANOVA. When a significant F value was found by ANOVA, Scheffe's test for multiple comparisons was performed to identify differences among the groups. $P < 0.05$ was considered to be indicative of significance.

RESULTS

Effect of Magnolia Bark Extract on Catecholamine Secretion from Bovine Adrenal Chromaffin Cells

We first examined the effects of the crude extract of magnolia bark (magnoliae cortex), which was obtained by treatment of *M. obovata* Thunberg with hot water, on the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by ACh. When the adrenal chromaffin cells were preincubated with the extract for 10 min and then incubated with ACh (50 μM), the ACh-evoked secretion was greatly diminished. The inhibition of ACh-evoked secretion by magnolia bark extract was dependent on its concentration (200–900 μg/mL) (Fig. 1). On the contrary, the extract at lower concentrations (100–400 μg/mL) did not alter the secretion induced by high K^+, which directly activates voltage-sensitive Ca^{2+} channels and results in Ca^{2+} influx into the cells through the channels and catecholamine secretion from the cells [10], but at higher concentrations (600–900 μg/mL), it slightly inhibited the secretion (Fig. 1). The spontaneous (basal) secretion of catecholamines from the non-stimulated cells was not affected by the extract (data not shown).

Effects of Several Ingredients from Magnolia Bark on ACh-Evoked Catecholamine Secretion from Chromaffin Cells

Magnolia bark contains many ingredients such as essential oils, diphenyl compounds, and alkaloids. Therefore, the effects of six kinds of ingredients, namely magnolol and honokiol (diphenyl compounds) and β-eudesmol, α- and β-pinenes, and bornyl acetate (essential oils) (Fig. 2), on ACh-evoked secretion were examined. Magnolol, honokiol, β-eudesmol, and bornyl acetate (30 μM) reduced the secretion significantly, whereas the α- and β-pinenes (30 μM) had only a slight effect (Fig. 3). β-Eudesmol was the most effective inhibitor, and the inhibitory potencies of the

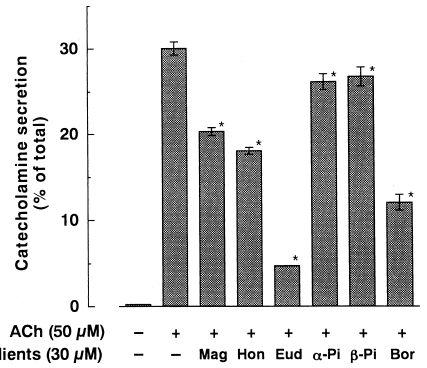

FIG. 3. Effects of various ingredients of magnolia bark on catecholamine secretion. The cultured cells were washed twice with prewarmed KRH buffer and preincubated with or without magnolol (Mag), honokiol (Hon), β-eudesmol (Eud), α-pinene (α-Pi), β-pinene (β-Pi), or bornyl acetate (Bor) (30 μM) in KRH buffer for 10 min. Then the cells were incubated with or without the compounds mentioned above in the presence or absence of ACh (50 μM) for 7 min. Catecholamines secreted from the cells into the medium were determined as described in Materials and Methods and were expressed as a percentage of total cellular catecholamines (39.6 ± 1.6 μg/2 × 10^6 cells). Values are means ± SD from four experiments. Key: (*) $P <$ 0.01 compared with the ACh-evoked secretion.

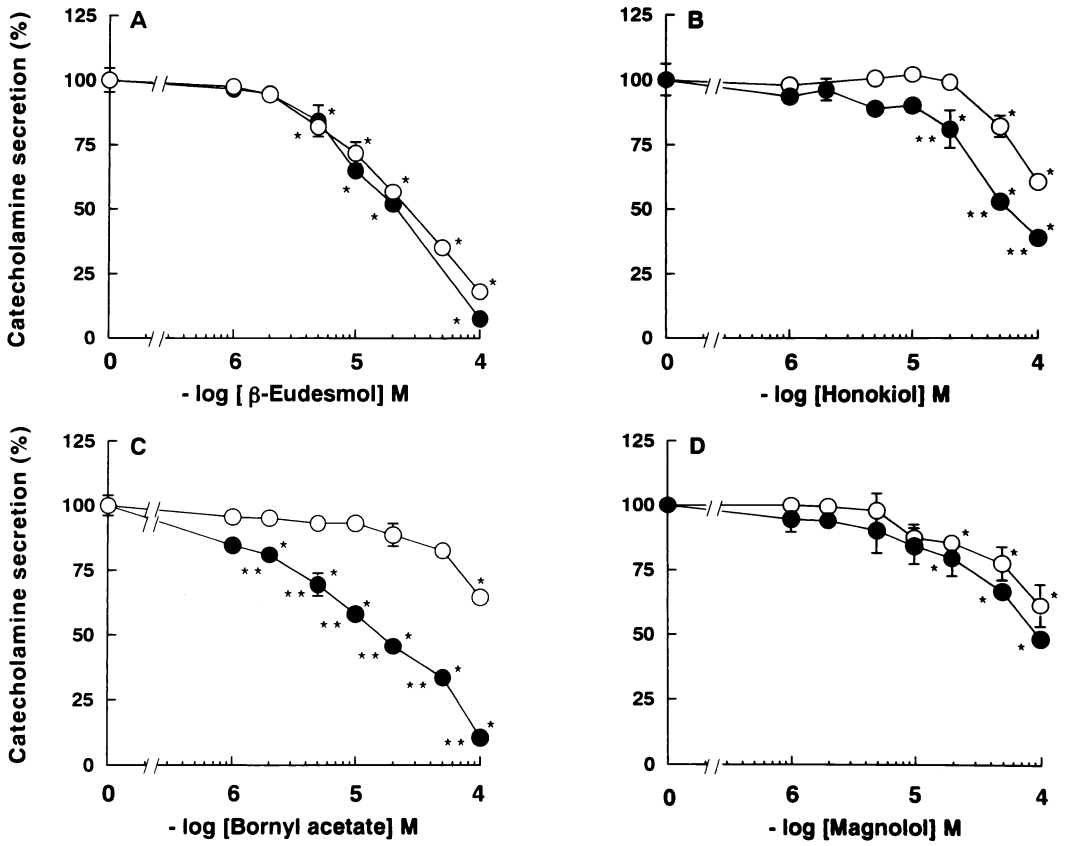

FIG. 4. Effects of β-eudesmol (A), honokiol (B), bornyl acetate (C), and magnolol (D) on ACh- or high K^+-induced secretion of catecholamines. The cultured cells were washed twice with prewarmed KRH buffer and preincubated with different concentrations of β-eudesmol, honokiol, bornyl acetate, or magnolol (0–100 μM) in KRH buffer for 10 min. Then the cells were incubated with different concentrations of β-eudesmol, honokiol, bornyl acetate, or magnolol (0–100 μM) mentioned above in the presence or absence of 50 μM ACh (●) or high K^+ (○). Catecholamines secreted from the cells into the medium were determined as described in Materials and Methods. Basal values (secretions) were subtracted from the data, and the ACh- or the high K^+-induced response was assigned the value of 100%. The ACh-induced and the high K^+-induced secretions were 12.03 ± 0.38 and 8.30 ± 0.25 μg catecholamines/2 × 10^6 cells (A), 11.95 ± 0.33 and 8.40 ± 0.33 μg catecholamines/2 × 10^6 cells (B), 12.50 ± 0.48 and 7.99 ± 0.31 μg catecholamines/2 × 10^6 cells (C), and 11.19 ± 0.53 and 8.05 ± 0.41 μg catecholamines/2 × 10^6 cells (D), respectively. The basal secretion was 0.10 ± 0.05 (A), 0.13 ± 0.02 (B), 0.12 ± 0.03 (C), and 0.14 ± 0.05 (D) μg catecholamines/2 × 10^6 cells. Values are means ± SD from four experiments. Key: (*) $P < 0.005$ compared with the corresponding control value; and (**) $P < 0.01$ compared with the corresponding high K^+-induced secretion.

ingredients (%) were as follows: β-eudesmol (85) > bornyl acetate (61) > honokiol (40) > magnolol (23).

Effects of β-Eudesmol and Honokiol on Catecholamine Secretion Induced by ACh and High K^+

Since the inhibition by β-eudesmol was the greatest among the ingredients of magnolia bark tested in this study, and since honokiol is a substance found in high proportions in the bark and was a relatively strong inhibitor, we investigated the inhibitory property of these compounds on the secretion of catecholamines. When the chromaffin cells were stimulated with ACh in the presence of various concentrations of these compounds, the secretion was diminished greatly by β-eudesmol or honokiol; inhibition by β-eudesmol and honokiol was detectable at 5 and 20 μM, and reached about 48% at 20 μM, and 47% at 50 μM, respectively (Fig. 4, A and B). Thus, inhibition by β-eudesmol and honokiol was concentration-dependent.

β-Eudesmol reduced high K^+-induced secretion in a concentration-dependent manner; the inhibition was detectable at 5 μM, and at 20 μM it was about 43% (Fig. 4A). Thus, the concentration–response curves for β-eudesmol inhibition of ACh-evoked and of high K^+-induced secretion were similar. On the other hand, honokiol at lower concentrations (1–20 μM) had no effect on secretion, but at higher concentrations (50–100 μM), it reduced secretion slightly (Fig. 4B).

Effects of β-Eudesmol and Honokiol on Na^+ and Ca^{2+} Influxes into Chromaffin Cells

Na^+ and Ca^{2+} influxes into chromaffin cells through the nicotinic ACh receptor-operated cation and voltage-sensi-

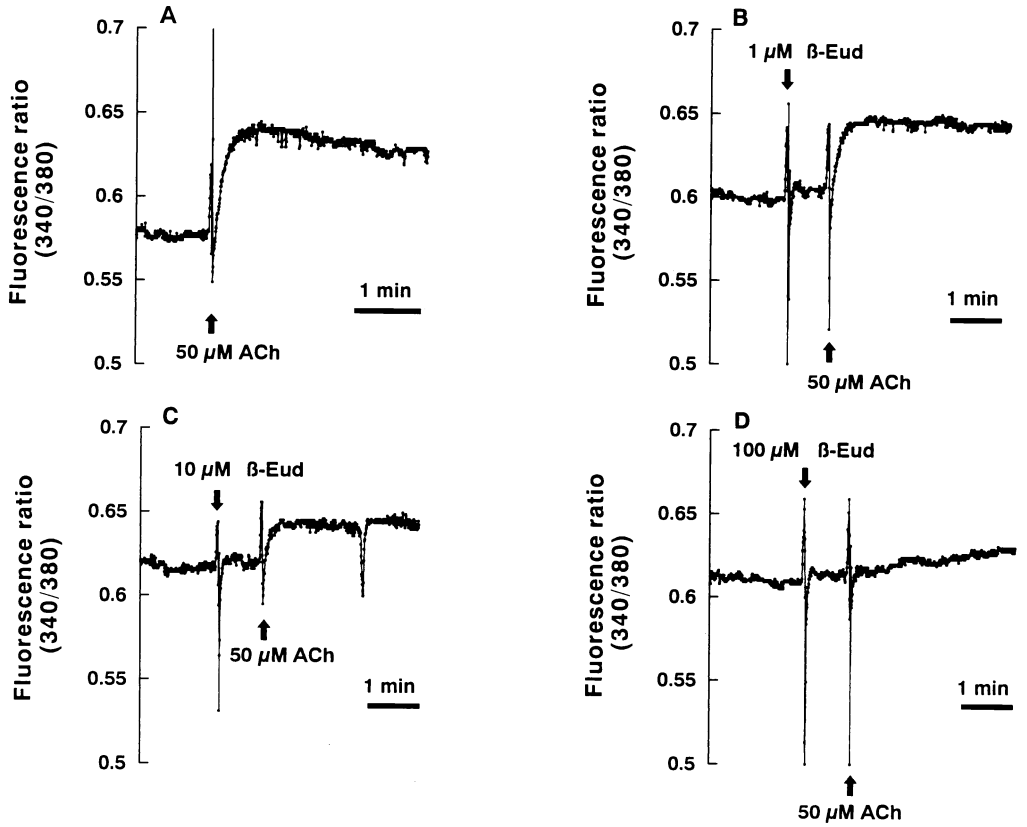

FIG. 5. Effect of β-eudesmol on ACh-induced increase in $[Na^+]_i$. The SBFI-loaded cells were preincubated with KRH buffer for 10 min at 37° in the fluorescence meter and then incubated with different concentrations of β-eudesmol (β-Eud) (0–100 μM) for 1 min. ACh (50 μM) was then added to the cuvette in the fluorescence meter. The fluorescence was recorded before and after the addition of the test agent. The change in $[Na^+]_i$ was expressed as the ratio of the fluorescence at an excitation wavelength of 340 nm to that of 380 nm. Data are from a representative sample of three experiments.

tive Ca^{2+} channels, respectively, are essential for the ACh-evoked secretion of catecholamines from the cells. As shown in Figs. 5A and 6A, the addition of 50 μM ACh to the SBFI-loaded cells led to an increase in the fluorescence ratio (340/380 nm) due to the external Na^+ influx into the cells [11]. β-Eudesmol and honokiol (1–100 and 20–100 μM) inhibited the ACh-induced Na^+ influx (Figs. 5, B–D, and 6, B–D). Furthermore, β-eudesmol (5–100 μM) diminished both ACh (at 50 μM)-induced and high K^+-induced $^{45}Ca^{2+}$ influxes (Fig. 7A). On the other hand, honokiol inhibited the $^{45}Ca^{2+}$ influx induced by ACh (20–100 μM) more strongly than that induced by high K^+ (100 μM) (Fig. 7B).

Effects of Bornyl Acetate and Magnolol on Catecholamine Secretion Induced by ACh or High K^+

The effects of other components of magnolia bark, bornyl acetate and magnolol, on ACh-evoked or high K^+-induced secretion of catecholamines were examined. Bornyl acetate strongly inhibited ACh-evoked secretion in a concentration-dependent manner (2–100 μM) (Fig. 4C), but it only slightly diminished high K^+-induced secretion (50–100 μM). On the other hand, magnolol was less potent than bornyl acetate against ACh-evoked secretion but was essentially equipotent against the responses to ACh and high K^+ (20–100 μM) (Fig. 4D).

DISCUSSION

The bark of *M. obovata* Thunberg is prescribed in many Chinese traditional medicines against anxiety. Actually, the extract of magnolia bark with ester has been reported to show depressant actions on the central nervous system, i.e. sedation, loss of righting reflex, and depression of drug-induced convulsions [1]. We demonstrated that the crude extract obtained by hot water treatment of magnolia bark, which contains both hydrophilic and hydrophobic substances, inhibited the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by ACh (Fig. 1). This suggests that magnolia bark contains some effective ingredients inhibiting the activity of the sympathetic nervous system in addition to suppressing that of the central nervous system. The inhibition is considered presumably to be a result of the influences on nicotinic ACh receptor-operated cation channels and voltage-sensitive Ca^{2+} channels. However, nicotinic receptors were more sensitive to the extract than were Ca^{2+} channels (Fig. 1).

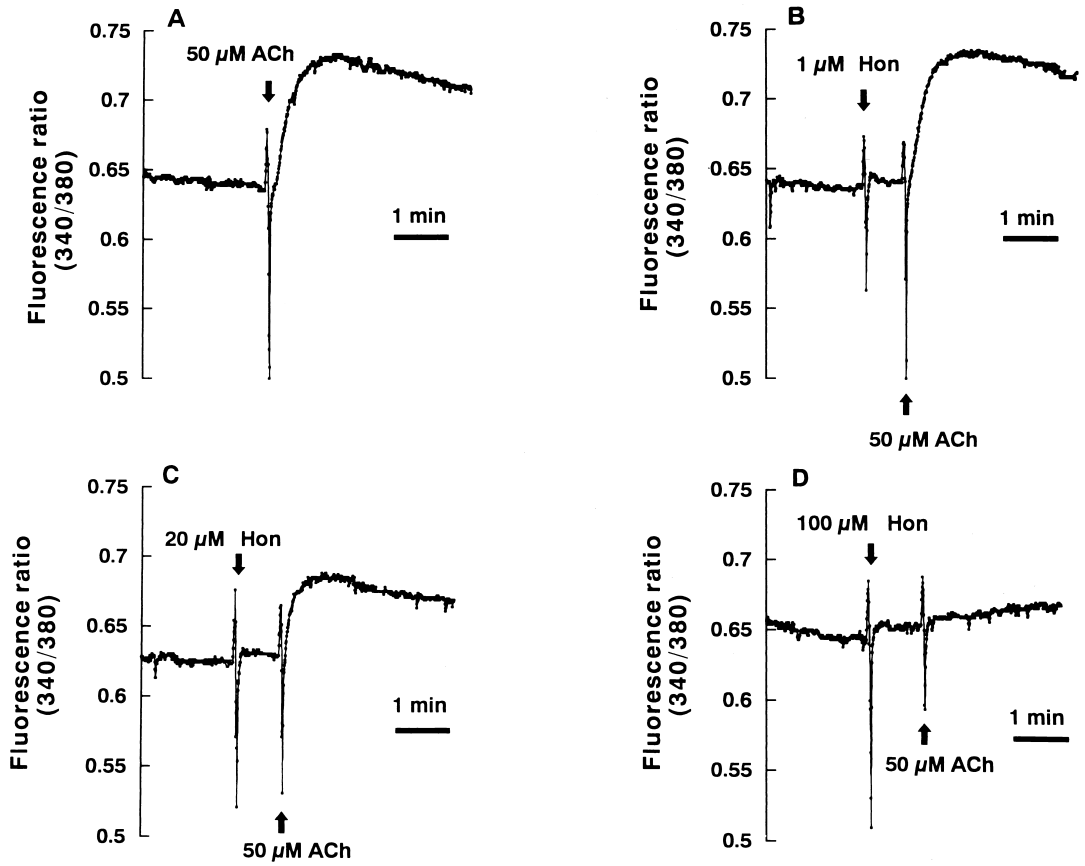

FIG. 6. Effect of honokiol on ACh-induced increase in $[Na^+]_i$. The SBFI-loaded cells were preincubated with KRH buffer for 10 min at 37° in the fluorescence meter and then incubated with different concentrations of honokiol (Hon) (0–100 μM) for 1 min. ACh (50 μM) was then added to the cuvette in the fluorescence meter. The fluorescence was recorded before and after the addition of the test agent. The change in $[Na^+]_i$ was expressed as a ratio of the fluorescence at an excitation wavelength of 340 to that of 380 nm. Data are from a representative sample of three experiments.

The major components in magnolia bark have been reported to be honokiol and magnolol as neolignane derivatives (diphenyl compounds), β-eudesmol as a sesquiterpenoid alcohol, caryophyllene as a sesquiterpenoid, and bornyl acetate as a monoterpenoid, although their content ratios are changed depending on where or when the magnolia bark is harvested. Since the contents of honokiol and magnolol in the dry magnolia bark were 0.52 ± 0.26 and 1.86 ± 0.66% (w/w), respectively, the crude extract from the bark used in this study should contain at least 1.6% honokiol and 5.6% magnolol (1 g extract corresponds to 10 g dry bark, and the recoveries of components into the extract are at least 30%). Therefore, the concentrations of honokiol (MW 266) and magnolol (MW 266) in 400 μg/mL of the extract are approximately 24 and 84 μM, respectively. On the other hand, although we do not have exact data for the contents of β-eudesmol and bornyl acetate in the dry bark, other bark that was harvested in the same area and season as ours has been reported to contain 0.25% β-eudesmol (MW 222) and 0.03% bornyl acetate (MW 196) [12], which are estimated as about 13 μM β-eudesmol and 2 μM bornyl acetate in 400 μg/mL of the extract. Thus, the ingredients at these concentrations can contribute to the inhibitory effect of the extract of magnolia bark on the ACh-evoked secretion of catecholamines (Figs. 1 and 4).

β-Eudesmol showed the greatest inhibition of the ACh-evoked secretion of catecholamines in the ingredients tested in this study (Fig. 3). It diminished not only ACh-induced Na^+ and Ca^{2+} influxes and secretion, but also the high K^+-induced Ca^{2+} influx and secretion at a similar potency (Figs. 4A, 5, and 7A). It has been demonstrated, using the cell-attached patch-clamp technique, that β-eudesmol blocks the nicotinic ACh receptor-operated cation channels in both the open and closed conformation at the neuromuscular junction [13]. Furthermore, in rat hippocampal slices, β-eudesmol has been shown to depress high K^+-induced electrographic seizure activity [3]. Thus, β-eudesmol regulates both the nicotinic ACh-operated cation and voltage-sensitive Ca^{2+} channels at the same potency. However, the inhibitory effect of magnolia bark extract on high K^+-induced secretion was less than that of the ACh-evoked secretion (Fig. 1). Moreover, β-eudesmol at 13 μM also inhibited high K^+-induced secretion significantly (Fig. 4A), but the extract at 400 μg/mL did not inhibit it (Fig. 1).

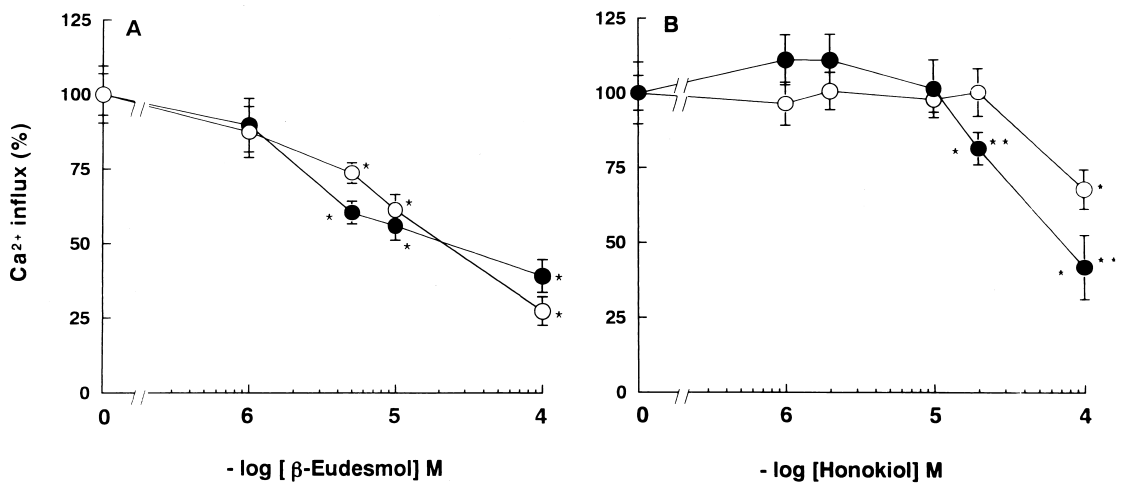

FIG. 7. Effects of β-eudesmol (A) and honokiol (B) on ACh- or high K^+-induced Ca^{2+} influx into the cells. The cultured cells were washed twice with prewarmed KRH buffer and preincubated with different concentrations of β-eudesmol or honokiol (0–100 μM) in KRH buffer for 10 min. The cells were incubated with the above-mentioned concentrations of β-eudesmol or honokiol (0–100 μM) in KRH buffer containing 37 KBq (1 μCi) $^{45}CaCl_2$ in the presence or absence of ACh (●) or high K^+ (○) for 7 min. The amount of Ca^{2+} influx into the cells was determined as described in Materials and Methods. Basal values (basal influxes) were subtracted from the data, and the ACh- or the high K^+-induced response was assigned the value of 100%. The ACh- and high K^+-induced Ca^{2+} influxes were 9.79 ± 0.80 and 10.68 ± 0.64 (A) and 9.87 ± 0.53 and 10.01 ± 0.59 (B) nmol/2 × 10^6 cells, respectively, and the basal influx was 1.42 ± 0.31 (A) and 1.59 ± 0.48 (B) nmol/2 x 10^6 cells. Values are means ± SD from four experiments. Key: (*) $P < 0.01$ compared with the corresponding control value; and (**) $P < 0.01$ compared with the corresponding high K^+-induced secretion.

Honokiol inhibited ACh-evoked Ca^{2+} influx and catecholamine secretion more strongly than high K^+-induced Ca^{2+} influx and secretion (Figs. 4B and 7B). Liu *et al.* [14] have also shown similar results in bovine adrenal chromaffin cells. On the other hand, magnolol similarly diminished both ACh-evoked and high K^+-induced secretion (Fig. 4D). Honokiol and magnolol are isomers. Therefore, it is very interesting that the effects of these diphenyl compounds on catecholamine secretion are distinct. Magnolol and honokiol are known to have many pharmacological effects. They show evident central depressant effects [2] in addition to antimicrobial and antifungal activities [15, 16]. A very recent report revealed that magnolol and honokiol are anxiolytic agents [17]. Furthermore, these ingredients have been suggested to antagonize voltage-sensitive Ca^{2+} channels [18, 19]. This antagonism may be associated with the central depressant actions of the diphenyl compounds. However, in this study, honokiol (but not magnolol) was a more potent inhibitor of ACh-evoked secretion than of high K^+-induced secretion.

Another terpenoid, bornyl acetate, greatly inhibited ACh-evoked secretion, but it only slightly suppressed high K^+-induced secretion (Fig. 4C). This is the first report showing the effect of bornyl acetate on nervous systems. Accordingly, honokiol and bornyl acetate are probably at least active ingredients responsible for the inhibition of ACh-evoked catecholamine secretion by the extract of magnolia bark. However, as mentioned above, amounts of β-eudesmol (above 13 μM) and magnolol (above 84 μM) sufficient to inhibit secretion are contained in the magnolia bark extract used in this study (above 400 μg/mL). Therefore, to evaluate the significant contribution of β-eudesmol and magnolol to the inhibitory effect, we will need to conduct a further study comparing the differences of affinity for the nicotinic receptor and the Ca^{2+} channels among the components in magnolia bark. Furthermore, since many very slight amounts of other compounds that were not used in this study exist in magnolia, the possibility that those components may strongly inhibit ACh-evoked secretion cannot be denied completely. We have to test their influences on the secretion.

Taken together, magnolia bark contains various components that inhibited the secretion of catecholamines from bovine adrenal chromaffin cells by affecting the nicotinic ACh receptor-operated cation channels and voltage-sensitive Ca^{2+} channels. Therefore, the bark seems to suppress not only the function of the central nervous system but also that of the peripheral nervous systems. Thus, magnolia bark is a treasury of active ingredients suppressing nervous activity.

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