JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

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To study the effects of tea components on ionotropic γ-aminobutyric acid (GABA) receptor response, ionotropic GABA receptors ($GABA_A$ receptors) were expressed in *Xenopus* oocytes by injecting cRNAs synthesized from cloned cDNAs of the α_1 and β_1 subunits of the bovine receptors, and their electrical responses were measured by a voltage clamping method. Extracts of green tea, black tea, and oolong tea in an aqueous solution induced the GABA-elicited response, which showed that these teas contain GABA, whereas coffee does not. Caffeine weakly inhibited the response in a competitive manner (K_i = 15 mM), and (+)-catechin inhibited it in a noncompetitive one (K_i = 1.7 mM). Especially, two catechin derivatives, (−)-epicatechin gallate and (−)-epigallocatechin gallate, inhibited the response strongly. Alcohols such as leaf alcohol or linalool potentiated the response, possibly because their binding to the potentiation site enhances the GABA-binding affinity to $GABA_A$ receptors when they bind. Extracts of green tea made with ethyl ether, which must contain lipophilic components of green tea, inhibited the response elicited by GABA, possibly because the amounts of caffeine and catechin derivatives were much larger than fragrant alcohols in such extracts of tea.

KEYWORDS: Caffeine; catechin; $GABA_A$ receptor; green tea; *Xenopus* oocyte

INTRODUCTION

Tea is one of the major beverages consumed by humans. Enormous amounts of tea (*Camellia sinensis*) processed to green tea, black tea, or oolong tea are consumed all over the world, because drinking tea relieves drowsiness, stress, or neuralgia in humans. Chemical analyses of tea components have revealed that it contains healthy compounds: amino acids such as γ-aminobutyric acid (GABA) or theanine, vitamins such as vitamin C or E, caffeine, catechin, and pigments (*1*, *2*). Reportedly, various components of tea cause alertness, heart stimulation, antioxidant, anticancer, or antibiotic effects. It is also known that some tea components decrease blood pressure and blood sugar or control the amount of cholesterol in the blood (*1*, *2*).

It has been reported that components of foods or drinks act on receptors, channels, or enzymes in the brain and modulate human consciousness (*3*). For example, nicotine in tobacco binds to nicotinic acetylcholine receptors in the brain and modulates human consciousness. Ethanol in liquors potentiates the response of $GABA_A$ receptors (*4*), whereas it inhibits that of NMDA receptors (*5*). It also opens G-protein-coupled inwardly rectifying K^+ channels (*6*, *7*). Capsaicin in hot chilli peppers opens heat-activated ion channels (warm receptors) (*8*), whereas menthol in spearmint opens cold receptors (*9*). Caffeine in tea or coffee is known to work as a central nervous system stimulant (*3*). To account for the effects of caffeine on neurons, the following theories have been proposed (*10*): (a) inhibition of cellular phosphodiesterase activity, resulting in an increased concentration of cAMP; (b) increased free Ca^{2+} concentration inside nerve cells through ryanodine receptor opening; and (c) antagonism of adenosine actions on purine receptors. Thus, it is important to know whether tea components affect the neural transmission in the brain and change peoples' moods or consciousness. It is especially important to clarify their effects on ionotropic GABA receptor ($GABA_A$ receptor) responses, because many mood-defining drugs are thought to target $GABA_A$ receptors in the brain.

The various ionotropic neurotransmitter receptors are known to have been evolved from one common ancestral gene (*11*). These receptors have competitive inhibitors, known as antagonists, which bind to the same site as the agonist does and also share a common noncompetitive inhibition site that interacts with various lipid-dependent hydrophobic compounds, probably at the interface between the receptors and membrane lipids (*12*), although the inhibition by these compounds is not strong (*13*). The $GABA_A$ receptors have a complex pharmacology (*12*, *14*), with binding sites for direct GABA agonists and antagonists together with multiple allosteric sites for benzodiazepine

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10.1021/jf011607h CCC: $22.00
Published on Web 05/31/2002

tranquilizers, for the barbiturate central nervous system depressants, for both synthetic and endogenous steroids (*15*), for general anesthetics (*16*), and for ethanol (*4*). These structurally diverse compounds enhance the response of $GABA_A$ receptors in the presence of low concentrations of GABA. In previous papers (*17*−*20*), we reported expressing $GABA_A$ receptors in *Xenopus* oocytes by injecting rat whole brain mRNAs or cRNAs prepared from cDNAs of bovine $GABA_A$ receptor subunits and showed the inhibition or the potentiation of the responses of these receptors caused by various compounds such as alcohols and phenol derivatives, which are present in food additives or essential oils. A simple kinetic model for the potentiation of $GABA_A$ receptor responses was proposed previously (*21*). Because *Xenopus* oocytes, which are round and have a diameter of >1 mm, are larger, more stable, and simpler in shape than neurons, electrophysiological measurements of the responses of the receptors expressed in oocytes can be made easily and repetitively for a long period.

In this study, we expressed the $GABA_A$ receptors in *Xenopus* oocytes by injecting cRNAs synthesized from cloned cDNA of α_1 and β_1 subunits of the bovine $GABA_A$ receptors, because addition of the γ_2 subunit to the injected cRNAs did not cause any differences in the potentiation or the inhibition of the $GABA_A$ receptor response caused by many compounds. The effects of tea extracts and the components of the extracts on the responses of the $GABA_A$ receptors were examined electrophysiologically. Tea extract in aqueous solution induced the response of $GABA_A$ receptors, probably because tea contains GABA. The tea components caffeine and catechin inhibited the response of $GABA_A$ receptors, whereas other components of tea, the fragrant higher alcohols, potentiated the response. Thus, tea contains various components that potentiate or inhibit the responses of $GABA_A$ receptors.

MATERIALS AND METHODS

Materials. γ-Aminobutyric acid (GABA), linalool, benzyl alcohol, phenylethyl alcohol, and dimethyl sulfide were purchased from Nacalai Tesque, Kyoto, Japan. (3*Z*)-Hexen-1-ol (leaf alcohol) was purchased from Sigma Chemical Co., St. Louis, MO. Nerolidol was purchased from Tokyokasei, Tokyo, Japan. Caffeine was purchased from Katayama Chemical Co., Ltd. Tokyo, Japan. (+)-Catechin, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg) were supplied from Tokyo Food Techno Co. Ltd., Tokyo, Japan. All chemicals were of guaranteed reagent quality. Green tea was a gift from the foundation of Kyoto Green Tea. Black tea (Nittoh, Tokyo, Japan), oolong tea (Kotanikokuhunn, Kochi, Japan), and coffee (AGF, Tokyo, Japan) were purchased from a local grocery shop.

One gram of tea or coffee was extracted in 20 mL of hot frog normal Ringer solution (115 mM NaCl, 1 mM KCl, and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) for 3 min. After filtration, the extract was diluted 10-fold with the normal Ringer solution and applied to oocytes expressing the α_1 and β_1 subunits of the bovine $GABA_A$ receptors. The responses elicited by the diluted extract in the $GABA_A$ receptor-expressed oocyte were compared to those elicited by 10 μM GABA.

Twenty grams of green tea was shaken (speed = 110 times/min) in 200 mL of distilled water for 60 min at room temperature. One hundred millliliters of ethyl ether was added to the tea extract, and the mixture was shaken vigorously for 2 min. Then the upper ethyl ether phase was separated from the aqueous phase, and ethyl ether was removed by evaporation. The tea extract was dissolved in 200 μL of ethanol. The effect of this tea extract on the GABA-elicited response of the bovine $GABA_A$ receptors was examined by addition of the extract to GABA solution used to treat oocytes.

Preparation of cRNA and *Xenopus* Oocytes. 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 Center, U.K.

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. They 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. (*22*). The oocytes were microinjected with cRNAs in sterilized water and then incubated in a modified Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$, and 0.41 mM $CaCl_2$ in 5 mM Tris at pH 7.6] containing 25 mg/L of penicillin and 50 mg/L of streptomycin at 15−18 °C for 2−7 days before the electrophysiological measurements.

Electrophysiological Measurements. The membrane current of the receptors evoked by GABA was measured by 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 solution by using a gravity feed system, usually at a flow rate of ~2 mL/min (*23*).

Measurement of the Receptor Response. GABA was dissolved in frog normal Ringer solution. To examine the effect of the extract or tea components on the GABA-elicited response, each test compound was added to the solutions. One or the other of the solutions was selected by switching a cock in the flow system. The control response was obtained by perfusing GABA solution without any compound and was taken as 100%. The effect of a given compound on the response of the receptors was measured by using a mixture of GABA and the compound; in some cases, the compound was added for 1 min before coapplication with GABA when desensitization of the receptors was significantly induced before equilibrium of the compound binding was attained (*24*). The measurement was repeated several times with the same oocyte, and control values were measured after every two or three measurements. Values of data were usually the means from four experiments. To eliminate desensitization of the receptors, the oocyte was washed for >10 min in frog normal Ringer solution before the next measurement, because desensitization of the ionotropic $GABA_A$ receptors is a reversible process and the receptors usually recover after ~10 min of washing (*25*). Student's *t* test was used to evaluate the significance in the mean values, compared with the control.

RESULTS

$GABA_A$ receptors expressed in *Xenopus* oocytes by injecting cRNAs of the α_1 and β_1 subunits of bovine $GABA_A$ receptors were used to examine the effects of the compounds on the $GABA_A$ receptor response. Only cRNAs of the α_1 and β_1 subunits were injected because addition of the γ_2 subunit cRNA to the injected α_1 and β_1 subunit cRNAs did not cause any differences in the potentiation or inhibition of the responses by the compounds. Aqueous extracts of green tea, black tea, or oolong tea induced the response, indicating the presence of GABA-like compound(s) in these teas, whereas the extract of coffee did not (**Figure 1a**). The response of GABA-like compound(s) increased with the extraction time and reached a plateau in 10 min. About 70% of the maximum response was observed after 1 min of extraction (data not shown) (*1*).

Figure 1b shows a typical example of the potentiation and inhibition of the $GABA_A$ receptor response by tea components, leaf alcohol and catechin. **Figure 2** shows the effect of various tea components at 1 mM on the response of $GABA_A$ receptors elicited by 1 μM GABA. Caffeine and catechin inhibited the $GABA_A$ receptor response, whereas fragrant higher alcohols potentiated the response. Dimethyl sulfide had little effect on the GABA receptor-mediated response.

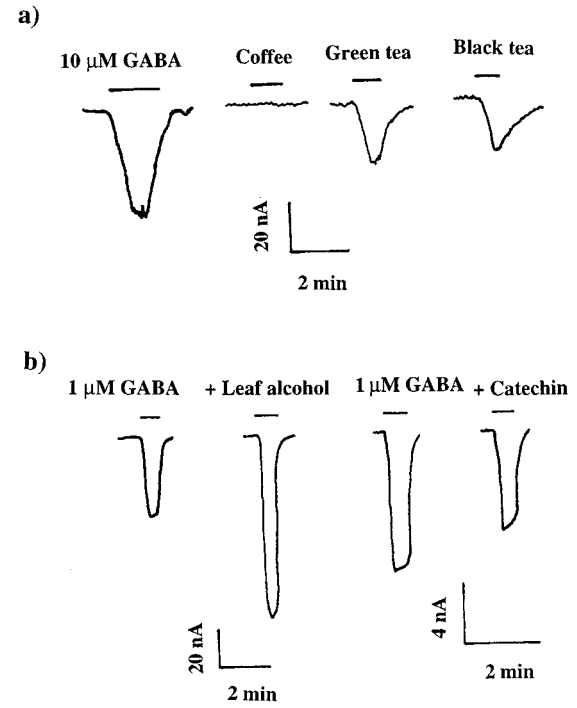

Figure 1. (a) $GABA_A$ receptor responses elicited by an aqueous extract of green tea and black tea. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs of the α_1 and β_1 subunits of the bovine receptors. All traces were obtained with a voltage clamp at −40 mV. An inward current is shown as a downward curve. The upper bars indicate the application of the samples. All responses were obtained from the same injected oocyte. (b) Examples of the potentiation or the inhibition of GABA-elicited current by leaf alcohol or catechin. The concentrations of GABA, leaf alcohol, and catechin were 1 μM, 1 mM, and 1 mM, respectively. Each pair of the responses was obtained from the same injected oocyte, but the responses of different pairs were from different injected oocytes.

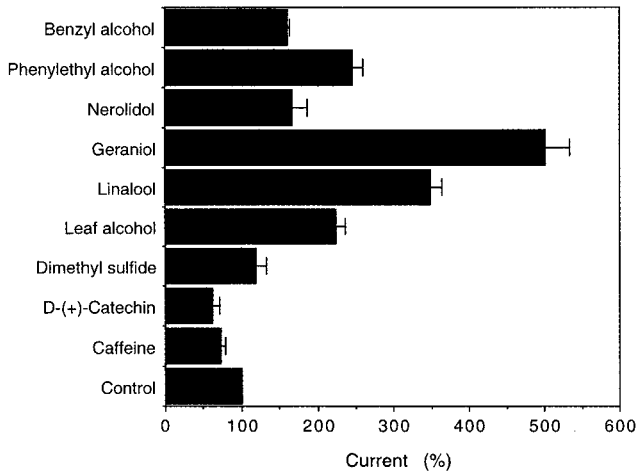

Figure 2. Effect of tea components at 1 mM on the response of $GABA_A$ receptors elicited by 1 μM GABA. The response elicited by 1 μM GABA without any tea component was taken to be 100%. $p < 0.05$ between the control value and the value in the presence of the component, by Student's *t* test.

To clarify the mechanisms of inhibition by caffeine and catechin, the responses of $GABA_A$ receptors were measured at various concentrations of both GABA and the inhibitor. As expected, the inhibition of the responses by both inhibitors increased with their concentrations (**Figure 3a**). **Figure 3b**

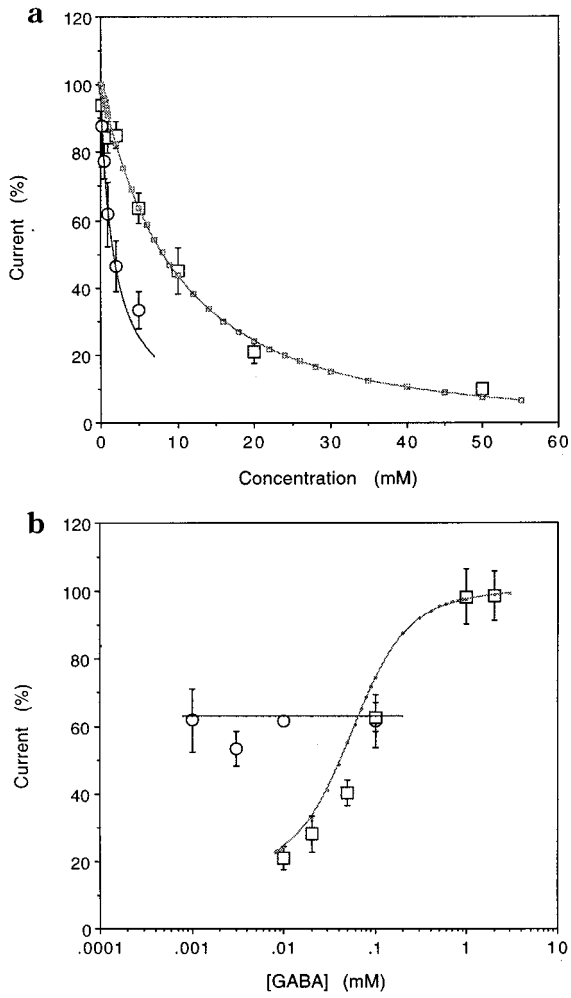

Figure 3. (a) Dose–response of inhibition of caffeine (□) and catechin (○). The effects of caffeine were measured in the presence of 10 μM GABA, and the theoretical curve was drawn by using the competitive inhibition constant of 15 mM. The effects of catechin were measured in the presence of 1 μM GABA, and the theoretical curve was drawn by using the noncompetitive inhibition constant of 1.7 mM. $p < 0.05$ between the control value and the value in the presence of the component, by Student's *t* test. (b) Effects of GABA concentration on the inhibition of $GABA_A$ receptor response in the presence of 20 mM caffeine (□) and 1 mM catechin (○). The theoretical curve for caffeine was drawn by using the competitive inhibition constant of 15 mM and the dissociation constant (K_1) of 59 μM between GABA and the receptor, and that for catechin was drawn by using the noncompetitive inhibition constant of 1.7 mM. $p < 0.05$ between the control value and the value in the presence of the component, by Student's *t* test.

shows that the inhibition by caffeine depended on the GABA concentration, indicating competitive inhibition. The competitive inhibition constant of caffeine was estimated to be 15 mM on the basis of the minimal model proposed before (*26*). On the other hand, the inhibition by catechin was independent of the GABA concentration, indicating a noncompetitive inhibition mechanism. The noncompetitive inhibition constant of catechin were estimated to be 1.7 mM. Because ECg and EGCg, the major tea polyphenols with a triphenol structure, generally have stronger biological activities than catechin (*2*, *27*), their effects on the $GABA_A$ receptor-elicited response were examined by varying the concentrations of both GABA and the compounds. Because the inhibition of the GABA receptor-mediated response by catechin derivatives was almost independent of the GABA concentration (**Figure 4a**), these derivatives inhibited the

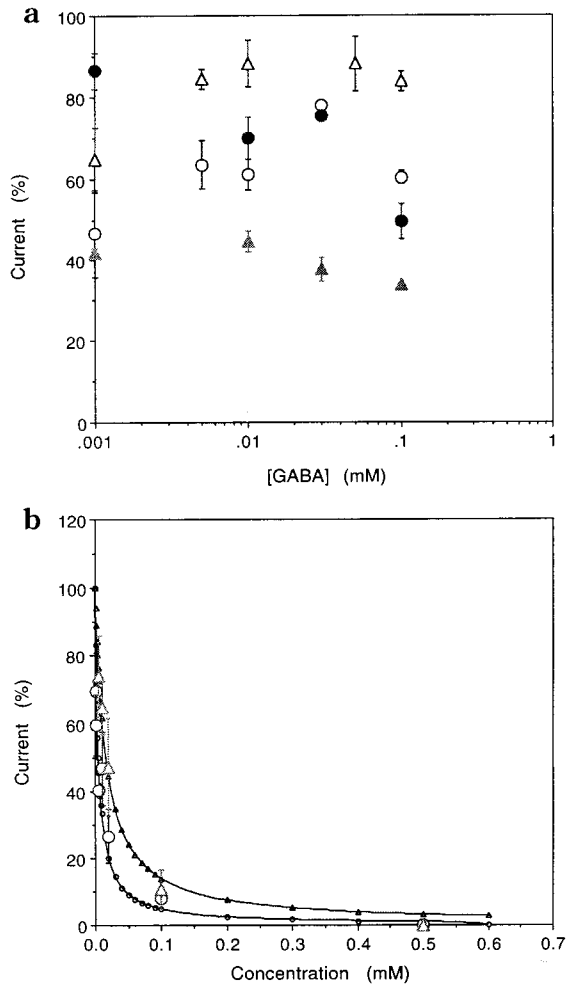

Figure 4. (a) Effects of GABA concentration on the inhibition of $GABA_A$ receptor response in the presence of 10 μM ECg (○), 10 μM EGCg (△), 1 mM EC (●), or 1 mM EGC (▲). $p < 0.05$ between the control value and the value in the presence of the component, by Student's t test. (b) Dose–response of inhibition of ECg (○) and EGCg (△) in the presence of 1 μM GABA. The IC_{50} values of ECg and EGCg were estimated to be 5.5 and 16 μM, respectively. $p < 0.05$ between the control value and the value in the presence of the component, by Student's t test.

Table 1. Inhibition Constants (K_i) of Catechin Derivatives for $GABA_A$ Receptors When the Noncompetitive Inhibition Mechanism Is Assumed[a]

compound	K_i (mM)	compound	K_i (mM)
catechin	1.7	ECg	0.019
EC	2.4	EGCg	0.062
EGC	0.65		

[a] Inhibition constants were calculated from the results in **Figure 4a**. However, data in the presence of 1 μM GABA were omitted when the inhibition constants for ECg and EGCg were calculated.

response in a noncompetitive manner. Their noncompetitive inhibition constants are summarized in **Table 1**. As expected, ECg and EGCg inhibited the response more strongly than other derivatives. However, ECg and EGCg inhibited the response slightly more strongly at 1 μM GABA concentration than at higher GABA concentrations (**Figure 4a**). Therefore, we measured the dose−inhibition relationship in the presence of 1 μM GABA (**Figure 4b**) and thereby estimated the concentrations for half-maximal inhibition (IC_{50}) to be 5.5 μM for ECg and 16 μM for EGCg. These IC_{50} values were smaller than the

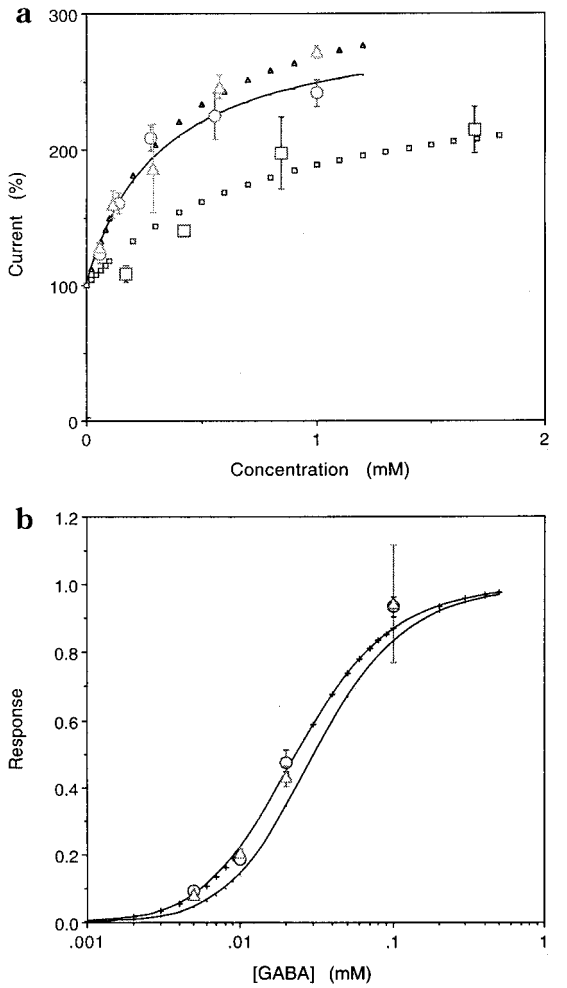

Figure 5. (a) Dose–potentiation of linalool (○), geraniol (△), and leaf alcohol (□). The responses of $GABA_A$ receptors were measured in the presence of 5 μM GABA. The theoretical curves were drawn by using the following constants: $K_1 = 59$ μM; $K_p = 0.32$ mM, $V_m = 296\%$, and $K_{1p} = 31$ μM for linalool (—); $K_p = 0.34$ mM, $V_m = 332\%$, and $K_{1p} = 29$ μM for geraniol (smaller triangles); and $K_p = 0.78$ mM, $V_m = 258\%$, and $K_{1p} = 34$ μM for leaf alcohol (smaller boxes). $p < 0.05$ between the control value and the value in the presence of the component, by Student's t test. (b) Effects of GABA concentration on the potentiation of $GABA_A$ receptor response in the presence of 0.28 mM linalool (○) and 0.29 mM geraniol (△). Theoretical curves were calculated by using the values in (a), but only the curve for linalool is shown here because both curves were very similar. The maximum response elicited by a high concentration of GABA without any compound was taken as 1.

K_i values estimated above. Moreover, inhibition by these compounds showed much variation, depending on both the frogs used and the oocytes used from a given frog.

To clarify the mechanism of potentiation by fragrant alcohols, the responses of $GABA_A$ receptors were measured at different concentrations of both GABA and the alcohols. The potentiation by alcohols increased gradually with their concentrations and finally reached a saturation level (**Figure 5a**). The dissociation constant (K_p) and the maximum potentiation (V_m) when the potentiation site of all receptors was occupied by the alcohol were estimated from the results shown in **Figure 5a**. The dissociation constant of GABA (K_{1p}) when the potentiation site of the receptors was occupied by the alcohol was also estimated as shown in **Figure 5a** (*21*). **Figure 5b** shows the effect of GABA concentration on the alcohol-elicited potentiation and reveals a shift of the GABA dose−response curve to a lower

Table 2. Comparison of the Effects of Leaf Alcohol, Caffeine, Catechin, and ECg on 1 μM GABA-Elicited Response with the Effect of a Mixture of Leaf Alcohol and the Other Compound

compound(s)	response (%)
control	100
+ 1 mM leaf alcohol	223.7 ± 12.0
+ 1 mM caffeine	72.9 ± 6.3
+ 1 mM leaf alcohol and 1 mM caffeine	149.6 ± 6.9
+ 1 mM catechin	61.8 ± 9.5
+ 1 mM leaf alcohol and 1 mM catechin	125.2 ± 6.7
+ 0.02 mM ECg	26.5 ± 8.1
+ 1 mM leaf alcohol and 0.02 mM ECg	79.8 ± 6.4

[a] The response elicited by 1 μM GABA was taken as the control (100%). $p < 0.01$ by Student's t test for all data.

concentration, indicating the enhancement of GABA binding to $GABA_A$ receptors (*28*, *29*).

Because green tea includes compounds that both potentiate and inhibit the response of $GABA_A$ receptors, we examined the effect of an aqueous extract of green tea on the response. One gram of dry green tea was extracted in 20 mL of hot frog normal Ringer solution for 3 min. Addition of this extract to 100 μM GABA solution inhibited the response of $GABA_A$ receptors [addition of 0.1% (v/v) extract; 71.2 ± 11.0% ($n = 4$), addition of 0.5% (v/v) extract; 57.2 ± 7.8% ($n = 3$), $p < 0.01$ by Student's t test]. Although addition of the extract perturbs the GABA concentration slightly (**Figure 1a**), it is negligible, because 100 μM GABA induces almost maximum current in GABA dose−response curve.

Although hydrophilic compounds are selectively taken into the brain through the blood−brain barrier by transporters, lipophilic compounds go through the blood−brain barrier easily to the brain and thus may modulate the responses of $GABA_A$ receptors. Therefore, we extracted lipophilic tea components from tea aqueous solution with ethyl ether and dissolved the components in ethanol to examine their effects on the $GABA_A$ receptor responses. This tea extract did not induce any $GABA_A$ receptor responses but inhibited the 10 μM GABA-elicited response of the receptors dose dependently [0.2 μL/mL extract; 89.5 ± 6.7% ($n = 5$), 1 μL/mL extract; 72.7 ± 14.2% ($n = 4$), 2 μL/mL extract; 64.9 ± 7.2% ($n = 4$), $p < 0.03$ by Student's t test].

To examine the effect of fragrant tea components on the $GABA_A$ receptor response in the presence of caffeine, catechin, or ECg, the GABA-elicited responses in the presence of the mixture of leaf alcohol and caffeine, catechin, or ECg were compared with those in the presence of only each compound alone (**Table 2**). As expected, addition of leaf alcohol decreased the inhibition of the $GABA_A$ receptor response by caffeine, catechin, or ECg.

DISCUSSION

As expected, aqueous extracts of green tea, black tea, and oolong tea induced the response of $GABA_A$ receptors that had been expressed in *Xenopus* oocytes by injecting cRNAs of α_1 and β_1 subunits of the bovine $GABA_A$ receptors, probably because of the presence of GABA-like compound(s) in the tea. Because an aqueous extract of green tea also contained a high amount of inhibitors of the receptors such as caffeine or catechin derivatives, the amount of GABA-like compound(s) in the extract must be more than that estimated from the comparison of the electrical response with the control. Reportedly, some special types of tea that contain large amounts of GABA decreased the blood pressure of rats and humans when they drank this tea every day for a few months (*1*). It is unlikely that GABA goes into the brain directly and activates $GABA_A$ receptors, because GABA, which is soluble in aqueous solutions, is selectively taken into the brain through the blood−brain barrier by GABA transporters. However, a high concentration of GABA in the blood may lead to an increase of the GABA concentration in the brain.

Caffeine is known to have a stimulating effect on the central nervous system through effects on phosphodiesterase, ryanodine receptor, and purine receptors (*3*, *10*). Our experiments showed that caffeine also inhibited the responses of $GABA_A$ receptors competitively. Catechin, which acts as an antioxidant and is effective in preventing cancer or aging (*2*), also inhibited the $GABA_A$ receptor response noncompetitively. ECg and EGCg especially caused strong but variable inhibition of the response, perhaps because their binding site in the receptors is located at the interface between the receptors and the membrane lipids (*11*) or because these compounds also bind to the lipid bilayers with high affinity and may perturb the membrane structure (*30*). Moreover, they inhibited the response slightly more strongly at 1 μM GABA concentration than at higher GABA concentrations. Therefore, these compounds may not only bind strongly to a noncompetitive inhibition site but also bind weakly to GABA binding sites. Although polyphenols can be absorbed to some extent through the intestinal epithelium and are detectable in various organs, the concentration of polyphenols in the blood plasma and organs is low. Nakagawa et al. (*31*) have reported that the plasma polyphenol concentration after the ingestion of a normal amount of tea (375 mg of EGCg intake) was 4.3 μM, which is slightly lower than the K_i or IC_{50} values. Because $GABA_A$ receptors are the main inhibitory neurotransmitter receptors in the brain, the stimulating effect of tea may result in part from the inhibition of the $GABA_A$ receptor responses by these compounds. It is noteworthy that phenol derivatives change their effect on the $GABA_A$ receptor responses from potentiation to inhibition as the number of their hydroxyl or phenyl groups is increased (*20*), although most monophenol compounds potentiate the response (*32*).

Some fragrant tea components such as higher alcohols potentiate the responses of $GABA_A$ receptors. These compounds may bind to the potentiation site of the receptor (*21*, *33*) and increase the GABA-binding affinity to the receptor as general anesthetics do (*28*, *29*). The dissociation constant (K_1) of the complex between GABA and the receptor decreased from 59 μM to about half of that value in the presence of a saturating amount of these fragrant compounds. However, some fragrant compounds such as dimethyl sulfide caused little effect on the response of $GABA_A$ receptors. The accumulation of essential oil components in the mouse brain when they were given by means of percutaneous or vapor exposure absorption was reported recently (*34*, *35*). The direct effects of fragrant compounds on $GABA_A$ receptors were suggested by a study showing that the inhalation of chamomile and lemon oil vapor decreased restriction-stress-induced increases in the plasma adrenocorticotropic hormone (ACTH) levels in ovariectomized rats, as did diazepam, a benzodiazepine derivative (*36*). It has also been reported that anticonflict effects of rose oil and its components were observed in a mouse behavior test (*37*, *38*).

Because tea includes compounds that both potentiate and inhibit the response of $GABA_A$ receptors, a tea extract in hot frog normal Ringer solution was applied to the receptors. Even a small amount (0.1%) of this extract inhibited the response of

the receptors. However, hydrophilic compounds are selectively taken into the brain through the blood−brain barrier by the transporters. Therefore, a tea extract made with ethyl ether, which thus included lipophilic compounds and could easily pass the blood−brain barrier, was also applied to the receptors. This tea extract also inhibited the response of the receptor, possibly because of the presence of large amounts of inhibitory compounds in the tea extract made using ethyl ether. Thus, if one drinks tea, the lipophilic compounds extracted with ethyl ether will be taken into the brain, inhibit the $GABA_A$ receptor response on the whole, and stimulate the human mind. However, fragrant compounds such as higher alcohols will help to prevent the overly stimulating effects of caffeine and catechin, which have other important activities described before (*1*, *2*). In fact, inhibition of the $GABA_A$ receptor response of caffeine or catechin was prevented in part by the addition of leaf alcohol, although the concentration of higher alcohols must be much lower than that of caffeine or catechin under physiological conditions. Thus, drinking tea will induce complex effects on the human body which are different from those of drugs that induce the strong but simple effects on the body and which may also cause harmful secondary effects. In the future, it is necessary to clarify how high of a concentration of these compounds reaches the synapses of the brain, which possibly depends on both the amount of uptake through the blood−brain barrier and their stability in the body.

ABBREVIATIONS USED

EC, (−)-epicatechin; ECg, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin gallate; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate.

ACKNOWLEDGMENT

We thank Prof. Eric A. Barnard of the MCR Center in the United Kingdom for the gift of cDNAs of the $GABA_A$ receptor subunits from bovine brain. We also thank Prof. Ryuzo Shingai of Iwate University for the preparation of the cDNAs.

LITERATURE CITED

(1) Yamanishi, T. *Science of Tea*; Shokabo: Tokyo, Japan, 1992 (in Japanese).
(2) Ina, K.; Sakata, K.; Tomita, I.; Isemura, M. *Tea Components and Their Function*; Kougakusyuppan: Kawasaki, Japan, 2001 (in Japanese).
(3) Kanarek, R. B.; Marks-Kaufman, R. *Nutrition and Behavior: New Perspectives*; Chapman and Hall: New York, 1991.
(4) Wafford, K. A.; Burnett, D. M.; Dunwiddie, T. V.; Harris, R. A. Genetic differences in the ethanol sensitivity of $GABA_A$ receptors expressed in *Xenopus* oocytes. *Science* **1990**, *249*, 291−293.
(5) Lovinger, D. M.; White, D.; Weight, F. F. Ethanol inhibits NMDA-activated ion current in hipocampal neurons. *Science* **1989**, *243*, 1721−1724.
(6) Lewohl, J. M.; Wilson, W. R.; Mayfield, R. D.; Brozowski, S. J.; Morrisett, R. A.; Harris, R. A. G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. *Nat. Neurosci.* **1999**, *2*, 1084−1090.
(7) Kobayashi, T.; Ikeda, K.; Kojima, H.; Niki, H.; Yano, R.; Yoshioka, T.; Kumanishi, Y. Ethanol opens G-protein-activated inwardly rectifying K^+ channels. *Nat. Neurosci.* **1999**, *2*, 1091−1097.
(8) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **1997**, *389*, 816−824.
(9) McKemy, D. D.; Neuhausser, W. M.; Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* **2002**, *416*, 52−58.
(10) Cardinali, D. P. Methylxanthines: possible mechanisms of action in brain. *Trends Pharmacol. Sci.* **1980**, *1*, 405−407.
(11) Nicholls, D. G. *Proteins, Transmitters and Synapses*; Blackwell Scientific Publications: Oxford, U.K., 1994; pp 155−199.
(12) Changeux, J. P.; Devillers-Thiery, A.; Chemouilli, P. Acetylcholine receptor: an allosteric protein. *Science* **1984**, *225*, 1335−1345.
(13) Aoshima, H.; Mitsusada, N.; Nishino, T. Effects of aliphatic alcohols and food additives on nicotinic acetylcholine receptors in *Xenopus* oocytes. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1776−1779.
(14) Chebib, M.; Johnston, A. R. GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. *J. Med. Chem.* **2000**, *43*, 1427−1447.
(15) Rupprecht, R.; Holsboer, F. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci.* **1999**, *22*, 410−416.
(16) Olsen, R. W. The molecular mechanism of action of general anesthetics: structural aspects of interactions with $GABA_A$ receptors. *Toxicol. Lett.* **1998**, *100−101*, 193−201.
(17) Aoshima, H. Potentiation and inhibition of ionotropic neurotransmitter receptors expressed in *Xenopus* oocyte by linoleic acid and its hydroperoxide. *J. Neurochem.* **1996**, *66*, 1300−1305.
(18) Aoshima, H.; Tenpaku, Y. Modulation of GABA receptors expressed in *Xenopus* oocytes by 13-L-hydroxylinoleic acid and food additives. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 2051−2057.
(19) Aoshima, H.; Hamamoto, K. Potentiation of $GABA_A$ receptors expressed in *Xenopus* oocytes by perfume and phytoncid. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 743−748.
(20) Aoshima, H.; Hossain, S. J.; Imamura, K.; Shingai, R. Effects of bisphenol A and its derivatives on the response of $GABA_A$ receptors expressed in *Xenopus* oocytes. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 2070−2077.
(21) Aoshima, H.; Hossain, S. J.; Hamamoto, K.; Yokoyama, T.; Yamada, M.; Shingai, R. Kinetic analyses of alcohol-induced potentiation of the response of $GABA_A$ receptors composed of α_1 and β_1 subunits. *J. Biochem.* **2001**, *130*, 703−709.
(22) Kusano, K.; Miledi, R.; Stinnarkre, J. Cholinergic and catecholaminergic receptors in the *Xenopus* oocytes membrane. *J. Physiol.* **1982**, *328*, 143−170.
(23) Kobayashi, S.; Aoshima, H. Time-course of the induction of acetylcholine receptors in *Xenopus* oocytes injected with mRNA from *Electrophorus electricus* electroplax. *Dev. Brain Res.* **1986**, *24*, 211−216.
(24) Aoshima, H.; Inoue, Y.; Hori, K. Inhibition of ionotropic neurotransmitter receptors by antagonists: strategy to estimate the association and the dissociation rate constant of antagonists with very strong affinity to the receptors. *J. Biochem.* **1992**, *112*, 495−502.
(25) Aoshima, H.; Anan, M.; Ishii, H.; Iio, H.; Kobayashi, S. Minimal model to account for the membrane conductance increase and desensitization of γ-aminobutyric acid receptors synthesized in the *Xenopus* oocytes injected with rat brain mRNA. *Biochemistry* **1987**, *26*, 4811−4816.
(26) Aoshima, H. Inhibition schemes for acetylcholine receptor-mediated ion translocation in the presence of various kinds of inhibitors. *J. Biochem.* **1983**, *94*, 1739−1751.
(27) Kobayashi, Y.; Suzuki, M.; Satsu, H.; Arai, S.; Hara, Y.; Suzuki, K.; Miyamoto, Y.; Sjimizu, M. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epitherial cells by a competitive mechanism. *J. Agric. Food Chem.* **2000**, *48*, 5618−5623.

(28) Jones, M. Y.; Brooks, P. A.; Harrison, N. L. Enhancement of γ-aminobutyric acid-activated Cl^- currents in cultured rat hippocampal neurons by three volatile anaesthetics. *J. Physiol.* **1992**, *449*, 279−293.

(29) Franks, N. P.; Lieb, W. R. Molecular and cellular mechanisms of general anaesthesia. *Nature* **1994**, *367*, 607−614.

(30) Hashimoto, T.; Kumazawa, S.; Nanjo, F.; Hara, Y.; Nakayama, T. Interaction of tea catechins with lipid bilayers investigated with liposome systems. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 2252−2255.

(31) Nakagawa, K.; Okuda, S.; Miyazawa, T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1981−1985.

(32) Krasowski, M. D.; Jenkins, A.; Flood, P.; Kung, A. Y.; Hopfinger, A. J.; Harrison, N. L. General anesthetic potencies of a series of propofol analogs correlate with potency for potentiation of γ-aminobutyric acid (GABA) current at the $GABA_A$ receptor but not with lipid solubility. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 338−351.

(33) Mihic, S. J.; Ye, Q.; Wick, M. J.; Koltchine, V. V.; Krasowski, M. D.; Finn, S. E.; Mascia, M. P.; Valenzuela, C. F.; Hanson, K. K.; Greenblatt, E. P.; Harris, R. A.; Harrison, N. L. Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. *Nature* **1997**, *389*, 385−389.

(34) Inoue, S.; Yamaguchi, H. Systemic absorption of essential oils in mice by holistic vapor exposure. *Aroma Res.* **2000**, *1* (3), 72−79.

(35) Inoue, S.; Yamaguchi, H. Systemic absorption and metabolism of essential oils in rats by holistic vapor exposure. *Aroma Res.* **2000**, *1* (4), 77−81.

(36) Yamada, K.; Miura, T.; Mimaki, Y.; Sashida, Y. Effect of inhalation of chamomile oil vapor on plasma ACTH level in oavari-ectomized-rat under restriction stress. *Biol. Pharm. Bull.* **1996**, *19*, 1244−1246.

(37) Umezu, T. Anti-conflict effects of plant-derived essential oils. *Pharmacol. Biochem. Behav.* **1999**, *64*, 35−40.

(38) Umezu, T. Behavioral effects of plant-derived essential oils in the Geller type conflict test in mice. *Jpn. J. Pharmacol.* **2000**, *83*, 150−153.

Received for review December 7, 2001. Revised manuscript received April 17, 2002. Accepted April 17, 2002. This work was supported in part by the Foundation of Kyoto Prefecture for Green Tea Research in Japan.

JF011607H