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Effects of Coffee Components on the Response of $GABA_A$ Receptors Expressed in *Xenopus* Oocytes

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The effects of both coffee components and coffee extract on the electrical responses of $GABA_A$ receptors expressed in *Xenopus* oocytes were studied by injecting cRNAs of the α_1 and β_1 subunits of the bovine receptors. The aqueous extract of coffee dose-dependently inhibited the GABA-elicited responses, whereas the lipophilic extract of coffee by diethyl ether slightly potentiated it at low doses (0.1−0.4 μL/mL) but showed inhibition at high doses (0.5−0.8 μL/mL). Theophylline inhibited the response in a noncompetitive mechanism (K_i = 0.55 mM), whereas theobromine and trigonelline hydrochloride inhibited it in a competitive manner, K_i = 3.8 and 13 mM, respectively. Benzothiazole, catechol, 2,4-dimethylstyrene, guaiacol, 1-octen-3-ol, sotolone, and 2,3,5-trimethylphenol potentiated the responses significantly. Potentiation elicited by guaiacol and sotolone was independent of GABA concentrations, whereas that by 1-octen-3-ol was dependent. When 1-octen-3-ol (100 mg/kg) was orally administered to mice prior to intraperitoneal administration of pentobarbital, the sleeping time of mice induced by pentobarbital increased significantly.

KEYWORDS: Coffee; fragrance; $GABA_A$ receptor; theobromine; theophylline; *Xenopus* oocyte

INTRODUCTION

Coffee is the most popular beverage in the present day world. Enormous amounts of coffee are processed as different brands and consumed by many people, because drinking coffee wakes us up and relieves drowsiness, stress, and neuralgia. Chemical analyses of coffee plants (*Coffea* sp.) have revealed many chemical components (*1*, *2*), which have both beneficial (*3*−*5*) and harmful (*6*−*8*) effects on human health. Reportedly, some components of coffee cause alertness, heart stimulation, antioxidant, antitumor, antibiotic, or hypotensive effects.

The γ-aminobutyric acid type A ($GABA_A$) receptors are hetero-oligomeric proteins, responsible for most fast inhibitory neurotransmission in the central nervous system, and are involved in the control of many neurological states such as anxiety, wakefulness, and seizures. Numerous natural and synthetic compounds interact with the $GABA_A$ receptors. The $GABA_A$ receptors have a complex pharmacology (*9*, *10*) with binding sites for direct GABA agonists and antagonists together with multiple allosteric sites for benzodiazepine tranquilizers, for the barbiturate central nervous system depressants, for both synthetic and endogenous steroids (*11*), for general anesthetics (*12*), and for ethanol (*13*). These structurally diverse compounds enhance the response of $GABA_A$ receptors in the presence of a low concentration of GABA. In previous papers (*14*−*19*), we reported that the inhibition or the potentiation of the responses of $GABA_A$ receptors was caused by the components of tea, fragrances of whiskey, and various components present in food additives or essential oils such as alcohol and phenol derivatives. A simple kinetic model for the potentiation of $GABA_A$ receptor responses was proposed previously (*20*).

Various components of foods or beverages act on receptors, channels, or enzymes in the brain and modulate human consciousness (*21*). For example, nicotine binds to nicotinic acetylcholine receptors; ethanol acts on the $GABA_A$ receptors (*13*), the NMDA receptors (*22*), and K^+ channels (*23*, *24*); capsaicin opens warm receptors (*25*), whereas menthol opens cold receptors (*26*). It is known that caffeine, the major alkaloid of coffee and tea, acts as a central nervous system stimulant (*21*) and significantly affects cognitive performance, mood, and thirst (*27*). The psychostimulant action of caffeine comes from the blockade of adenosine A_{2A} receptors (*28*), due to the involvement of DARPP-32 and its phosphorylation (*29*). Moreover, caffeine also enhances the potentials of A_{2A} antagonists and can be used as a neuroprotective agent against the nerve degeneration associated with neurodegenerative (Parkinson's) disease (*30*). Thus, it is important to explore the bioactivity of other components of coffee and to know whether components of coffee modulate the neural transmission in the brain and change peoples' moods or consciousness, because various reports have shown that many components of coffee

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are well absorbed into the human body, enter into blood circulation, and may reach the central nervous system. It is especially important to clarify their effects on the responses of ionotropic GABA receptor ($GABA_A$ receptors), because many mood-defining drugs are thought to target $GABA_A$ receptors in the brain. Therefore, in the present study, by using the two-electrode voltage-clamping method, we investigated the effects of coffee extracts and the components of coffee on the responses of the ionotropic $GABA_A$ receptors expressed in *Xenopus* oocytes, which are composed of the α_1 and β_1 subunits of the bovine $GABA_A$ receptors.

MATERIALS AND METHODS

Materials. GABA was purchased from Nacalai Tesque, Kyoto, Japan. Theophylline, theobromine, allantoin, chlorogenic acid, 1,3-dimethyluric acid, and 1-methylxanthine were purchased from Sigma Chemical Co., St. Louis, MO. Limonene, 2,4-dimethylstyrene, pyrrole, and catechol were purchased from Aldrich Chemical Co. Trigonelline hydrochloride, 2,3,5-trimethylphenol, 2-methylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2-ethyl-3-methylpyrazine, maltol (3-hydroxy-2-methyl-4-pyrone), benzothiazole, 1-octen-3-ol, 2-methyl-3-buten-2-ol, 3-methyl-2-buten-1-ol, *cis*-2-penten-1-ol, and 1-penten-3-ol were purchased from Tokyokasei, Tokyo, Japan. (*E*)-2-Hexen-1-ol was purchased from Taiyo Corp., Tokyo, Japan. Hydroquinone and guaiacol were purchased from Sigma Aldrich Co. Sotolone [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone] was purchased from Takasago, Tokyo, Japan. All chemicals were of guaranteed reagent quality and were used without further purification. Green tea was a gift from the foundation of Kyoto Green Tea, Japan. Coffee (AGF, Tokyo, Japan), black tea (Nittoh, Tokyo, Japan), and oolong tea (Kotanikokuhunn, Kochi, Japan) were purchased from a local supermarket.

For the preparation of aqueous extract, 1 g of coffee, black tea, green tea, or oolong tea was extacted in 20 mL of hot frog normal Ringer's solution (115 mM NaCl, 1 mM KCl, and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) for 1 min. Thereafter, the extract was passed through filter paper (Advantec 1, 90 mm, Toyo Roshi Kaisha, Ltd.), and the filtrate volume was adjusted to 20 mL by adding the Ringer's solution; this mixture was then applied to the noninjected oocytes to account for the nonspecific currents elicited by the aqueous extracts of these beverages. In the case of coffee, the aqueous extract was also applied together with 0.25 μM GABA to oocytes expressing the α_1 and β_1 subunits of the bovine $GABA_A$ receptors. The responses elicited by the mixture of 0.25 μM GABA and different volumes (5−100 μL/mL) of the aqueous extract of coffee were compared to those elicited by 0.25 μM GABA.

For the preparation of diethyl ether or pentane extract, 10 g of coffee was extracted in 200 mL of hot frog normal Ringer's solution. To this was added 100 mL of diethyl ether or pentane followed by 1 min of vigorous shaking; then the upper diethyl ether or pentane phase was separated from the aqueous phase by a separating funnel. The solvent was evaporated by an evaporator, and finally the solid was dissolved in 100 μL of ethanol and stored at 4 °C. The effect of these coffee extracts on the GABA-elicited response of the bovine $GABA_A$ receptors was examined by the addition of the extracts to GABA solution to treat oocytes.

Preparation of cRNAs of α_1 and β_1 Subunits of the Bovine $GABA_A$ Receptors. The cRNAs of the α_1 and β_1 subunits of the bovine $GABA_A$ receptors were synthesized from cloned cDNAs of bovine brain receptors using RNA polymerase according to standard procedures. The cloned cDNAs were gifts from Prof. Eric A. Barnard of the MRC Centre.

Preparation of *Xenopus* Oocytes. Adult female frogs (*Xenopus laevis*) were purchased from Hamamatsu Seibutsu Kyozai, Co., Hamamatsu, Japan. The oocytes were dissected from the ovaries of adult female frogs that had been kept in ice for 1 h. After this, oocytes were manually detached from the inner ovarian epithelium and follicular envelope after incubation in a collagenase (type I, 1 mg/mL; Sigma) solution for 1 h according to the procedure of Kusano et al. (*31*). These oocytes were microinjected with the cRNAs in sterilized water and then incubated in modified Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 0.82 mM $MgSO_4$ in 7.5 mM Tris, pH 7.6] containing 25 mg/L penicillin and 50 mg/L streptomycin at 15−18 °C for 2−7 days before electrophysiological measurements.

Electrophysiological Measurements. The membrane current of the receptors evoked by GABA was measured by using the voltage clamping method with a voltage clamp amplifier (CEZ-1100; Nihon Kohden Kogyo, Tokyo, Japan). An oocyte was placed on the net of a small chamber (0.3 mL) and impaled with two microelectrodes filled with 3 M KCl, one for monitoring the membrane potential and the other for passing current for clamping the membrane potential, usually at −40 mV. The oocyte placed on the net was continuously perfused from the bottom with frog normal Ringer's solution by the gravity feed system, at a flow rate of 2 mL/min (*32*).

Measurement of the Receptor Response. GABA was dissolved in frog normal Ringer's solution. To examine the effects of extracts or coffee components on the GABA-elicited response, each test compound was added to the solutions. The solution was changed by switching a cock of the flow system. The control response was obtained by perfusing the GABA solution without extract or any compound and was taken as 100%. The effect of the extract or a given compound on the response of the receptors was measured by using a mixture of GABA and the extract or compound; in some cases, the compound was added 1 min before coapplication with GABA when desensitization of the receptors was significantly induced before equilibrium of the compound binding was attained (*33*). The measurement was repeated several times with the same oocyte, and the control values were obtained every two or three measurements. Values of data were usually the means from four experiments. To eliminate the desensitization of the receptors, the oocyte was washed for >10 min in frog normal Ringer's solution before the next measurement, because desensitization of the $GABA_A$ receptors is a reversible process and the receptors usually recover after 10 min of washing (*34*).

Student's *t* test was used to evaluate the significance in the mean values, compared with the control.

Effect of 1-Octen-3-ol on Pentobarbital-Induced Sleeping Time in Mice. Male ddY mice (Japan SLC Co., Shizuoka, Japan) were obtained at the age of 28 days. They were housed five per cage under standardized light−dark cycle conditions (lights on at 7:00 a.m., off at 7:00 p.m.) at 24 ± 1 °C and 60 ± 10% humidity with food and water ad libitum. All animals received humane care in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacology Society.

1-Octen-3-ol (20 or 100 mg/kg) was administered to mice orally 30 min prior to the administration of pentobarbital. Pentobarbital (50 mg/kg) was administered to mice intraperitoneally. Sleeping time was calculated as the time between the disappearance of the righting reflex and the recovery of the righting reflex.

RESULTS

The $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs composed of the α_1 and β_1 subunits of the bovine $GABA_A$ receptors to examine the effects of coffee components on the electrical response of $GABA_A$ receptors. The electrical responses of the receptors were measured by the two-electrode voltage-clamping method. **Figure 1** shows typical examples of the potentiation and the inhibition of the $GABA_A$ receptor-elicited responses by the aqueous extract and the components of coffee, theophylline and 1-octen-3-ol.

The aqueous extract of coffee inhibited the 0.25 μM GABA-induced responses dose-dependently (**Figure 2a**). However, the inhibition of the $GABA_A$ receptor response by the aqueous extract showed little dependence on GABA concentration, suggesting a noncompetitive inhibition mechanism. Although hydrophilic components are selectively taken into the brain through the blood−brain barrier by transporters, lipophilic components go through the blood−brain barrier easily to the

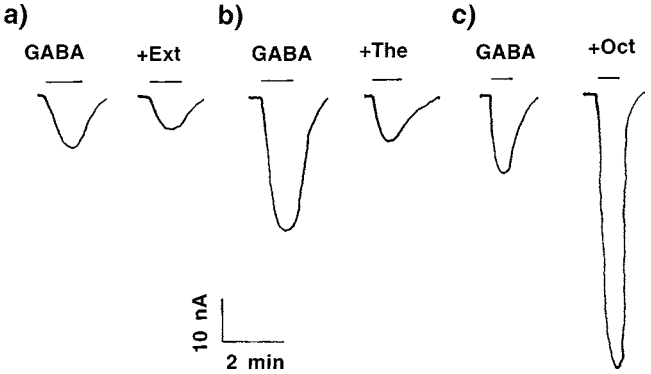

Figure 1. Effects of the aqueous extract of coffee (Ext), theophylline (The), or 1-octen-3-ol (Oct) on the GABA-elicited currents: (**a**) GABA, 0.25 μM GABA; Ext, 30 μL/mL extract; (**b**) GABA, 5 μM GABA; The, 1 mM theophylline; (**c**) GABA, 5 μM GABA; Oct, 0.5 mM 1-octen-3-ol. All traces were obtained with a voltage clamp at −40 mV. An inward current is shown as a downward curve. The upper bars show when GABA or the mixture of GABA and the compound was applied. Both responses in a given panel were obtained from the same injected oocyte, but the responses in panels **a**–**c** from different oocytes.

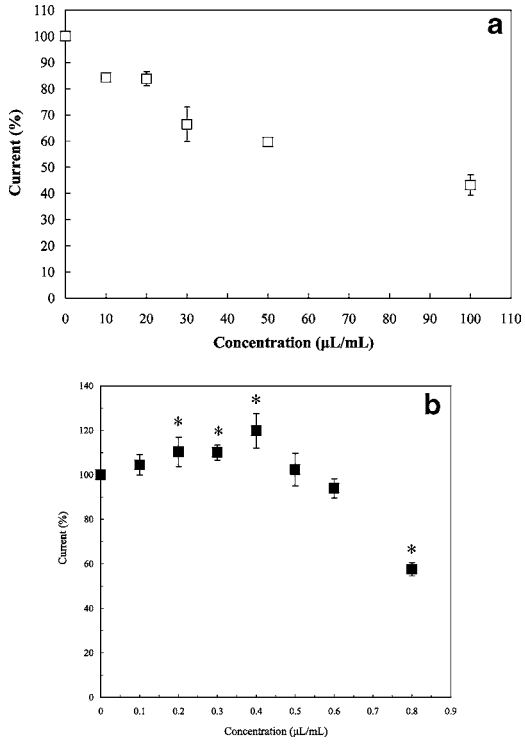

Figure 2. (**a**) Dose–inhibition curve of the aqueous extract of coffee (μL/mL) in the presence of 0.25 μM GABA. The response induced by 0.25 μM GABA without the extract was taken to be 100%. Data are mean ± SD (bars) values from four experiments. $p < 0.05$ between the control and the value in the presence of the extract, by Student's *t* test. (**b**) Effect of the diethyl ether extract of coffee (μL/mL) on the $GABA_A$ receptor response. The response induced by 0.25 μM GABA without the extract was taken to be 100%. Data are mean ± SD (bars) values from four experiments. *, $p < 0.05$ between the control and the value in the presence of the extract, by Student's *t* test.

brain and thereby may modulate the responses of $GABA_A$ receptors. Therefore, we extracted coffee components from the aqueous extract of coffee with diethyl ether or pentane, which therefore had the lipophilic components and could easily pass the blood−brain barrier, and dissolved these components in

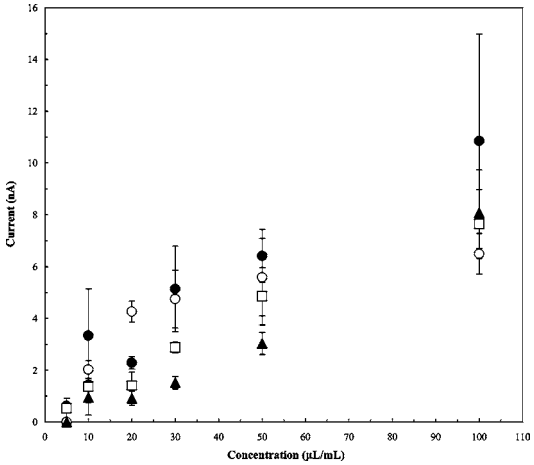

Figure 3. Nonspecific currents in noninjected *Xenopus* oocytes caused by aqueous extract of coffee (▲), green tea (□), oolong tea (○), and black tea (●). Peak currents were measured by applying various concentrations of extracts in frog normal Ringer's solution to noninjected oocytes for 1 min. Data are mean ± SD (bars) values from six experiments.

ethanol after evaporation of diethyl ether or pentane. The diethyl ether extract of coffee at low concentrations (0.1−0.4 μL/mL) showed slight potentiation of the 0.25 μM GABA-elicited $GABA_A$ receptor responses, whereas high concentrations (0.5−0.8 μL/mL) of the extract inhibited them (**Figure 2b**). The pentane extract of coffee also potentiated the response similarly.

We also examined the nonspecific electrical currents evoked by the aqueous extracts of coffee, green tea, black tea, or oolong tea. These extracts (5−100 μL/mL) evoked nonspecific currents dose-dependently even in noninjected oocytes (**Figure 3**), possibly because they perturbed the membranes of oocytes nonspecifically. These nonspecific currents showed much variation in size, depending on the individual frogs and oocytes. If the nonspecific currents were not negligible, they were subtracted from the responses caused by a mixture of GABA and the aqueous extract when the effects were examined. Black tea aqueous extract evoked the highest magnitude of nonspecific current, 10.85 ± 4.13 nA, at the concentration of 100 μL/mL. However, diethyl ether extract of coffee at 0.5 μL/mL did not evoke any detectable current.

Figure 4 shows the effect of various coffee compounds at 0.5 mM on the responses of $GABA_A$ receptors composed of α_1 and β_1 subunits elicited by 0.25 μM GABA. Among the compounds, allantoin, chlorogenic acid, 3-hydroxy-2-methyl-4-pyrone (maltol), theobromine, theophylline, and 2,3,5-trimethylpyrazine significantly inhibited the $GABA_A$ receptor responses, whereas benzothiazole, catechol, 2,4-dimethylstyrene, guaiacol, 1-octen-3-ol, sotolone, and 2,3,5-trimethylphenol potentiated the responses significantly, and others had little or no effect on the responses. In our previous paper on tea (*18*), we reported the inhibition of the $GABA_A$ receptor-mediated responses by catechin derivatives and caffeine, which is common in both tea and coffee, and the potentiation by fragrant higher alcohols.

Because theophylline and theobromine showed inhibition of the $GABA_A$ receptor responses, we investigated the molecular mechanisms underlying the inhibition of the responses of $GABA_A$ receptors composed of α_1 and β_1 subunits by these compounds. The responses were measured at different concentrations of both GABA and the compounds. As expected, the inhibition of the responses by these inhibitors increased with their concentrations. Therefore, we measured the dose−inhibition relationship (**Figure 5a**) of these compounds. We also

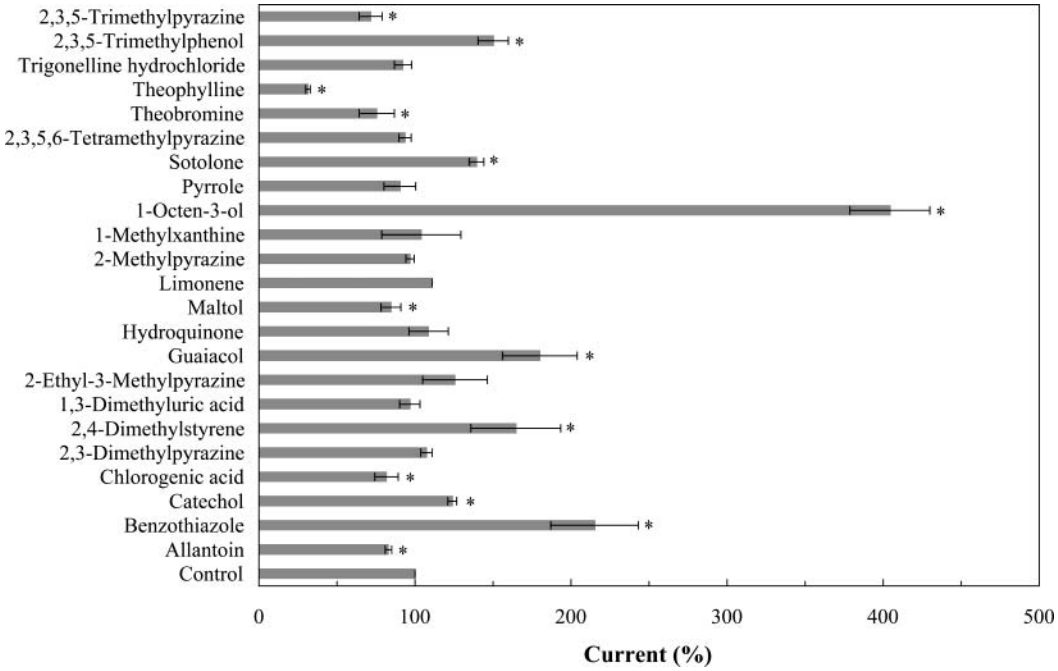

Figure 4. Effect of coffee components at 0.5 mM on the 0.25 μM GABA-elicited response. The response evoked by 0.25 μM GABA without any component was taken to be 100%. Data are mean ± SD (bars) values from four experiments. *, $p < 0.05$ between the control and the value in the presence of the component, by Student's *t* test.

investigated the dose−inhibition of trigonelline hydrochloride, a component of coffee, which shows antitumor activity (*35*). This component weakly inhibited the responses of $GABA_A$ receptors, with an estimated inhibition constant (K_i) of 13 mM, and the inhibition mechanism was competitive (data not shown). The inhibition by trigonelline hydrochloride was similar to that by caffeine, the competitive inhibition constant of which was estimated to be ~15 mM (*18*). **Figure 5b** shows that the inhibition by theophylline is independent of the GABA concentrations, indicating a noncompetitive inhibition mechanism. The noncompetitive inhibition constant (K_i) of theophylline was estimated to be 0.55 mM. However, inhibition by theobromine was dependent on the GABA concentrations, suggesting a competitive inhibition mechanism. The competitive inhibition constant (K_i) of theobromine was estimated to be 3.8 mM when the GABA concentration was 0.25 μM. However, the inhibition constant of theobromine did not explain the results in **Figure 5b** well, which suggests the possibility that a complicated mechanism is involved in the inhibition by theobromine.

We examined the mechanism of potentiation of the responses of $GABA_A$ receptors composed of α_1 and β_1 subunits by fragrant compounds in coffee, such as 1-octen-3-ol, guaiacol, and sotolone. The responses were measured at different concentrations of both GABA and the compounds. The potentiations by fragrant compounds increased gradually with the compounds' concentrations and reached a saturation level (**Figure 6a**). The dissociation constant (K_p) and the maximum potentiation (V_m) when the potentiation site of all receptors was occupied by the fragrance compound were estimated to be 0.76 mM and 688% for 1-octen-3-ol, 0.16 mM and 194% for guaiacol, and 8.6 mM and 963% for sotolone, respectively, from the results in **Figure 6a**. **Figure 6b** shows the effect of GABA concentrations on the 1-octen-3-ol-, guaiacol-, and sotolone-elicited potentiation. In the case of 1-octen-3-ol, the GABA dose−response curve shifted to a lower concentration, indicating the enhancement of GABA binding to $GABA_A$ receptors (*36*, *37*). The dissociation constant of GABA (K_{1p}) when the potentiation site of the receptors was occupied by 1-octen-3-ol was also estimated as shown in **Figure 6a** (*20*). In contrast, guaiacol- and sotolone-induced potentiation of $GABA_A$ receptors was independent of GABA concentrations.

Because 1-octen-3-ol potentiated the response of $GABA_A$ receptors more prominently, we investigated the effects of some of the unsaturated alcohols having shorter carbon chain length. These compounds at 0.5 mM also significantly potentiated the responses of $GABA_A$ receptors, but less than 1-octen-3-ol did, as shown in **Figure 7**. These results are similar to our previous observations that alcohol-induced potentiation of the $GABA_A$ receptor-mediated response increased with the increased chain length of the normal alcohols (*20*).

1-Octen-3-ol was the most potent activator of the $GABA_A$ receptor-elicited response. Therefore, we investigated the effect of 1-octen-3-ol on the sleeping time of mice induced by pentobarbital, an agent that induces sleeping by the potentiation of the $GABA_A$ receptor-elicited response in the brain. **Figure 8** shows the effect of 1-octen-3-ol on the sleeping time of mice treated with 1-octen-3-ol prior to the administration of pentobarbital. 1-Octen-3-ol significantly increased the sleeping time induced by pentobarbital, suggesting that 1-octen-3-ol was absorbed into the brain and potentiated the $GABA_A$ receptor-elicited responses. Other alcohols also potentiate the $GABA_A$ receptor response, so they must cause a similar effect on the sleeping time (*38*).

To examine the effect of theophylline on the $GABA_A$ receptor response in the presence of 1-octen-3-ol or guaiacol, the GABA-elicited responses in the presence of the mixture of 1-octen-3-ol or guaiacol and theophylline were compared with those in the presence of only one compound alone (**Table 1**). As expected, the addition of 1-octen-3-ol or guaiacol decreased the inhibition of the response of $GABA_A$ receptors by theophylline.

DISCUSSION

In a previous paper (*18*), we found that aqueous extracts of various types of tea induced the response of $GABA_A$ receptors expressed in *Xenopus* oocytes, with injected cRNAs of α_1 and

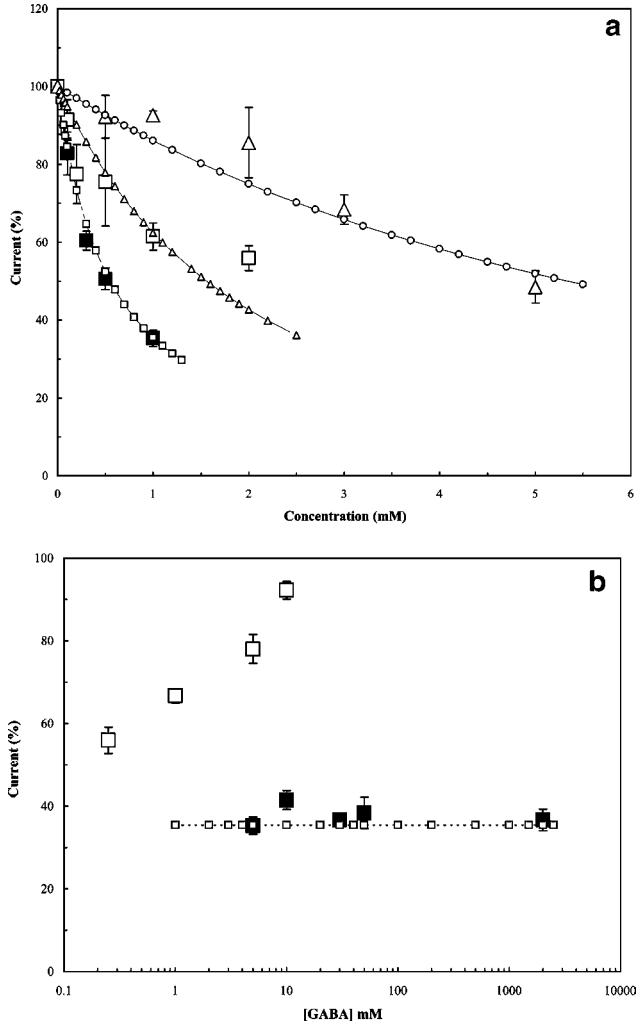

Figure 5. (**a**) Dose–response of inhibition of theophylline (■), theobromine (□), and trigonelline hydrochloride (△). The effect of theophylline was measured in the presence of 5 μM GABA, and the theoretical curve (small squares) was drawn using the noncompetitive inhibition constant of 0.55 mM. The effects of theobromine and trigonelline hydrochloride were measured in the presence of 0.25 μM GABA, and the theoretical curves (small triangles for theobromine; small circles for trigonelline hydrochloride) were drawn by using the competitive inhibition constants of 3.8 and 13 mM, respectively, and a dissociation constant (K_1) of 59 μM between GABA and the receptor. Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test. (**b**) Effects of GABA concentrations on the inhibition by 1 mM theophylline (■) and 2 mM theobromine (□). The theoretical curve for theophylline (small squares) was drawn by using the noncompetitive inhibition constant of 0.55 mM. $p < 0.05$ between the control and the value in the presence of the component, by Student's t test.

β_1 subunits of the bovine receptors, possibly because of the presence of a GABA-like compound(s) in the tea. In contrast, the aqueous extract of coffee induced no $GABA_A$ receptor response in the cRNA-injected oocyte. Therefore, we examined the effects of an aqueous extract of coffee on the $GABA_A$ receptor response induced by 0.25 μM GABA. The aqueous extract of coffee inhibited the electrical responses elicited by 0.25 μM GABA dose-dependently. Although coffee includes compounds that both potentiate and inhibit the response of $GABA_A$ receptors, the aqueous extract of coffee possibly includes larger amounts of noncompetitive inhibitory compounds than potentiators.

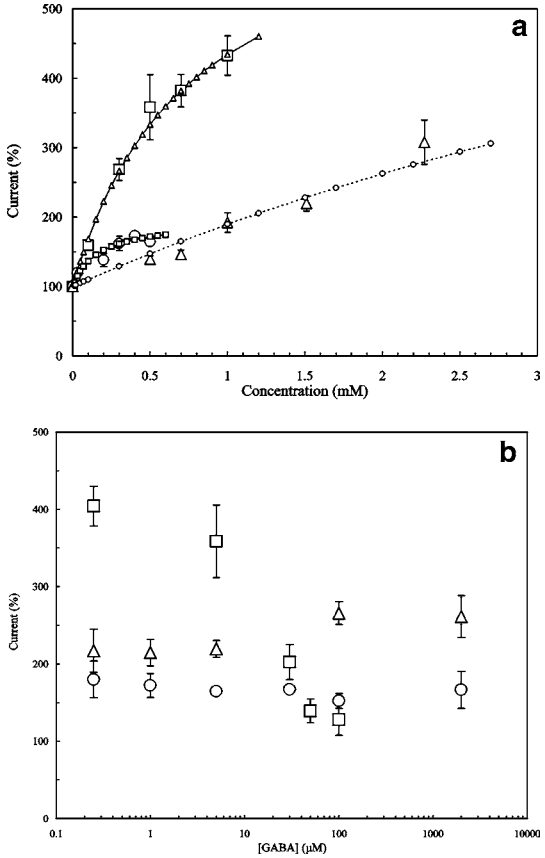

Figure 6. (**a**) Dose–potentiation of 1-octen-3-ol (□), guaiacol (○), and sotolone (△). The responses of $GABA_A$ receptors were measured in the presence of 5 μM GABA. The theoretical curves were drawn by using the following constants: $K_1 = 59$ μM; $K_p = 0.76$ mM, $V_m = 688\%$, and $K_{1p} = 0.021$ mM for 1-octen-3-ol (small triangles); $K_p = 0.16$ mM and $V_m = 194\%$ for guaiacol (small squares); and $K_p = 8.6$ mM and $V_m = 963\%$ for sotolone (small circles). Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test. (**b**) Effects of GABA concentrations on the potentiation of $GABA_A$ receptor response in the presence of 0.5 mM 1-octen-3-ol (□), guaiacol (○), and 1.51 mM sotolone (△). Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test.

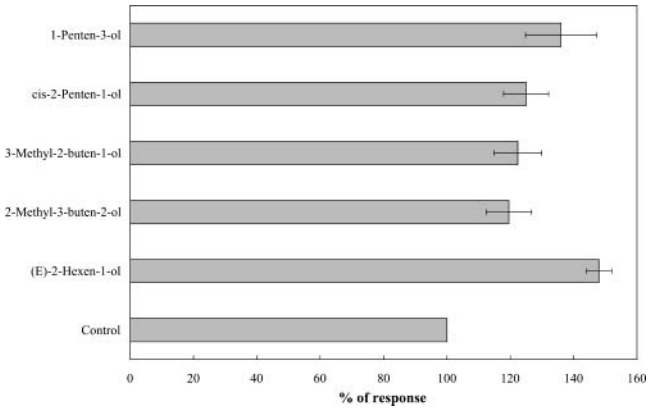

Figure 7. Effects of unsaturated alcohols on the potentiation of the $GABA_A$ receptor response. These compounds at 0.5 mM significantly potentiated the responses of $GABA_A$ receptors elicited by 0.25 μM GABA. $p < 0.05$ by Student's t test.

It is well-known that caffeine, the major component of coffee and tea, acts as a central nervous system stimulant through the effects on phosphodiesterase, ryanodine receptor, and purine

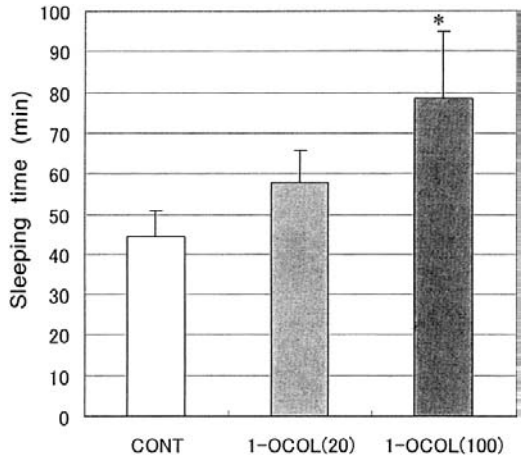

Figure 8. Effect of 1-octen-3-ol on pentobarbital-induced sleeping time in mice. 1-Octen-3-ol (20 or 100 mg/kg) was orally administered to mice 30 min prior to intraperitoneal administration of pentobarbital (50 mg/kg). Sleeping time was measured with or without the administration of 1-octen-3-ol. CONT means control (sleeping time by the administration of pentobarbital only); 1-OCOL(20) and 1-OCOL(100) indicate sleeping time by the administration of pentobarbital with 20 and 100 mg/kg 1-octen-3-ol, respectively. Each bar represents the mean ± SD obtained from four mice. $*$,$p < 0.05$ by Student's *t* test.

Table 1. Comparison of the Effects of 1-Octen-3-ol and Guaiacol on 5 μM GABA-Elicited Response[a] with the Effect of a Mixture of Theophylline and the Other Compound

compound (s)	response (%)
control	
+ 0.5 mM theophylline	50.6 ± 2.8
+ 0.5 mM 1-octen-3-ol	358.4 ± 46.9
+ 0.5 mM theophylline and 0.5 mM 1-octen-3-ol	115.0 ± 9.1
+ 0.5 mM guaiacol	164.7 ± 2.7
+ 0.5 mM theophylline and 0.5 mM guaiacol	76.9 ± 3.7

[a] The response elicited by 5 μM GABA was taken as the control (100%). $p < 0.05$ by Student's *t* test for all data.

receptors (*21*, *39*). Our previous paper (*18*) showed that a xanthin derivative, caffeine, also inhibited the $GABA_A$ receptors in a competitive mechanism. In this study, xanthin derivatives, theophylline and theobromine, which cause convulsions (*40*, *41*) at high doses when used as drug treatment for asthma, also inhibited the responses of $GABA_A$ receptors dose-dependently. The inhibition by theobromine was dependent on GABA concentration, whereas that by theophylline was independent, although Sugimoto et al. (*42*) reported that theophylline inhibited the response of $GABA_A$ receptors composed of $\alpha_1\beta_2\gamma_{2s}$ subunits in a GABA-concentration-dependent manner. Allantoin, chlorogenic acid, 3-hydroxy-2-methyl-4-pyrone (maltol), trigonelline hydrochloride, and 2,3,5-trimethylpyrazine also inhibited the $GABA_A$ receptor response slightly. It is thought that caffeine and its derivatives stimulate the central nervous system mainly by blocking the purine (A_1 and A_{2A}) receptors. However, the inhibition of the response of $GABA_A$ receptors, which are the main inhibitory neurotransmitter receptors, by xanthin derivatives, allantoin, chlorogenic acid, 3-hydroxy-2-methyl-4-pyrone (maltol), trigonelline hydrochloride, and 2,3,5-trimethylpyrazine, may also contribute in part to the central nervous system stimulation.

The lipophilic components of coffee were extracted by diethyl ether or pentane. These components possibly pass the blood−brain barrier easily to be taken into the brain. The diethyl ether extract of coffee potentiated the responses of the $GABA_A$ receptors slightly at low concentrations but inhibited the response at high concentrations. This extract of coffee possibly contains two types of components; components that potentiate the response with high affinity to the receptors and those that inhibit the response with low affinity to the receptor. The inhibitors in coffee are mainly xanthin derivatives ($K_i > 0.5$ mM). The potentiators in coffee are fragrant compounds such as benzothiazole, catechol, 2,4-dimethylstyrene, guaiacol, 1-octen-3-ol, and sotolone. 1-Octen-3-ol shifted the GABA dose−response curve to lower concentrations, indicating an increase of the GABA binding affinity to the receptors. However, the potentiation of the $GABA_A$ receptor response by guaiacol and sotolone was independent of GABA concentrations, so the compounds may shift the equilibrium between open and closed channel, from the closed to the open one, or may increase the single-channel current. It is necessary to examine the effects of these compounds on the single-channel current by a patch clamp method in the future. Potentiation of $GABA_A$ receptors by the higher alcohols of tea (*18*) and the fragrant compounds of whiskey (*19*) was also reported recently. The unsaturated alcohols used in this study also increased potentiation of the $GABA_A$ receptor-mediated response with the increased chain length, as did the normal alcohols (*20*). 1-Octen-3-ol increased the sleeping time of mice through the activation of $GABA_A$ receptor-elicited responses as shown in **Figure 8**. The direct effects of the fragrant compounds on $GABA_A$ receptors were suggested by a study showing that the inhalation of chamomile and lemon vapor decreased restriction-stress-induced increases in the plasma adrenocorticotropic hormone (ACTH) levels in the ovariectomized rats, as did diazepam, a benzodiazepine derivative (*43*). It is also reported that rose oil and its components showed anticonflict effects in mouse behavior tests (*44*, *45*). Moreover, an accumulation of essential oil components in the mouse brain was found when they were given by means of percutaneous or vapor-exposure absorption (*46*, *47*).

The fragrant components in coffee help to subdue the stimulating effects of the inhibitory components such as caffeine, theophylline, and theobromine. In fact, the inhibition of $GABA_A$ receptor response by theophylline was prevented by the addition of 1-octen-3-ol or guaiacol (**Table 1**), although the concentration of the fragrant components must be lower than the inhibitory components under physiological conditions. From the reported number of identified fragrant compounds in coffee and tea, it could be concluded that coffee consists of more fragrant compounds than tea. In addition, both coffee and tea have major common components that cause the inhibition of $GABA_A$ receptors such as caffeine, theophylline, and theobromine. However, polyphenol catechin derivatives of tea inhibit the $GABA_A$ receptors noncompetitively (*18*), whereas chlorogenic acid, a polyphenol in coffee, had little inhibitory effect. Thus, coffee has less inhibitory action on the $GABA_A$ receptors than tea. However, consumption of coffee induces complex effects on the human body and mind, which are different from those of drugs that induce strong but simple effects, which may also cause harmful secondary effects. In the future, it will be necessary to elucidate the concentrations at which these compounds reach the synapses of the brain and thereby influence the synaptic transmission.

Aqueous extracts of coffee, green tea, black tea, or oolong tea also induced electrical responses in noninjected oocytes. In our previous paper (*48*), we reported that polyphenols such as saponin and tannic acid evoked large electrical responses even in noninjected oocytes, but catechin and its derivatives evoked

small currents. These nonspecific currents may be due to membrane perturbation by the component(s) present in the aqueous extracts. This may lead to destruction of the membrane potential of epithelial cells in the small intestine and thus disturb the uptake of nutrients if concentrations are very high at the epithelial cells in the stomach or small intestine after consumption. In the future, it will be necessary to clarify whether these aqueous extracts also induce nonspecific currents in the epithelial cells under physiological condition and become detrimental to health.

ABBREVIATIONS USED

GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; SD, standard deviation.

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