Biosci. Biotechnol. Biochem., **68** (9), 1842–1848, 2004

Fragrances in Oolong Tea That Enhance the Response of $GABA_A$ Receptors

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Received January 21, 2004; Accepted June 10, 2004

We electrophysiologically investigated the effect of some fragrant compounds in oolong tea on the response of ionotropic γ-aminobutyric acid (GABA) receptors ($GABA_A$ receptors) which were expressed in *Xenopus* oocytes. Of the tested fragrances in oolong tea, *cis*-jasmone, jasmine lactone, linalool oxide and methyl jasmonate significantly potentiated the response. Among these, *cis*-jasmone and methyl jasmonate potently potentiated the response, having a respective dissociation constant of the compound (K_p) and maximum potentiation (V_m) of 0.49 mM and 322% for *cis*-jasmone, and 0.84 mM and 450% for methyl jasmonate. Inhalation of 0.1% *cis*-jasmone or methyl jasmonate significantly increased the sleeping time of mice induced by pentobarbital, suggesting that these fragrant compounds were absorbed by the brain and thereby potentiated the $GABA_A$ receptor response. Both of these compounds may therefore have a tranquillizing effect on the brain.

Key words: fragrance; ionotropic γ-aminobutyric acid ($GABA_A$) receptor; oolong tea; *Xenopus* oocyte

Oolong tea is one of the types of processed tea consumed by many people and is reportedly considered to have such benefits as anti-obesity, anti-cancer, anti-tumor and anti-oxidative effects on human health. It is also known that some components of tea decrease the blood pressure and blood sugar or control the amount of cholesterol in the blood.[1,2] Drinking tea wakes us up and relieves drowsiness, stress and neuralgia. Oolong tea has special sensory characteristics because it is semi-fermented. The manufacturing process involving fermentation gives oolong tea and black tea their unique floral, fruity and jasmine-like aroma which is supposed to originate from the presence of such fragrant compounds[1,2] as *cis*-jasmone, jasmine lactone, linalool oxide and methyl jasmonate. Jasmine derivatives are also present in jasmine tea, which is a typical flower tea originating in China.

The γ-aminobutyric acid type A ($GABA_A$) receptor is the major ligand-gated Cl^- ion channel conferring fast inhibitory synaptic transmission in the central nervous system. The enhancement of neural inhibition by GABA is a common therapeutic strategy for treating central nervous system (CNS) diseases such as anxiety disorders, sleep disturbances, muscle spasms and seizure disorders. The $GABA_A$ receptors have a complex pharmacology[3] with binding sites for direct GABA agonists and antagonists, together with multiple allosteric sites for benzodiazepine tranquilizers, for barbiturate central nervous system depressants, for both synthetic and endogenous steroids,[4] for general anesthetics[5] and for ethanol.[6] These structurally diverse compounds enhance the response of $GABA_A$ receptors in the presence of a low concentration of GABA. We have reported in previous papers the inhibition or potentiation of the response of $GABA_A$ receptors by the various components present in foods,[7] beverages[8,9] and essential oils used for aromatherapy,[10] and thereby found that the fragrant compounds generally potentiated the $GABA_A$ receptor responses. We also proposed a simple kinetic model for the potentiation of $GABA_A$ receptor responses.[10] The fragrant compounds that potentiate the $GABA_A$ receptor responses could be used as a relaxing agent or medication in aromatherapy. It is thus important to understand the action of the fragrances in oolong tea on the $GABA_A$ receptors. We used the two-electrode voltage-clamping method to investigate the effects of an oolong tea extract and its fragrances on the responses of the ionotropic $GABA_A$ receptors, which comprised the α_1 and β_1 subunits of the bovine receptors, expressed in *Xenopus* oocytes. We also measured the effects of *cis*-jasmone and methyl jasmonate on the sleeping time induced by pentobarbital in mice.

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Abbreviations: ACTH, adrenocorticotropic hormone; GABA, γ-aminobutyric acid; $GABA_A$ receptor, ionotropic GABA receptor

Materials and Methods

Materials. γ-Aminobutyric acid (GABA) was purchased from Nacalai Tesque (Kyoto, Japan). *Cis*-jasmone, jasmine lactone, linalool oxide and methyl jasmonate were each supplied by Suntory Ltd. (Osaka, Japan). All chemicals were of guaranteed reagent quality. Oolong tea (Transworks Co., Ltd., Japan) was purchased from a local super-market.

To prepare the diethyl ether extract, 10 g of oolong tea was extracted in 200 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). A 100-ml amount of diethyl ether was added to the extract, which was shaken vigorously for 1 min, before the upper ethyl ether phase was separated from the aqueous phase in a separating funnel. The solvent was removed in an evaporator, and the resulting solid was dissolved in 100 μl of ethanol and stored at 4 °C. The effect of this oolong tea extract on the GABA-elicited response of the bovine $GABA_A$ receptors was examined by adding the extract to a GABA solution to treat oocytes.

Preparation of cRNAs of α_1 and β_1 subunits of the bovine $GABA_A$ receptors. cRNAs of the α_1 and β_1 subunits of the bovine $GABA_A$ receptors were synthesized from cloned cDNAs of the bovine brain receptors by using RNA polymerase according to the standard procedure. The cloned cDNAs were presented by Prof. Eric A. Barnard of the MRC Center (London, U.K.).

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. The oocytes were then manually detached from the inner ovarian epithelium and follicular envelope after being incubated in a collagenase (type I, 1 mg/ml; Sigma) solution for 1 h. These oocytes were microinjected with the 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 $Ca(NO_3)_2$, 0.41 mM $CaCl_2$ and 0.82 mM $MgSO_4$ 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 mesh of a small chamber (0.3 ml) and impaled with two microelectrodes, each 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 mesh was continuously perfused from the bottom with a frog normal Ringer's solution with or without the compounds by the gravity feed system, usually at a flow rate of 2 ml/min.

Measurement of the receptor response. GABA was dissolved in a frog normal Ringer's solution. To examine the effect of an extract or fragrance of oolong tea on the GABA-elicited response, each test compound was added to the solution. The solution was changed by manipulating the cock in 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 an extract or compound; in some cases, the compound was added 1 min before its co-application with GABA when desensitization of the receptors had been significantly induced before binding equilibrium of the compound was attained.[11] The measurement was repeated several times with the same oocyte, and control values were obtained every two or three measurements. Each data value is usually the mean from four experiments. To eliminate any desensitization of the receptors, the oocyte was washed for more than 10 min in a frog normal Ringer's solution before the next measurement, because such desensitization of the $GABA_A$ receptors was a reversible process and the receptors usually recovered after 10 min of washing.

Measurement of the pentobarbital-induced sleep in mice. Male ddY mice at the age of 28 days (Japan SLC Co., Shizuoka, Japan) were housed five per cage under a standardized light-dark cycle (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 provided *ad libitum*. All animals received humane care in accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacology Society.

Pentobarbital-induced sleep was measured as previously reported by Matsumoto *et al.*,[12] the inhalation apparatus being a lidded cage ($L = 31.5 \times W = 21.0 \times H = 21.0$ cm).

The aroma of *cis*-jasmone or methyl jasmonate was generated by bubbling it with an air-pump. Air containing the fragrant compound was introduced into the cage. Male ddY strain male mice (7 weeks old) were exposed to the aroma 30 min prior to the administration of pentobarbital, because it possibly took some time before the aroma was absorbed into the blood through respiration. Sodium pentobarbital (50 mg/kg) was administered to the mice intraperitoneally (ip). The sleeping time was measured while the mice were inhaling the aroma, and is calculated as the time between the disappearance and recovery of the righting reflex.

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

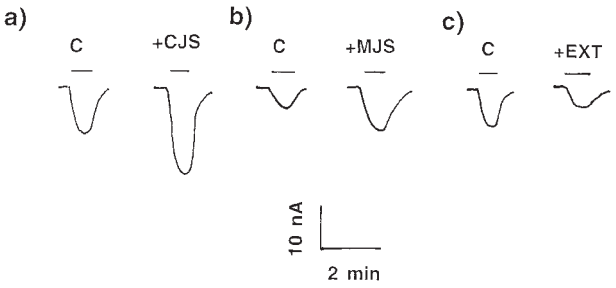

Fig. 1. Potentiation or Inhibition of the 0.25 μM GABA-Mediated Current of $GABA_A$ Receptors Expressed in *Xenopus* Oocytes by the 0.5 mM *cis*-Jasmone (a), Methyl Jasmonate (b) and Oolong Tea Extract (c).

All traces were usually obtained with a voltage clamp at −40 mV. An inward current is shown as a downward curve. The upper bars show when GABA or a 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), b) and c) were from different oocytes. The oolong tea extract was prepared as described in the Materials and Methods section. CJS, 0.5 mM *cis*-jasmone; MJS, 0.5 mM methyl jasmonate; EXT, 0.1 μl/ml of oolong tea extract.

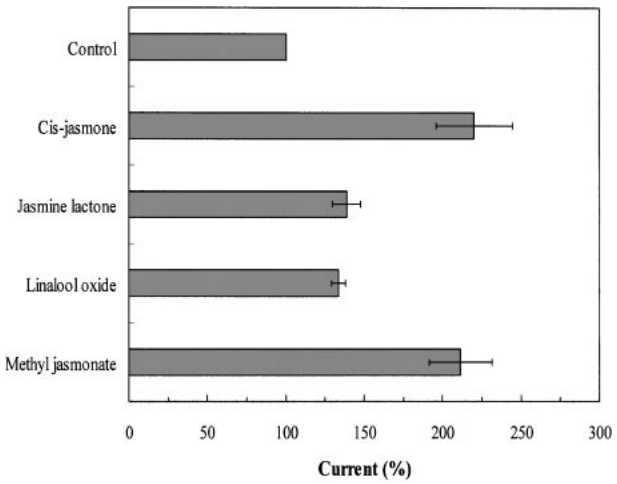

Fig. 2. Effects of the Major Components of Oolong Tea at 0.5 mM on the Response of $GABA_A$ Receptors Elicited by 0.25 μM GABA.

The response elicited by 0.25 μM GABA without any oolong tea component is taken to be 100%. Each value is the mean ± SD (bar) from at least four experiments. $p < 0.05$ between the control value and the value in the presence of a component by Student's *t* test.

Results

Potentiation of the $GABA_A$ receptor response by the fragrant compounds from oolong tea

Figures 1a and 1b show potentiation of the electrical GABA-elicited response of the $GABA_A$ receptors by *cis*-jasmone and methyl jasmonate at 0.5 mM. The receptors were expressed in *Xenopus* oocytes by injecting cRNAs synthesized from cloned cDNAs of the α_1 and β_1 subunits of bovine $GABA_A$ receptors. The electrical response was measured by using the two-electrode voltage-clamping method.

Figure 2 shows the effect of the fragrances at 0.5 mM on the response of the $GABA_A$ receptors composed of the α_1 and β_1 subunits elicited by 0.25 μM GABA. The examined fragrances, *cis*-jasmone, jasmine lactone, linalool oxide and methyl jasmonate, all significantly potentiated the response. These fragrances themselves caused no electrical responses in the oocytes expressing the $GABA_A$ receptors in the range of concentrations used (data no shown).

Dose-potentiation curve of cis-jasmone and methyl jasmonate

Since *cis*-jasmone and methyl jasmonate strongly potentiated the response of the $GABA_A$ receptors, we investigated the dose-potentiation relationship of both GABA and the fragrant compounds. Figure 3 shows that the potentiation gradually increased with increasing concentration of each compound and ultimately reached a saturation level. The dissociation constant for the compound (K_p) and maximum potentiation of the receptors (V_m), when the potentiation sites of all receptors were occupied by the compounds, were estimated by assuming a simple equilibrium condition between the compound and the receptor (Table 1). The

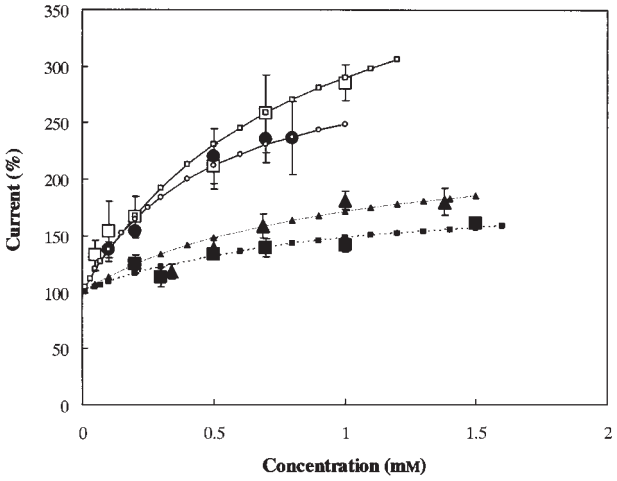

Fig. 3. Dose-potentiation of *cis*-Jasmone (●), Jasmine Lactone (▲), Methyl Jasmonate (□) and Linalool Oxide (■) in the Presence of 0.25 μM GABA.

The dissociation constant (K_p), maximum potentiation (V_m) and dissociation constant of GABA (K_{1p}) were estimated from these data. Each value is the mean ± SD (bar) from at least four experiments. $p < 0.05$ between the control value and the value in the presence of the component by Student's *t* test. The theoretical curves for *cis*-jasmone (small circles), jasmine lactone (small triangles), methyl jasmonate (small squares) and linalool oxide (black small squers) were drawn by using the constants shown in Table 1.

dissociation constant of GABA (K_{1p}), when the potentiation site of the receptor was fully occupied with the compound, was also estimated on the basis of the simple kinetic model that has been proposed.[10] Figure 4 shows the effect of GABA concentration on the *cis*-jasmone- and methyl jasmonate-elicited potentiation of the receptors. The addition of these compounds shifted the GABA dose-response curve to a lower concentration,

Table 1. Estimated Dissociation Constant (K_p), Maximum Potentiation (V_m) and Dissociation Constant of GABA (K_{1p}) When All the Potentiation Sites Were Occupied by the Fragrant Compound

Compound	K_p (mM)	V_m (%)	K_{1p} (μM)
Control			59
Cis-jasmone	0.49	322	33
Jasmine lactone	0.94	239	38
Linalool oxide	0.93	193	43
Methyl jasmonate	0.84	450	28

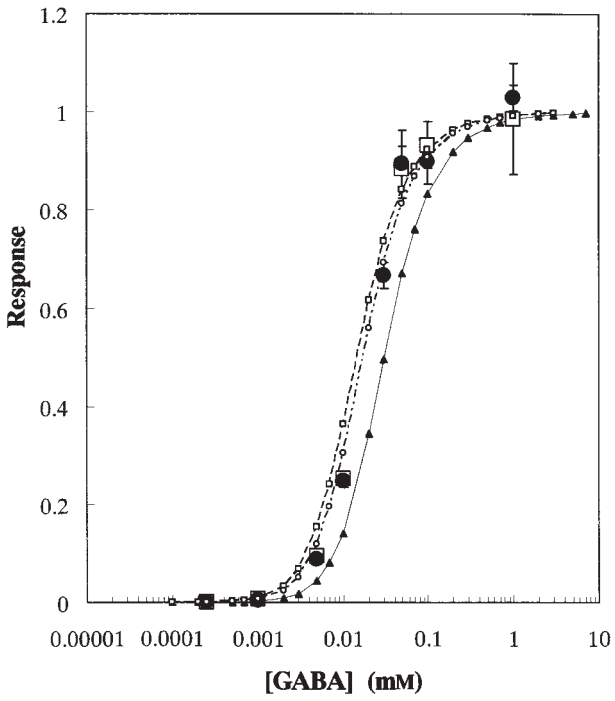

Fig. 4. Effects of GABA Concentration on the Potentiation of $GABA_A$ Receptor Response in the Presence of *cis*-Jasmone (●) and Methyl Jasmonate (□) at a Concentration of 0.5 mM.

The theoretical curves (small circles for *cis*-jasmone; small squares for methyl jasmonate) were calculated by using the values in Table 1. The maximum response elicited by a high concentration of GABA without any compound was taken as 1.

indicating enhanced GABA binding to the $GABA_A$ receptors.[13,14]

Effect of cis-jasmone and methyl jasmonate on the sleeping time induced by pentobarbital in mice

As shown in Figs. 2 and 3 that *cis*-jasmone and methyl jasmonate potently potentiated the $GABA_A$ receptor response, we attempted to learn whether these compounds act on the $GABA_A$ receptor *in vivo*. It is known that pentobarbital induces sleep by potentiating the $GABA_A$ receptor response.[12] Figure 5 shows the effect of *cis*-jasmone and methyl jasmonate on the sleeping time of mice induced by an injection of pentobarbital. Both the compounds significantly prolonged the sleeping time in mice induced by pentobarbital in comparison with the control (pentobarbital alone).

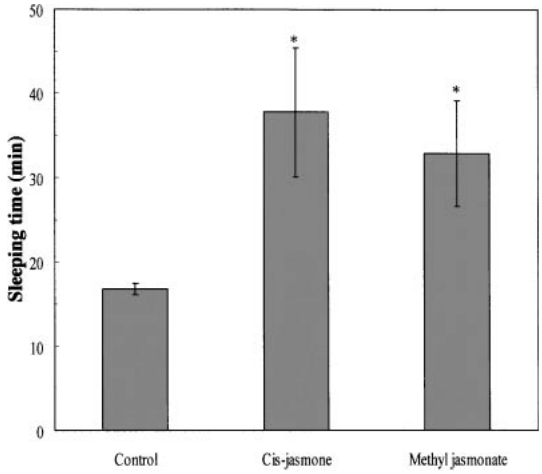

Fig. 5. Effects of *cis*-Jasmone and Methyl Jasmonate on the Sleeping Time Induced by Pentobarbital in Mice.

Sodium pentobarbital (50 mg/kg) was injected intraperitoneally 30 min after the inhalation of *cis*-jasmone or methyl jasmonate at 0.1%. Each value is the mean ± SD (bar) from four experiments. $^*p < 0.05$ between the control value and the value in the presence of the component by Student's *t* test.

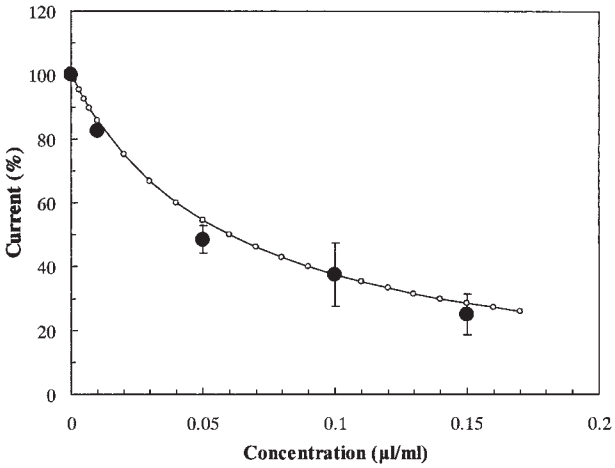

Fig. 6. Dose-response Characteristics for the Inhibition of Diethyl Ether Extract of Oolong Tea in the Presence of 0.25 μM GABA.

The theoretical curve (small circles) was drawn by using the noncompetitive inhibition constant of 0.058 μl/ml (v/v). Each value is the mean ± SD (bar) from at least four experiments. $p < 0.05$ between the control value and the value in the presence of the component by Student's *t* test.

Inhibition of the $GABA_A$ receptor-elicited response by a lipophilic extract of oolong tea

Lipophilic components easily pass through the blood-brain barrier to the brain and may thereby modulate the response of the $GABA_A$ receptors. The diethyl ether extract of oolong tea, which would have contained the lipophilic components, dose-dependently inhibited the 0.25 μM GABA-elicited $GABA_A$ receptor response (Figs. 1c and 6). We also found that the inhibition by

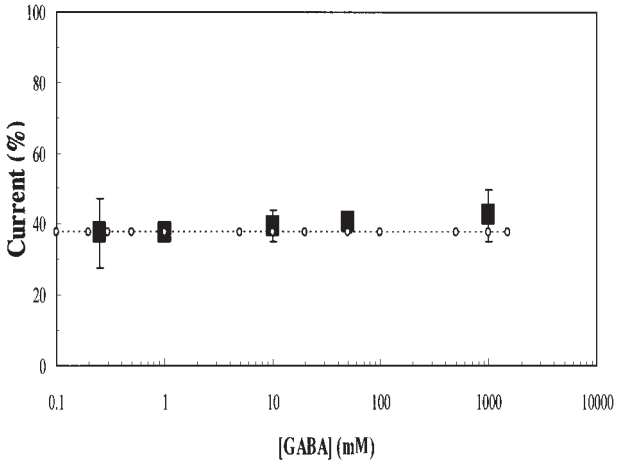

Fig. 7. Effects of GABA Concentrations on the Inhibition by 0.1 μl/ml (v/v) Diethyl Ether Extract of Oolong Tea.

The theoretical curve was drawn by using the non-competitive inhibition constant of 0.058 μl/ml (v/v). $p < 0.05$ between the control and the value in the presence of the extract by Student's t test.

the diethyl ether extract of oolong tea was independent of the GABA concentration, indicating a non-competitive inhibition mechanism (Fig. 7).

Discussion

The fragrances in essential oils for aromatherapy and phytocids emanating from trees of the forest have been used to induce mental tranquility or relaxation and to aid sleep in humans.[13] It has been reported that the fragrance of whiskey[14] and wine[15] has an effect on human brain functions, altering the mood and relaxing consciousness. The effect of the green odor of green tea, a mixture of *trans*-2-hexenal and *cis*-3-hexenol, on rats and monkeys has recently been reported. The green odor attenuated stress-induced elevation of the plasma adrenocorticotropic hormone (ACTH) and body temperature in rats.[16] Positron emission tomography has shown that the green odor activated the anterior cingulated gyrus in monkeys.[17] A power spectral analysis of the heart rate variability showed that the jasmine tea odor affected the autonomic nervous system of humans, depending on both the odor concentration and the subject's preference for the odor.[18] These effects of fragrances on rats, monkeys and humans are assumed to have been induced by stimulation of their olfactory system.

We have reported in previous papers[8–10,19,20] that the fragrant compounds in essential oils, perfumes, tea, coffee and whiskey potentiated the response of the $GABA_A$ receptors, and proposed that they might have contributed in part to altering moods and relaxing consciousness through their direct potentiation of the receptors, since it is known that potentiation of the $GABA_A$ receptor response by various drugs induces anxiolytic, anticonvulsant and sedative activity.[3] We further found that the fragrant compounds in whiskey played an important role in the potentiation of the $GABA_A$ receptor response and possibly in the sedative effect of whiskey.[21]

We have attempted in this present study to learn the effects of the fragrant compounds in oolong tea on the response of $GABA_A$ receptors, because GABA is the major fast inhibitory neurotransmitter in the central nervous system of the brain. All the examined compounds significantly potentiated the response of the $GABA_A$ receptors expressed in *Xenopus* oocytes (Fig. 2), although they induced no electrical response in the oocytes. This is possibly because the binding of these compounds to the potentiation site of the receptor increased the GABA-binding affinity[10] to the receptor as general anesthetics do.[22,23] The dissociation constant (K_1) of the complex between GABA and the receptor decreased from the control value in the presence of a saturating amount of each fragrant compound (Table 1), which is similar to the results in the previous reports on other fragrances of tea[8] and whiskey,[9] and also of essential oils[10] used for aromatherapy. It was hypothesized in the previous report[20] that fragrance compounds might be absorbed into the blood and carried to the brain through the blood-brain barrier, and then potentiate the $GABA_A$ receptor-mediated response which would have a tranquillizing effect on the brain. Our experiments in Fig. 5 suggest that *cis*-jasmone and methyl jasmonate had a tranquillizing effect on the mouse brain, possibly through potentiation of the $GABA_A$ receptor response, since pentobarbital is known to induce sleep by potentiating the $GABA_A$ receptor response and these fragrances worked additively, although the possibility cannot be excluded that these fragrant compounds inhibited pentobarbital decomposition in the liver and increased the mouse sleeping time. The direct effect of fragrant compounds on the $GABA_A$ receptors has been suggested by a study showing that inhaling chamomile and lemon oil vapor decreased the restriction-stress-induced increase in the plasma ACTH level of ovariectomized rats, as did diazepam, a benzodiazepine derivative.[24] Recent reports have also demonstrated that rose oil had an anti-anxiety-like effect in a mouse behavior test when it was applied to the mouse subcutaneously or intraperitoneally.[25,26] This effect of rose oil on the mouse behavior must have been through the central nervous system, and not through the olfactory system of the mouse. It has been reported that essential oil components were accumulated in the mouse brain when their vapors were exposed to rats.[27,28] Lipophilic compounds were reportedly transported to the brain not only through the lungs, but also through the nasal cavity.[29] However, it is necessary to prove in the future that the fragrant compounds in oolong tea are transported to the human brain and modulate the human mood and consciousness.[18]

Oolong tea contains not only the fragrant compounds measured in this paper, but also other fragrant compounds such as geraniol, linalool and benzyl alcohol

produced from their glycosides by β-primeverosidase.[1,2,30,31] These fragrant compounds strongly potentiated the $GABA_A$ receptor responses.[10,20] However, the diethyl ether extract of oolong tea strongly inhibited the response of the $GABA_A$ receptors. The inhibitory effect on the $GABA_A$ receptor response reportedly[8,19] mainly comes from xanthine and chatechin derivatives such as caffeine, (−)-epicatechin gallate and (−)-epigallocatechin gallate in tea. The oolong tea extract had these compounds in much greater quantity than the fragrant compounds. Inhibition of the response of the $GABA_A$ receptors causes excitation of the central nervous system which eventually stimulates the mind. These xanthine derivatives are also known to stimulate the central nervous system through the inhibition of adenosine receptors.[32,33] Therefore, drinking oolong tea will possibly induce a complex effect on the body and also on the mind. Fragrant compounds would induce a tranquillizing effect, while the lipophilic inhibitory ($GABA_A$ receptor) components of oolong tea would have an exciting effect on the brain. These fragrant compounds may be important in preventing the overly stimulating effects of the xanthine and catechin derivatives in teas, as has been proposed in previous papers.[8,19] These hypotheses are proposed on the basis of animal experiments, so further experiments on the human are necessary to prove the hypotheses in the future.[18]

Acknowledgments

We thank Prof. Eric A. Barnard of the MCR Center in the UK for presenting cDNAs of the $GABA_A$ receptor subunits from bovine brain.

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