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Inhibition of acetylcholine-mediated effects by borneol

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Abstract

We previously reported that the aqueous extract from a medicinal plant *Dryobalanops aromatica* specifically inhibits the nicotinic acetylcholine receptor (nAChR) (Oh *et al.* Pharmacol Res 2000;42(6):559–64). Here, the effect of borneol, the main constituent of *D. aromatica*, on nAChR activity was investigated in bovine adrenal chromaffin cells. Borneol inhibited a nAChR agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP)-induced calcium increase with a half maximal inhibitory concentration (IC_{50}) of 56 ± 9 μM. In contrast, borneol did not affect the calcium increases induced by high K^+, veratridine, and bradykinin. The sodium increase induced by DMPP was also inhibited by borneol with similar potency (49 ± 12 μM), suggesting that the activity of nAChRs is inhibited by borneol. Borneol inhibited DMPP-induced secretion of [^{3}H]norepinephrine with an IC_{50} of 70 ± 12 μM. Carbon-fiber amperometry also confirmed the inhibition of DMPP-induced exocytosis by borneol in single chromaffin cells. [^{3}H]nicotine binding, however, was not affected by borneol. The inhibitory effect by borneol is more potent than the effect by lidocaine, a commonly used local anesthetic. The data suggest that borneol specifically inhibits the nAChR-mediated effects in a noncompetitive way.

Keywords: Borneol; Nicotinic acetylcholine receptor; Lidocaine; Bovine adrenal chromaffin cells

1. Introduction

Catecholamines such as dopamine, norepinephrine, and epinephrine are formed in brain, chromaffin cells, sympathetic nerves, and sympathetic ganglia and play an important role in stress and emotional behavior (for review, see [1]). Many kinds of psychotropic drugs are known to act on catecholamine-containing neurons [2]. Therefore, the compounds that modulate catecholamine secretion can be used as potential therapeutic drugs for the treatment of affective disorders. Bovine adrenal chromaffin cells are neuroendocrine cells and have been widely used as a model system for the study of catecholamine secretion [3,4]. We have screened the effects of several extracts from medicinal plants on catecholamine secretion and found that the water extract from *Dryobalanops aromatica* specifically inhibits nicotinic stimulation-induced catecholamine [5]. Since borneol (Fig. 1), a monoterpenoid alcohol whose biological function has not been studied well is the main component of the medicinal plant, we investigated the effect of borneol on the activity of nAChRs. We found that borneol specifically inhibits nAChR-mediated effects in a noncompetitive way, thereby leading to the inhibition of nAChR-mediated calcium increase and catecholamine secretion. In addition, inhibitory effects of borneol and lidocaine were compared. This is the first study that concretely investigated the action of borneol in the cellular signal transduction system.

2. Materials and methods

2.1. Materials

Borneol, DMPP, bradykinin, and ionomycin were purchased from Sigma Chemical Co. Fura-2/AM, SBFI/AM,

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Abbreviations: DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; IC_{50}, half maximal inhibitory concentration; PLC, phospholipase C; [^{3}H]NE, [^{3}H]norepinephrine; nAChR, nicotinic acetylcholine receptor.

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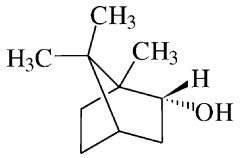

Fig. 1. Structure of borneol.

and Pluronic F-127 were purchased from Molecular Probes, Inc. [^{3}H]NE and [^{3}H]nicotine were purchased from NEN Life Science Products.

2.2. Preparation of chromaffin cells

Chromaffin cells were isolated from bovine adrenal medulla by two-step collagenase digestion as previously described [3]. For the measurement of [^{3}H]NE secretion and the [^{3}H]nicotine binding assay, cells were plated in 24-well plates at a density of 5×10^5 cells/well. Chromaffin cells transferred to 100 mm culture dishes (1×10^7 cells per dish) were used to measure cytosolic-free calcium and sodium concentrations. The cells were maintained in Dulbecco's modified Eagle medium/F-12 (Life Technologies, Inc.) containing 10% bovine calf serum (Hyclone) and 1% antibiotics (Life Technologies, Inc.). Chromaffin cells were incubated in a humidified atmosphere of 5% CO_2/95% air at 37° for 3–7 days before use.

2.3. $[Ca^{2+}]_i$ measurement

Cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined with the help of the fluorescent Ca^{2+} indicator fura-2 as reported previously [6]. Briefly, the chromaffin cell suspension was incubated with fresh serum-free DMEM/F-12 medium containing fura-2/AM (3 μM) for 40 min at 37° with continuous stirring. The cells were then washed with Locke's solution and left at room temperature until use. Sulfinpyrazone (250 μM) was added to all solutions to prevent dye leakage. Fluorescence ratios were measured by an alternative wavelength time scanning method (dual excitation at 340 and 380 nm; emission at 500 nm).

2.4. $[Na^+]_i$ measurement

Cytosolic-free Na^+ concentration ($[Na^+]_i$) was measured using the fluorescent Na^+ indicator SBFI as previously described in the report by Park *et al.* [7]. In brief, the chromaffin cell suspension was incubated in fresh DMEM/F-12 medium containing 15 μM SBFI/AM, 10% bovine calf serum, and 0.2% Pluronic F-127 for 2 hr at 37° with continuous stirring. The cells were then washed twice with fresh DMEM/F-12 medium and left at room temperature until use. Sulfinpyrazone (250 μM) was added to all solutions to prevent dye leakage. Fluorescence ratios were taken with alternate excitation at 340 and 380 nm and emission at 530 nm. Changes in $[Na^+]_i$ are presented as fluorescence ratios.

2.5. Measurement of [^{3}H]NE secretion

Catecholamine secretion from chromaffin cells was measured in 24-well plates following the method reported by Park *et al.* [4]. In brief, cells were loaded with [^{3}H]NE (1 μCi/mL; 68 pmol/mL) by incubation in DMEM/F-12 containing 0.01% ascorbic acid for 1 hr at 37° in 5% CO_2/95% air. The cells were washed with Ca^{2+}-free Locke's solution and were incubated with fresh Locke's solution for 10 min to measure basal secretion. The cells were subsequently stimulated with the drugs under test for 10 min. After the incubation, the medium was removed from each well and transferred to a scintillation vial. Finally, residual catecholamine in the cells was extracted by addition of 10% trichloroacetic acid, and the extract was transferred to a scintillation vial. The radioactivity in each vial was measured with a scintillation counter. The amount of [^{3}H]NE secreted was calculated as percentage of total [^{3}H]NE content. Net stimulated secretion was obtained by subtracting the basal secretion from the stimulated secretion.

2.6. Amperometric measurement

Recordings were performed at room temperature as described by Kim *et al.* [8]. Chromaffin cells were buffered with amine-free solution containing (in mM): 137.5 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 10 HEPES, pH 7.3 titrated by NaOH. Carbon-fiber electrodes were fabricated from 5 to 11 μm carbon fibers (PAN T650 or P25; Amoco Performance Products) and polypropylene 10 μL micropipettor tips. A carbon-fiber electrode, backfilled with 3 M KCl to connect to the headstage, was attached to a single cell. Measurements were begun after this electrode current fell below 10 pA. The amperometric current, generated by oxidation of catecholamines, was measured using an Axopatch 200 B amplifier (Axon Instruments, Inc.) and operated in the voltage-clamp mode at a holding potential of +650 mV. Amperometric signals were low-pass filtered at 1 kHz, then sampled 0.5 kHz. For data acquisition and analysis, pClamp 8 software (Axon Instruments, Inc.) was used as well as IGOR software (WaveMetrics) especially for visualizing large amounts of numeric data. Solutions were exchanged by a local perfusion system that allows complete exchange of medium bathing the cells within 2 sec.

2.7. [^{3}H]Nicotine binding analysis

Binding of [^{3}H]nicotine to intact cells was measured as previously described by Kilpatrik *et al.* [9]. Intact chromaffin cells in 24-well plates (5×10^5 cells/well) were washed twice with Locke's solution and incubated with 20 nM [^{3}H]nicotine and indicated concentrations of borneol for 40 min at 25°. Then the cells were washed three times with 1 mL ice-cold Ca^{2+}-free Locke's solution

containing 100 μM EGTA. Finally, the cells were lysed and scraped in 0.5 mL of 5% trichloroacetic acid and the radioactivity was measured by liquid scintillation counting. Nonspecific binding, determined by coincubation with 1 mM nicotine, amounted to less than 20% of total binding, and was routinely subtracted from the total binding. The binding data were analyzed and expressed as percentage of total binding.

2.8. Statistical analysis

All quantitative data were expressed as mean ± SEM. IC_{50} values were calculated with the Microcal Origin for Windows program.

3. Results

3.1. Inhibitory effects of borneol on nAChR-mediated calcium increase

Effect of borneol on DMPP-induced calcium increase was first investigated. Borneol (up to 300 μM) by itself had no effect on $[Ca^{2+}]_i$ (data not shown), whereas DMPP-induced calcium increase (trace 'a' in Fig. 2A) was inhibited by borneol (trace 'b' in Fig. 2A) in a concentration-dependent manner with an IC_{50} of 56 ± 9 μM (Fig. 2B). Since the initial calcium rises by DMPP treatment are similar in the absence or presence of borneol, it is likely that borneol may not affect calcium entry kinetics but number of active calcium channels. Incubation of cells with 300 μM borneol caused complete inhibition of the DMPP-induced calcium increase. In order to assess the mechanism of borneol action we investigated the effects of different concentrations of DMPP in the presence of 70 μM borneol. DMPP evoked greater calcium increases as the concentrations of DMPP increased, reaching a maximum at 30 μM (open boxes in Fig. 2C). Borneol inhibited DMPP-induced calcium increase with similar potency at all tested DMPP concentrations, making the concentration response to the nicotinic agonist shift downward (closed boxes in Fig. 2C). The result suggests that borneol does not act in a competitive manner. The time course of the borneol effect on the DMPP-induced calcium increase was also examined to obtain a clue for the action mechanism of borneol. When chromaffin cells were simultaneously treated with DMPP and 100 μM borneol, the initial peak height was decreased by 74%, compared to the DMPP treatment alone. With various incubation times of borneol, the inhibition was similar (Fig. 2D), suggesting that the effect of borneol is almost instantaneous. Therefore, it seems that borneol directly acts on nAChRs in the plasma membrane rather than via generation of second messengers.

To prove the presence of nAChRs in chromaffin cells and its activation by DMPP, we used specific antagonist and agonist of nAChR. Treatment of bovine adrenal chromaffin cells with 20 μM D-tubocurarine, which is a specific blocker of nAChRs [9,10], completely inhibited the DMPP-induced calcium increase (data not shown). In addition, borneol inhibited the calcium increase induced by nicotine that is a specific agonist of nAChR by approximately 65% (365 ± 7 nM by 100 μM nicotine in the presence of 70 μM borneol, compared with 1037 ± 17 nM by 100 μM nicotine alone). These results suggest that DMPP specifically activates nAChRs and that borneol inhibits the nAChR-mediated calcium increase.

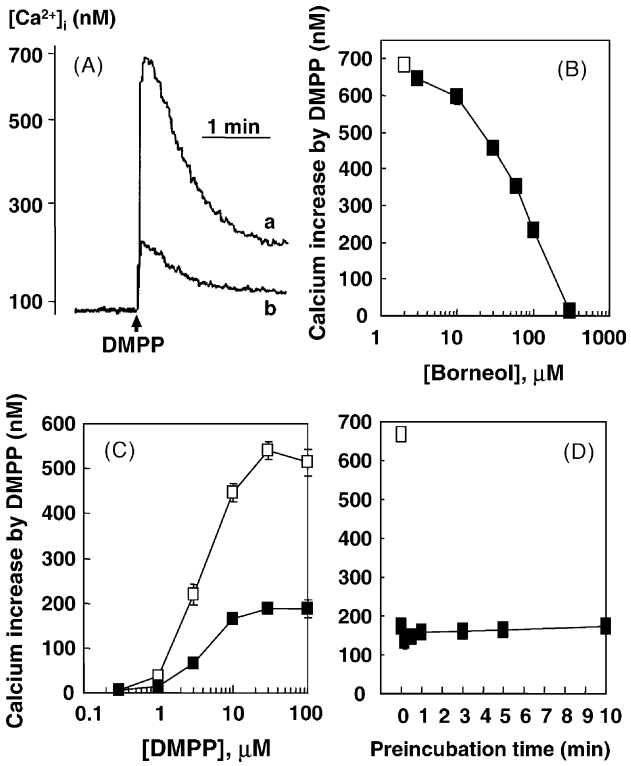

Fig. 2. Inhibitory effect of borneol on $[Ca^{2+}]_i$ elevation in chromaffin cells. (A) The intracellular $[Ca^{2+}]_i$ rise induced by 10 μM DMPP was measured in the absence (trace a) or presence (trace b) of 100 μM borneol. Cells were incubated with borneol for 3 min before stimulation with DMPP. The experiments were performed three times independently, and the typical Ca^{2+} traces are presented. (B) The calcium increase induced by 10 μM DMPP was measured 3 min after preincubation with the indicated concentrations of borneol (closed box). The peak height of each stimulation was compared to that of the control calcium increase caused by DMPP alone (open box). Data are the means ± SEM (bars) values of triplicate measurements. (C) The calcium increase induced by the indicated concentrations of DMPP was measured in the absence (open box) or presence (closed box) of 70 μM borneol. Data are the means ± SEM (bars) values of triplicate measurements. (D) Chromaffin cells were preincubated for the indicated times with 100 μM borneol and then stimulated with 10 μM DMPP (closed box). Incubation with zero time means that borneol and DMPP were treated simultaneously. The peak height of each stimulation was compared to that of the control calcium increase caused by DMPP alone (open box). Data are the means ± SEM (bars) values of triplicate measurements.

3.2. Specificity of borneol action

Specificity of borneol-induced inhibitory effects was examined by testing the effects of borneol on calcium

Table 1
Lack of borneol effect on $[Ca^{2+}]_i$ rises induced by other reagents than DMPP

Agonist	Calcium increase (nM)	
	−Borneol	+Borneol
50 mM K^+	530 ± 7	535 ± 8
Veratridine	88 ± 2	84 ± 4
Bradykinin	277 ± 8	266 ± 10

Chromaffin cells were incubated with borneol (100 μM) for 3 min. Then the cells were stimulated with 50 mM K^+, 100 μM veratridine, or 5 μM bradykinin. Net increase in $[Ca^{2+}]_i$ was obtained by subtracting the basal level of $[Ca^{2+}]_i$ from the peak height after stimulation. Data are the means ± SEM (bars) values of triplicate measurements.

channels, sodium channels, and PLC-linked receptor signaling. As shown in Table 1, calcium increase induced by 50 mM K^+ was not inhibited by pretreatment with 100 μM borneol, suggesting that calcium channels are not affected by borneol. Veratridine is a plant alkaloid that opens voltage-sensitive sodium channels by binding to the pharmacological site 2 on the sodium channels and slowing its inactivation [11]. In bovine adrenal chromaffin cells, veratridine-induced activation of sodium channels is known to cause membrane depolarization [12,13] thereby leading to slow and weak calcium increase through voltage-sensitive calcium channels [14]. The calcium increase by veratridine was not affected by borneol, either. Bradykinin is known to activate PLC-linked B2 bradykinin receptors in bovine adrenal chromaffin cells [15,16]. Borneol at 100 μM did not cause any effect on the bradykinin-evoked calcium increase. The data together suggest that borneol has no significant inhibitory effect on calcium channels, sodium channels, and PLC-linked receptors. Therefore, it seems that the effect of borneol on nAChRs is highly specific.

3.3. Inhibitory effect of borneol on the sodium influx through the nAChR

Since both calcium channels and nAChRs are activated by nicotinic stimulation [4], inhibition of DMPP-induced $[Ca^{2+}]_i$ rise can be resulted from the inhibition of nAChRs or calcium channels. In order to directly verify whether nAChRs are inhibited by borneol, we tested the effect of borneol on DMPP-induced sodium increase that occurs only through nAChRs. As shown in Fig. 3A, DMPP induced an increase in cytosolic sodium. Borneol inhibited the DMPP-induced sodium increase in a concentration-dependent manner with an IC_{50} of 49 ± 12 μM (Fig. 3B), and 300 μM borneol completely inhibited the DMPP effect (trace 'b' in Fig. 3A). The results suggest that the inhibition of DMPP-induced calcium and sodium increases by borneol resulted from the direct inhibition of nAChRs. In addition, since the initial sodium rises by DMPP treatment in the absence or presence of borneol look similar, it is likely that borneol may not affect sodium entry kinetics but number of nAChRs.

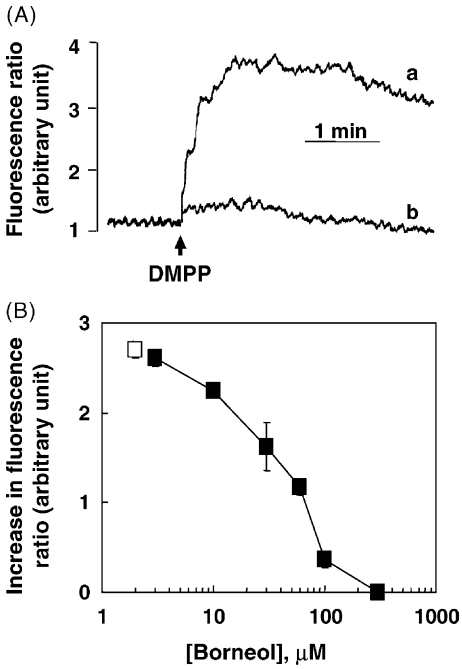

Fig. 3. Inhibitory effect of borneol on sodium increase in chromaffin cells. (A) The intracellular sodium increase induced by 10 μM DMPP was measured in the absence (trace a) or presence (trace b) of 100 μM borneol. The experiments were performed three times independently, and the results were reproducible. Typical Na^+ traces are presented. (B) The sodium increase induced by 10 μM DMPP was measured 3 min after preincubation with the indicated concentrations of borneol (closed box). The peak height of each stimulation was compared to that of the control sodium increase caused by DMPP alone (open box). Data are the means ± SEM (bars) values of triplicate measurements.

3.4. Inhibitory effect of borneol on nAChR-mediated exocytosis

Since calcium increase is an essential step in catecholamine secretion, the inhibition of calcium increase by

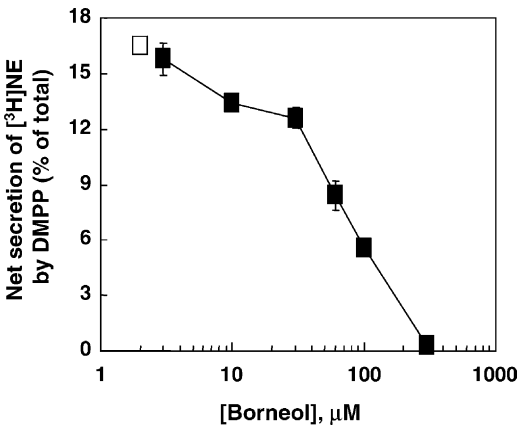

Fig. 4. Inhibitory effect of borneol on [^{3}H]NE secretion in chromaffin cells. [^{3}H]NE-loaded chromaffin cells were treated with 10 μM DMPP in the presence of the indicated concentrations of borneol (closed box). Secretion of [^{3}H]NE induced by DMPP in the absence of borneol is presented (open box). The secreted [^{3}H]NE was measured as described Section 2 and is expressed as percentage of total [^{3}H]NE. Data are the means ± SEM (bars) values of triplicate measurements.

borneol is likely to lead to decreased catecholamine secretion. In order to study the effects of borneol on catecholamine secretion, we treated [^{3}H]NE-loaded chromaffin cells with borneol. Borneol (up to 300 μM) by itself did not induce [^{3}H]NE secretion (data not shown). Stimulation of chromaffin cells with 10 μM DMPP raised [^{3}H]NE secretion by 16.5 ± 0.4% of the total endogenous content (open box in Fig. 4). Borneol decreased the DMPP-induced [^{3}H]NE secretion in a concentration-dependent manner with an IC_{50} of 70 ± 12 μM (closed boxes in Fig. 4). Borneol at 300 μM completely blocked the DMPP-induced [^{3}H]NE secretion.

To better understand how borneol inhibits secretory response evoked by nicotinic stimulation, exocytosis from single bovine adrenal chromaffin cells was measured using the amperometric method. When a brief pulse (20 sec) of DMPP was applied to a single chromaffin cell, a fast and transient increase in current occurred (Fig. 5A). When cells were subjected to repetitive stimulation with DMPP up to four times for 20 sec with an interval of 2 min, similar amounts of exocytosis were observed at each stimulation without significant rundown (data not shown). Presence of 60 μM borneol 100 sec before and during the DMPP pulse for 20 sec reduced the catecholamine secretion by

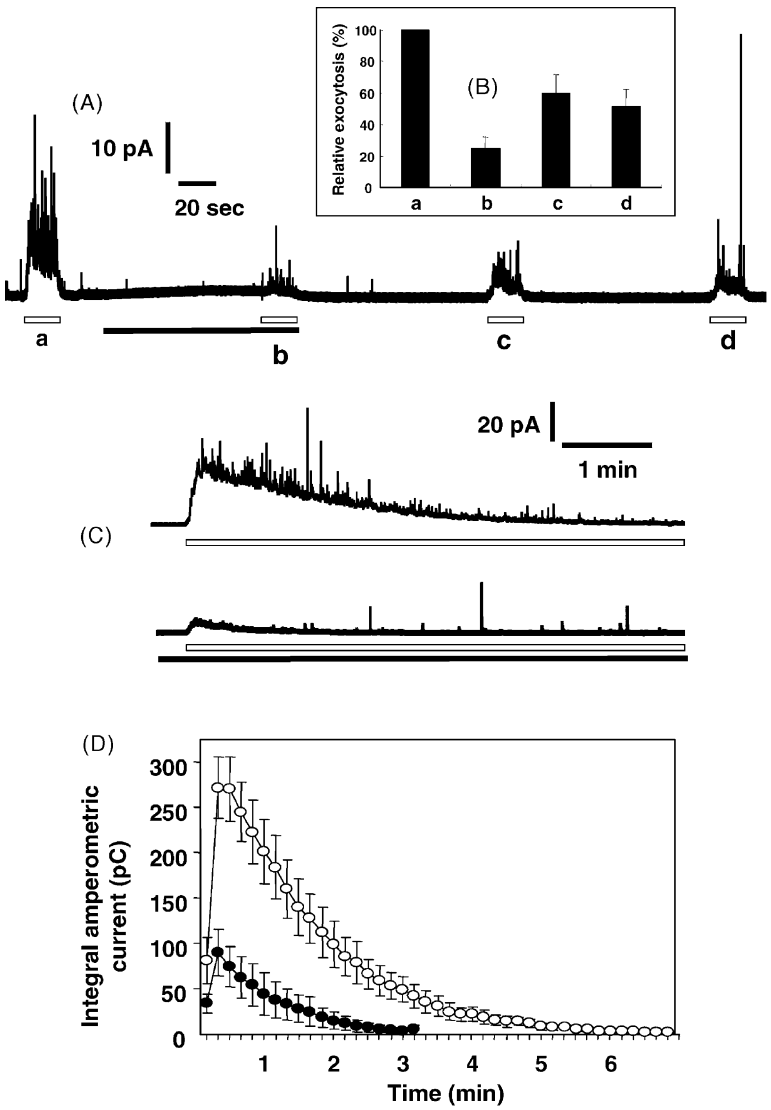

Fig. 5. Inhibitory effect of borneol on catecholamine secretion in single chromaffin cells. (A) Chromaffin cells were repetitively stimulated with 5 μM DMPP for 20 sec with an interval of 2 min in the absence or presence of 60 μM borneol. White and black bars represent application of DMPP and borneol, respectively. The experiment was performed three times independently, and the results were reproducible. Typical amperometric current trace is presented. (B) Total amperometric currents induced by the 20-sec DMPP pulse in (A) were integrated and represented as percentage of the first one. Data are the means ± SEM (bars) values of triplicate measurements. (C) Catecholamine secretion evoked by DMPP in single chromaffin cells was monitored using amperometry in the absence (N = 7) or presence (N = 9) of 60 μM borneol. White and black bars represent application of DMPP and borneol, respectively. The results were reproducible, and typical amperometric current traces are presented. (D) In the results of (C), amperometric currents obtained for every successive 10 sec starting from the addition of DMPP in the bath were integrated and the values are represented as the means ± SEM (bars). Open circles and closed circles represent the integrated currents in the absence and presence of borneol, respectively.

75.2 ± 7.1% (Fig. 5A and B). The borneol-induced inhibitory effect was partially reversible after washout of borneol. Effect of borneol on catecholamine secretion induced by a longer stimulation with DMPP was also investigated. As shown in Fig. 5C, secretion was reached rapidly at its maximal level and decreased slowly as time goes. Most of catecholamine secretion occurred within 2–3 min after onset of secretion. Borneol inhibited the DMPP-induced secretion during stimulation. The amperometric experiment was repeated several times and rate of amperometric current generations were analyzed statistically to get kinetics of exocytosis. As can be seen in Fig. 5D, borneol inhibited both a rapid initial secretion and later sustained one without modifying kinetics, suggesting that the exocytotic process was not affected by borneol. And these results clearly indicate that borneol inhibits nAChR-mediated exocytosis in single chromaffin cells.

3.5. Lack of borneol effect on nicotine binding

Since the inhibitory effect of borneol is almost instantaneous and the activity of nAChR is directly inhibited, it is likely that borneol directly binds to nAChRs. Therefore, we tested whether the binding of [^{3}H]nicotine to nAChRs is inhibited by borneol. As shown in Fig. 6, borneol did not significantly compete for binding with [^{3}H]nicotine, suggesting that its binding site is distinct from that of agonist including nicotine and acetylcholine.

3.6. Comparison of inhibitory effects by borneol and lidocaine

It has been reported that local anesthetics such as lidocaine, procaine, and QX-222 inhibit the function of nAChRs in a noncompetitive manner [17–19]. Therefore,

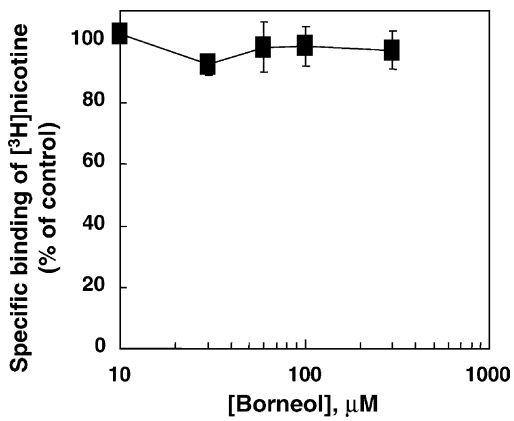

Fig. 6. Effect of borneol on [^{3}H]nicotine binding. Chromaffin cells were incubated with 20 nM [^{3}H]nicotine and various concentrations of borneol for 40 min at 25°. Specific binding of [^{3}H]nicotine is presented. Nonspecific binding was determined in the presence of 1 mM unlabeled nicotine. The experiments were performed three times independently, and the results were reproducible. Data are the means ± SEM (bars) values of triplicate measurements.

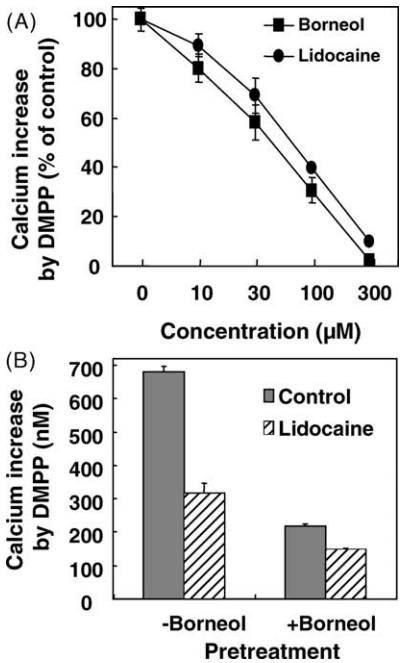

Fig. 7. Comparison of the inhibitory effects of borneol and lidocaine. (A) The calcium increase induced by 10 μM DMPP was measured 3 min after preincubation with the indicated concentrations of borneol (closed box) or lidocaine (closed circle). The peak height of each stimulation was compared to that of the control calcium increase caused by DMPP alone. Data are the means ± SEM (bars) values of triplicate measurements. (B) The calcium increase induced by 10 μM DMPP was measured 3 min after preincubation with 50 μM of borneol and/or lidocaine. Data are the means ± SEM (bars) values of triplicate measurements.

we compared the inhibitory potency of borneol with that of lidocaine under our experimental conditions. As shown in Fig. 7A, both borneol and lidocaine inhibited DMPP-induced calcium increase in which the inhibitory effect of borneol was more potent. On the other hand, lidocaine at 100 μM had no effect on calcium increases induced by 50 mM K^+, veratridine, and bradykinin, mimicking the effect of borneol (data not shown). Then we tested indirectly whether borneol shares binding sites on AChRs with lidocaine. If borneol and lidocaine do not share the binding sites, their inhibition of AChR activity would be independent. As shown in Fig. 7B, 50 μM lidocaine inhibited DMPP-induced calcium increase by 53.4 ± 4.4%. On the other hand, the same concentration of lidocaine inhibited the DMPP-induced response by 30.9 ± 0.9% in the presence of 50 μM borneol, indicating that inhibition by lidocaine became weaker in the presence of borneol. These results suggest that borneol and lidocaine may share the binding sites on nAChRs.

4. Discussion

Our data clearly indicate that borneol specifically inhibits the catecholamine secretion induced by nicotinic stimulation. The results which calcium increases induced by high K^+, veratridine, and bradykinin were not affected

by borneol suggest that borneol has no effect on calcium channels, sodium channels, and PLC-linked receptors. In contrast, the responses induced by nicotinic stimulation with DMPP were inhibited by borneol. Although calcium channels are activated in the activation process of nAChRs, both the inhibition of DMPP-induced sodium increase and the absence of inhibitory effect on high K^+-induced calcium increase clearly indicate that nAChRs, but not calcium channels, are the specific target of borneol. Lipid solubility and rapid onset of the inhibitory effect by borneol suggest that borneol directly interacts with nAChRs. Furthermore, downward shift of the concentration-dependent response to the nicotinic agonist in the presence of borneol and lack of inhibition by borneol in [^{3}H]nicotine binding to nAChRs indicate that borneol acts as a noncompetitive inhibitor of nAChR. In addition, similar initial calcium and sodium increases in the absence and presence of borneol suggest that borneol does not modify ion entry kinetics. This is supported by similar kinetics of catecholamine secretion obtained from amperometry. It is known that the nAChR as a representative allosteric protein has multiple, interconvertible conformations: a resting state, an open channel state and several desensitized states. A variety of pharmacological agents are known to modulate the transitions between the conformations by directly binding to several sites of nAChRs which are topographically distinct from the acetylcholine binding site (for review, see [20]). Many physiological or pharmacological effectors, such as divalent cations, neuropeptides, local and general anesthetics, membrane potential, and protein phosphorylation, modify the properties of nAChRs even though they do not significantly affect the binding of agonist such as acetylcholine and nicotine. These molecules, known as noncompetitive blockers, inhibit the ion channel gating activity of the nAChRs through mechanisms that differ from those of the competitive blockers [21]. Our results also suggest that borneol is likely to act as a noncompetitive blocker of nAChRs.

Borneol together with menthol and citronellol belongs to monoterpenoid alcohols that represent a class of natural compounds that are widely present in the environment as components of plants and are often concentrated in the production of oils, perfumes, and foods. In particular, borneol has been traditionally used as a substrate or inhibitor of liver UDP-glucuronosyltransferase (EC 2.4.1.17) [22–26] which catalyzes glucuronidation of drugs such as acetaminophen, morphine, and diazepam into highly polar conjugates, thus making them inactive and be excreted rapidly in the urine and feces [27]. In addition, borneol has been reported to have a marked antifungal activity against Candida albicans [28]. Underlying mechanism of the antifungal activity is not known. Interestingly, borneol has been used as a medicine by many Asian cultures. It is an important ingredient in many incense formulars. It may be helpful in helping to carry the other aromas and produce the needed alertness required in meditative practice. Furthermore, it has been traditionally known to restore consciousness and to relieve pain. It has been used to treat phlegm syncope, coma, and convulsion with musk and to treat swollen and sore throat and swollen and painful eyes with other medicinal compounds. In particular, borneol has been included in some traditional Chinese medicine for coronary heart disease [29,30]. However, little attention has been paid to the role of borneol as a bioactive material in cellular signal transduction systems.

The specific inhibitory effect on nAChR-mediated effects by borneol may contribute to understand the basic mechanism of borneol effects as a medicine in Asia. Furthermore, its specific action implicates that borneol may be used as a candidate for therapeutic agents. Clinical agents including local anesthetics [16] were shown to inhibit functions of nAChRs, although correlation between the inhibitory effect and its clinical effect is unclear yet. Most of these drugs, however, showed inhibitory effects on calcium channels [31–33] and PLC [34,35] as well as nAChRs. Therefore, highly selective inhibition of nAChRs by borneol could provide a great advantage for the development of medical reagents with an enhanced therapeutic effect and decreased side effects. Our results suggest that borneol and lidocaine both specifically inhibit the activity of nAChRs in bovine adrenal chromaffin cells. The two reagents are similar in that their inhibition is specific to nAChRs and noncompetitive. Furthermore, their inhibitions were not independent when cells were treated with them together. Therefore, it is likely that the mechanisms by which the two reagents inhibit the nAChR activity might be the same. It is possible that both reagents may share the binding sites on the nAChR. Determination of borneol's binding site on the nAChR and its potential clinical application will be interesting for further experiments. In addition, both can be used as useful tools for the study of nAChR-mediated signal transduction.

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