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# Effects of Beer and Hop on Ionotropic γ-Aminobutyric Acid Receptors

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Beer induced the response of the ionotropic γ-aminobutyric acid receptors ($GABA_A$ receptors) expressed in *Xenopus* oocytes, indicating the presence of γ-aminobutyric acid (GABA)-like activity. Furthermore, the pentane extract of the beer, hop (*Humulus lupulus L.*) oil, and myrcenol potentiated the $GABA_A$ receptor response elicited by GABA. The $GABA_A$ receptor responses were also potentiated by the addition of aliphatic esters, most of which are reported to be present in beer flavor. Aliphatic esters showed the tendency to decrease in the potentiation of the $GABA_A$ receptor response with an increase in their carbon chain length. When myrcenol was injected to mice prior to intraperitoneal administration of pentobarbital, the pentobarbital-induced sleeping time of mice increased additionally. Therefore, the beer contained not only GABA-like activity but also the modulator(s) of the $GABA_A$ receptor response.

**KEYWORDS: Beer; GABA; $GABA_A$ receptor; hop; myrcenol**

## INTRODUCTION

Most fragrant components in whiskey potentiated the response of ionotropic γ-aminobutyric acid receptors ($GABA_A$ receptors) expressed in *Xenopus* oocytes as reported in a previous paper (*1*). Whiskey itself also potentiated the $GABA_A$ receptor response more than ethanol at the same concentration as that of the whiskey (*2*). Inhalation of whiskey in mice increased the sleeping time induced by pentobarbital more than ethanol at the same concentration as that of the whiskey. The potentiation of the $GABA_A$ receptor response increased with the aging of the whiskey in oak barrels. These results suggest that not only ethanol but also minor fragrant components in whiskey play an important role in the potentiation of the $GABA_A$ receptor response and possibly the sedative effect of whiskey. During aging, the sharp or raw odor typical of fresh whiskey distillates is modified to a rounded, soft, and mellow one, suggesting changes and the production of flavor in whiskey (*3*), which possibly causes the potentiation of the $GABA_A$ receptor response. The similar changes in red wines are also expected during their aging in wood barrels.

Recently, we also found that a yellow Chinese medicinal liquor called He Jiu (or Chinese wine) in China, which is composed of a liquor fermented from rice, honey, and seeds of *Lycium barbarum*, a traditional Chinese medicine, had γ-aminobutyric acid (GABA)-like activity and compounds that have the ability to potentiate the $GABA_A$ receptor response (*4*).

Beer is a popular alcoholic beverage that is produced from malted barley, water, and hops (*5*). Hops (*Humulus lupulus* L.) are added to the beer to give the characteristic bitter taste to it. In this paper, the effects of beer, extract of beer with pentane (EXT), hop oils, and some beer fragrant components on the $GABA_A$ receptor response were examined by using *Xenopus* oocyte expression system and an electrophysiological method. At the beginning, $GABA_A$ receptors expressed in the oocyte by injecting rat whole brain mRNA were used for the measurements (*6*), but fragrances showed similar effects on the response of the receptors to those expressed by injecting cRNAs of the $\alpha_1$ and $\beta_1$ subunits of bovine $GABA_A$ receptors. So, $GABA_A$ receptors composed of the $\alpha_1$ and $\beta_1$ subunits were used in this experiment.

## MATERIALS AND METHODS

**Materials.** Beer (5.5% ethanol) and red wine (14% ethanol) were supplied from Suntory Ltd. (Osaka, Japan). Shokoshu (8% ethanol), sake (8% ethanol), shochyu (25% ethanol), and awamori (15% ethanol) were supplied from Takara Co. (Kyoto, Japan). Shokoshu and sake were brews produced from glutinous rice in China and nonglutinous rice in Japan, respectively. Shochyu and awamori were popular distilled liquors produced from plant starches in Japan. Varietal hop oils (Perle and Hallertauer Magnum) were purchased from T. Hasegawa Co., Ltd. (Tokyo, Japan). Myrcenol was purchased from Taiyokoryo Co., Ltd. (Osaka, Japan). Myrcene and α-humulene (α-caryophyllene) were purchased from Tokyo Kasei Kogyo, Co., Ltd. (Tokyo, Japan). Bicuculline, a competitive antagonist of $GABA_A$ receptor, was purchased from Sigma-Aldrich (Tokyo, Japan).

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For preparation of the EXT, a mixture of 150 mL of beer and 50 mL of pentane was shaken vigorously in a separating funnel for a few minutes and 30 mL of the upper pentane phase was taken to a round flask. Pentane was evaporated by an evaporator, and the solid was dissolved in 200 $\mu$L of ethanol. The effect of the beer extract on the $GABA_A$ receptor response was examined by the addition of EXT to GABA solution to treat oocytes expressing $GABA_A$ receptors. All chemicals were of guaranteed reagent quality.

**Preparation of cRNA and Xenopus Oocytes.** The cRNAs of the $\alpha_1$ and $\beta_1$ subunits of the bovine $GABA_A$ receptors were synthesized from cloned cDNAs of bovine brain receptors with RNA polymerase (Promega, Madison, WI) according to standard procedures (*7*). The cloned cDNAs were provided by Prof. Eric A. Barnard in the Medical Research Council Center (London, United Kingdom).

Adult female frogs (*Xenopus laevis*) were purchased from Hamamatsu Seibutsu Kyozai Co. (Hamamatsu, Japan). The oocytes were dissected from adult frog ovaries that had been kept in ice for 1 h. They were manually detached from the inner ovarian epithelium and follicular envelope after incubation in collagenase (type I, 1 mg/mL; Sigma) solution for 1 h according to the procedure of Kusano et al. (*8*). The oocytes were microinjected with 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 $Ca(NO_3)_2$, and 0.41 mM $CaCl_2$ in 5 mM Tris at pH 7.6] containing 25 mg/L penicillin and 50 mg/L streptomycin at 15−18 °C for 2−7 days before electrophysiological measurements (*9*).

**Electrophysiological Measurements of the Receptor Response.** 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) according to the procedure described in a previous paper (*10*). To examine the effect of liquors on the $GABA_A$ receptors, the liquors diluted by frog normal Ringer solution (115 mM NaCl, 1 mM KCl, and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) were applied to the oocyte expressing the $GABA_A$ receptors. The induced electrical responses were compared with the response caused by 0.25 $\mu$M GABA. To examine the effect of EXT, hop oils, and some fragrant components on the GABA-elicited response, they were added to 0.25 or 1 $\mu$M GABA solutions. One or the other of the solutions was selected by switching a valve in the flow system. The control response was obtained by perfusing 0.25 $\mu$M GABA solution without the extract or compounds and was taken as 100%. The effect of the extract or compounds on the response of the receptors was measured by using a mixture of GABA and the extract or the compounds. 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 expressed as the means of four experiments. A Student's *t*-test was used to evaluate the significance of differences between the mean values of the sample and those of the control.

**Measurement of 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 condition (lights on at 7:00 a.m., off at 7:00 p.m.) at 24 °C and 60% humidity with food and water ad libitum. All animals received care in accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacology Society.

Pentobarbital-induced sleep of mice was measured as previously reported by Matsumoto et al. (*11*). Myrcenol at various amounts and sodium pentobarbital (50 mg/kg) were administered to mice intraperitoneally (ip). The sleeping time was measured as the time between the disappearance of the righting reflex and the recovery of the righting reflex. Five measurements were done for each sample. A Student's *t*-test was used to evaluate the significance of differences between the mean values of the sample and those of the control.

## RESULTS

To examine the effect of beer on $GABA_A$ receptors expressed in *Xenopus* oocytes by injecting cRNAs of the $\alpha_1$ and $\beta_1$ subunits of bovine $GABA_A$ receptors, their expression was confirmed by application of 0.25 $\mu$M GABA (**Figure 1a**).

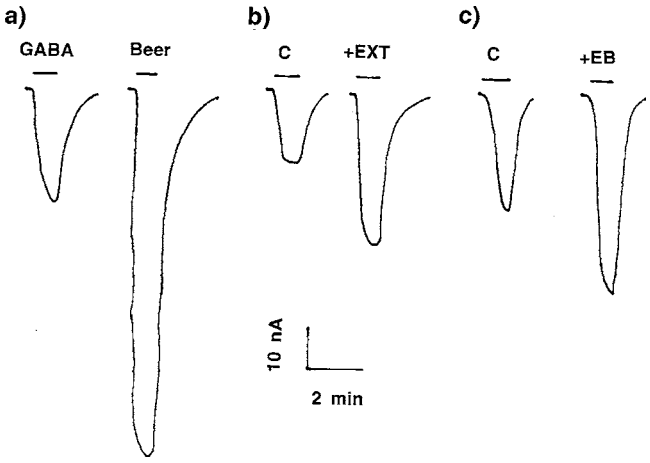


**Figure 1.** (**a**) $GABA_A$ receptor response elicited by 0.1% (v/v) beer as compared with that by 0.25 $\mu$M GABA. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs of the $\alpha_1$ and $\beta_1$ subunits of bovine $GABA_A$ receptors. The currents were measured with a voltage clamp at −40 mV. An inward current is shown as a downward curve. The upper bars show when 0.25 $\mu$M GABA or 0.1% (v/v) beer was applied. (**b**) Potentiation of $GABA_A$ receptor response by EXT. The preparation of the beer extract was described in detail in the Materials and Methods. Key: C, 0.25 $\mu$M GABA; +EXT, 0.25 $\mu$M GABA + 0.25% (v/v) beer extract. (**c**) Potentiation of $GABA_A$ receptor response by EB. Key: C, 1 $\mu$M GABA; +EB, 1 $\mu$M GABA + 1 mM EB. Each pair of the responses was obtained from the same injected oocyte, but the responses of different pairs were from a different injected oocyte.

Application of 0.1% (v/v) beer also induced a clear electrical response (**Figure 1a**). This response induced by beer was inhibited by bicuculline, a competitive inhibitor of $GABA_A$ receptors (data not shown). This result indicated the presence of GABA-like activity in the beer. The concentration of GABA in the beer was estimated to be about 0.8 mM equivalents from the comparison of the response with the control, when it was assumed that GABA-like activity came from GABA itself. For comparison, we examined the GABA-like activity of various types of liquors and found that brewed liquors such as wine, shokoshu, and sake had the GABA-like activity, while distilled liquors such as shochyu and awamori did not (**Figure 2**). The current caused by the $GABA_A$ receptors showed the variation in the size on each oocyte, but the ratios of the currents between the sample and the control were similar.

The potentiation of the $GABA_A$ receptor response by whiskey could be measured by adding whiskey itself to the GABA solution before (*2*), since whiskey contained no GABA. However, beer, brewed liquor, caused a high GABA-like activity as shown above. So, the EXT was prepared as described in the Materials and Methods to examine the presence of the modulators of $GABA_A$ receptors in beer. Although beer itself contained the GABA-like activity, EXT induced no electrical response in the $GABA_A$ receptor-expressed oocyte, indicating the absence of GABA-like activity in EXT. However, the addition of this extract caused the potentiation of the $GABA_A$ receptor response elicited by 0.25 $\mu$M GABA (**Figure 1b**). **Figure 3** shows the dose dependency of the potentiation in the $GABA_A$ receptor-elicited response by EXT. Because EXT contained ethanol, the potentiation by ethanol was subtracted from that by EXT in **Figure 3**. The dissociation constant ($K_p$) and the maximum potentiation of the receptor ($V_m$) when all potentiation sites of the receptors were occupied by the compounds were estimated to be 12.5 mL/L and 555%, respectively, with the assumption of a simple equilibrium between the receptors and the com-

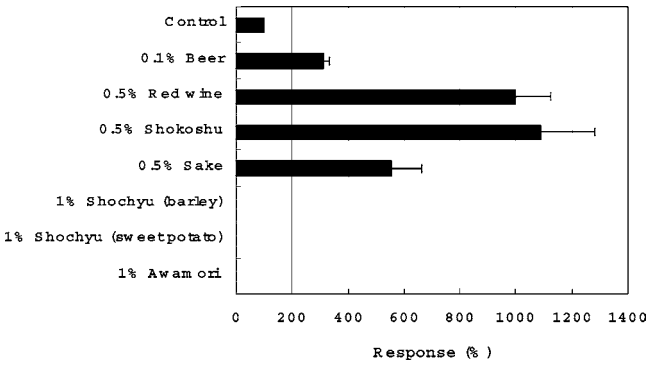


**Figure 2.** GABA-like activity of various liquors. Various liquors diluted in frog normal Ringer's solution were applied to $GABA_A$ receptors expressed in *Xenopus* oocytes. The response elicited by 0.25 $\mu$M GABA was taken as a standard (100%). Data are means ± SD (bars) values from three experiments. Beer, red wine, shokoshu, and sake are brewed liquors, and shochyu and awamori are distilled ones. The concentrations of distilled liquors were higher than those of brewed ones, just to show that distilled liquors do not contain GABA-like activity. $P < 0.01$ for the difference between the control and the brewed liquors by Student's *t*-test.

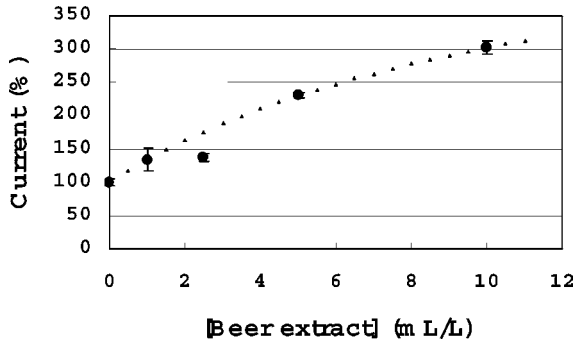


**Figure 3.** Dose potentiation of the EXT in the presence of 0.25 $\mu$M GABA. The EXT was prepared as described in the Materials and Methods. A theoretical curve (a small triangle one) was drawn by assuming a simple equilibrium between the compound and the receptor (*12*) and using the following constants: $K_p = 12.5$ mL/L and $V_m = 555\%$.

pounds (*12*). The similar potentiation of the $GABA_A$ receptor response by ethyl butanoate (EB) was also observed as shown in **Figure 1c**. The potentiation of the $GABA_A$ receptor response by EXT or EB was reversible: We obtained almost the same response as the control response after washing the oocytes with normal frog Ringer solution for several minutes (data not shown).

Because beer is produced from malted barley, water, and hops (*5*), we examined hop oils, α-humulene, myrcene, and mycenol on the $GABA_A$ receptor response (**Figure 4**). The potentiation of the $GABA_A$ receptor response by linalool, geraniol, and 1-octen-3-ol present in beer fragrance (*13*) has already been observed (*10*, *14*). α-Humulene and myrcene are typical hydrocarbons present in hops (*5*), and mycenol is produced from myrcene during boiling wort with hops. Mycenol as well as linalool, geraniol, and 1-octen-3-ol potentiated the $GABA_A$ receptor response significantly, and hop oils caused a small potentiation of the response induced by GABA. However, these compounds did not induce the response of the $GABA_A$ receptors; that is, they did not work as an agonist. **Figure 5** shows the dose dependency of the potentiation in the $GABA_A$ receptor response by myrcenol, from which the dissociation constant and the maximum potentiation of the receptor (*12*) were estimated to be 0.35 mM and 353%, respectively.

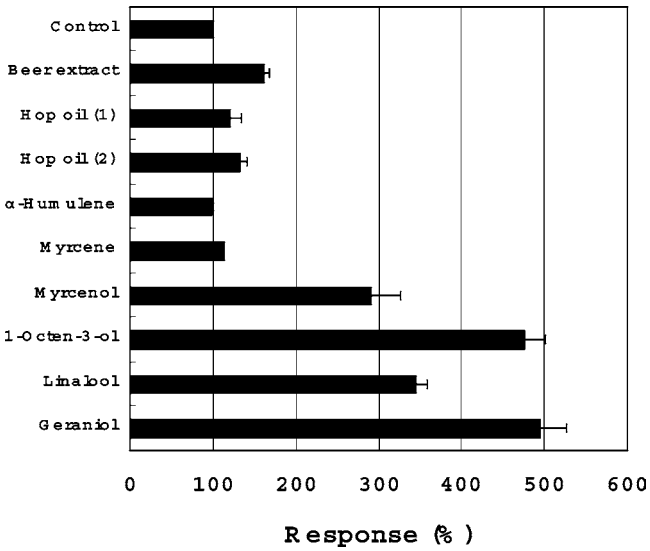


**Figure 4.** Effect of beer extract, hop oils, and various compounds present in the beer on the $GABA_A$ receptor response. The response elicited by 0.25 $\mu$M GABA was taken as a control (100%). EXT; 0.25% (v/v), hop oil (1); 0.05% (v/v), hop oil Perle, hop oil (2); 0.05% (v/v) hop oil Hallertauer Magnum, 1.2 mM myrcene, 1.1 mM mycenol, and 0.87 mM α-humulene. $P < 0.05$ for the difference between the control and the sample by Student's *t*-test. The data about 1 mM linalool, 1 mM geraniol, and 0.5 mM 1-octen-3-ol were taken from our previous papers (*14*, *15*).

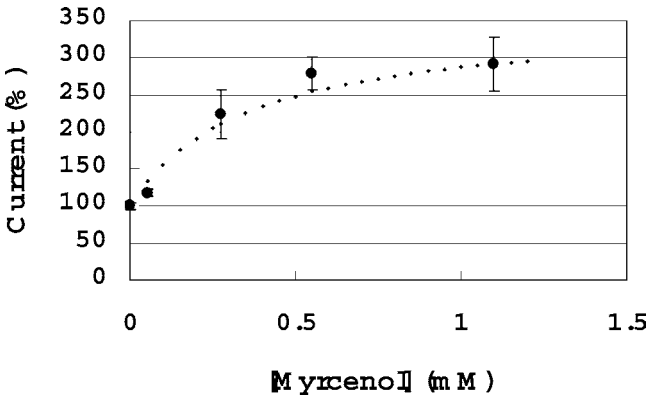


**Figure 5.** Dose potentiation of myrcenol in the presence of 0.25 $\mu$M GABA. A theoretical curve (dotted line) was drawn by assuming a simple equilibrium between the myrcenol and the receptor (*12*) and using the following constants: $K_p = 0.35$ mM and $V_m = 353\%$.

As we found in **Figures 4** and **5** that myrcenol potentiated the $GABA_A$ receptor response potently, we examined whether it acted on the $GABA_A$ receptor in vivo. It is known that pentobarbital induces sleep by potentiating the $GABA_A$ receptor response. **Figure 6** shows the effect of myrcenol on the sleeping time of mice induced by the injection of pentobarbital. Injection of myrcenol prolonged the sleeping time in mice induced by pentobarbital, as compared to the control (only pentobarbital injection). This finding suggests that myrcenol potentiates the $GABA_A$ receptor response in vivo as well.

Since the 1960s, many efforts have been made to clarify the flavor of beer by instrumental and sensory analysis (*5*, *13*, *15*, *16*). Beer contains various types of alcohols, esters, organic acids, aldehydes, ketones, hydrocarbons, and so on (*5*). In a previous paper (*12*), we found that both alcohols and aliphatic esters potentiated the $GABA_A$ receptor response and that aliphatic alcohols increased the potentiation of the $GABA_A$ receptor response with the increase of their carbon chain length. So, we examined the effect of various aliphatic esters on the

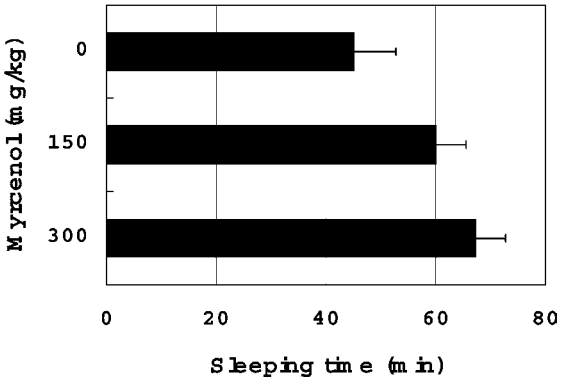


**Figure 6.** Effect of myrcenol on sleeping time induced by pentobarbital in mice. Sodium pentobarbital (50 mg/kg) was injected intraperitoneally 30 min after injection of myrcenol at 150 and 300 mg/kg. Data are means ± SD (bars) values from five experiments. $P < 0.05$ for the difference between the control value and the values injected with myrcenol by Student's *t*-test.

$GABA_A$ receptor response and found that the esters showed the tendency to decrease in the potentiation of the $GABA_A$ receptor response with an increase in their carbon chain length (**Figure 7a**,**b**). The potentiation of the $GABA_A$ receptor response by the esters increased with their concentration and reached the saturation (**Figure 8**). Both propyl acetate (▲) and ethyl propanoate (■) showed similar dose dependency. We also examined the effect of GABA concentration on the potentiation by the esters and found that the potentiation increased with the decrease of GABA concentration (data not shown) as observed in other fragrant compounds (*12*).

## DISCUSSION

Beer induced the response of $GABA_A$ receptors expressed in *Xenopus* oocytes, indicating the presence of GABA-like activity in the beer. Because brewed liquors usually have a GABA-like activity, GABA-like activity possibly comes from GABA in the germ of grains such as barley or rice, used for fermentation (*17*). However, no GABA-like activity was observed in distilled liquors such as whiskey (*2*), shochyu, or awamori. It has been reported that GABA-rich foods such as Gabaron tea (*18*) or yogurt decrease the blood pressure in rats and human hypertensives when taken for a few months, possibly because GABA acts on metabotropic GABA receptor ($GABA_B$ receptor), inhibiting noradrenaline release from sympathetic nerves (*19*). It is necessary to clarify in the future how GABA causes these effects and to what extent GABA in foods induces such effects in human subjects. However, it is unlikely that GABA in foods or beverages acts on GABA receptors in the brain, since GABA is selectively incorporated into the brain through the blood−brain barrier by GABA transporters.

In previous papers (*1*, *2*), we found that both whiskey itself and fragrant compounds in whiskey potentiated the $GABA_A$ receptor response. In this paper, the beer extract by pentane also potentiated the response, although we could not measure the potentiaiton of the response by beer itself as observed by whiskey, since beer itself contained a high GABA-like activity. It is reported that beer contains various fragrant compounds such as alcohols, esters, aldehydes, hydrocarbons, and so on (*5*). Brewers' yeast produces not only ethanol but also fusel alcohols such as various butanol and pentanol derivatives (*5*), which potentiated the $GABA_A$ receptor response (*12*). The potentiation by EXT is possibly caused at least in part by hops, which are boiled with sweet wort during the production process. It is

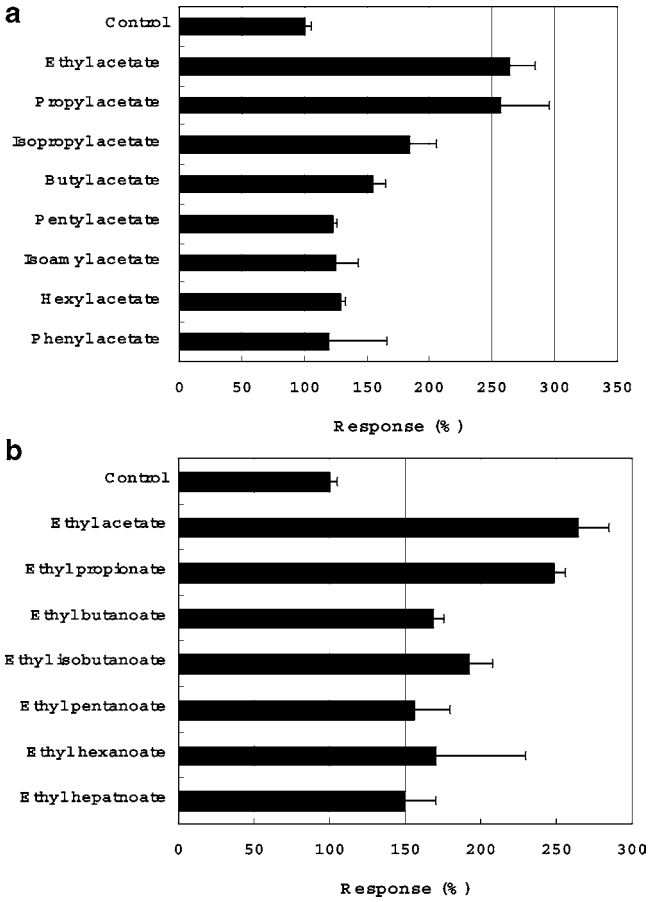


**Figure 7.** (**a**) Potentiation of the $GABA_A$ receptor response by various aliphatic esters with an acetyl group. The response elicited by 1 $\mu$M GABA was taken as a standard (100%), and the concentration of the esters was 1 mM. $P < 0.05$ for the difference between the control and the sample by Student's *t*-test. (**b**) Potentiation of the $GABA_A$ receptor response by various esters with an ethyl group. The response elicited by 1 $\mu$M GABA was taken as a standard (100%), and the concentration of esters was 1 mM. $P < 0.05$ for the difference between the control and the sample by Student's *t*-test.

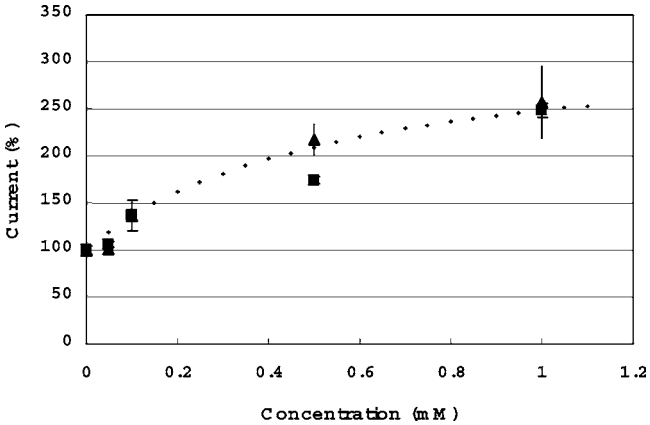


**Figure 8.** Dose potentiation of propyl acetate (▲) and ethyl propanate (■). A theoretical curve (dotted line) was drawn by assuming a simple equilibrium between the compound and the receptor (*12*) and using the following constants: $K_p = 0.55$ mM and $V_m = 330\%$.

known that hops include 1-octen-3-ol and terpenoids such as geraniol or linalool (*13*), which before potentiated the $GABA_A$ receptor response strongly (*14*, *15*). In fact, hop oil potentiated the response of the $GABA_A$ receptors. Moreover, hop cones have been used in folk medicine as a tranquilizer (*5*), suggesting

the potentiation of the $GABA_A$ receptor response. Reportedly, hop (*Humulus lupulus L.*) $CO_2$ extract caused a pentobarbital sleep-enhancing property and an antidepressant activity (*20*). However, Schellenberg et al. (*21*) reported that the fixed combination of valerian and hops acts via a central adenosine mechanism, which is possibly the reason for its sleep-inducing and -maintaining activity. So, further studies are necessary to clarify how the hop modulates mood.

In a previous paper (*12*), we examined the effect of carbon chain length of alcohols on the potentiation of $GABA_A$ receptor response and found that the potentiation of the receptors by alcohols increased with the increase of alcohol carbon chain length. Aliphatic esters are thought to be very important flavor-active compounds in beer. Reportedly, they are derived from the most abundant alcohol, ethanol, and various aliphatic acids or the most abundant acid, acetic acid, and various aliphatic alcohols during fermentation (*5*). It is very interesting that aliphatic esters decreased the potentiation with the increase of the carbon chain length, indicating that the lower esters such as ethyl acetate are important in the potentiation of the $GABA_A$ receptor response. However, esters with higher alcohols may induce the strong potentiation of the receptor response (*12*) when they are hydrolyzed by an esterase after absorption into the body. Hydrocarbons such as myrcene or α-humulene in beer caused little effect on the $GABA_A$ receptor response. However, if a hydrocarbon is oxidized to alcohol when boiling hops with wort, it will potentiate the response strongly as shown by myrcenol. The additive effect of myrcenol on the mouse sleeping time induced by pentobarbital was observed, which is well-known to induce sleep by potentiationg the $GABA_A$ receptor response. This result suggests that myrcenol also potentiates the $GABA_A$ receptor response as did 1-octen-3-ol, *cis*-jasmone, and methyl jasmonate (*14*, *22*) and modulates mood, although another possibility that cannot be excluded is that myrcenol inhibits the decomposition of pentobarbital in the liver and increases the mouse sleeping time.

It is reported that ethanol acts on $GABA_A$ receptors (*23*, *24*), N-methyl-D-aspartate (NMDA) receptors (*25*), and G-protein-coupled inwardly rectifying $K^+$ channels (*26*, *27*). Although ethanol, the main component of liquors, modulates our mood or consciousness, fragrant compounds in beer may also modulate our mood in part through the potentiation of the $GABA_A$ receptor response. The hydrophobic fragrant compounds will be carried to the brain through the blood−brain barrier and condensed in the membrane lipid of the brain. There, they might potentiate the $GABA_A$ receptor response in the brain, as we proposed in previous papers about whiskey (*1*, *2*). The direct effect of fragrant compounds on $GABA_A$ receptors was suggested by a study showing that inhaling chamomile, lemon, and lavender oil or linalool decreased restriction stress-induced increases in the plasma adrenocorticotropic hormone (ACTH) level of ovariectomized rats, as did diazepam, a benzodiazepine derivative (*28*, *29*). It was also reported that rose oil and its components showed anticonflict effects in a mouse behavior test (*30*, *31*). The accumulation of essential oil components in the mouse brain was found when they were given by means of percutaneous or vapor exposure absorption (*32*, *33*). It has also been reported that the fragrance in whiskey (*34*) and wine (*35*) has effects on human brain function, altering moods and consciousness.

Hops are added to the wort of barley malt possibly in order to give a bitter taste and good flavor to beer. However, the addition of hops will also give the potentiation of the $GABA_A$ receptor response to beer, which may be useful for mental health in humans. So, we can develop new beers by adding various herbs with much fragrance, and such a trial has already been done in European countries. Such effects are induced in both Chinese medicinal liquor, He Jiu, by adding seeds of *Lycium barbarum*, a traditional Chinese medicine (*4*), and whiskey, by aging it in oak barrels for a long period (*2*). Japanese liquors such as sake and shochyu are sometimes stored in wooden barrels for aging, which may also cause the effect in the liquors.

## ABBREVIATIONS USED

EB, ethyl butanoate; EXT, extract of beer with pentane; GABA, γ-aminobutyric acid; $GABA_A$ receptor, ionotropic γ-aminobutyric acid receptor; NMDA, N-methyl-D-aspartate.

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## LITERATURE CITED

(1) Hossain, S. J.; Aoshima, H.; Koda, H.; Kiso, Y. Potentiation of the ionotropic GABA receptor response by whiskey fragrance. *J. Agric. Food Chem.* **2002**, *50*, 6828−6834.

(2) Koda, H.; Hossain, S. J.; Kiso, Y.; Aoshima, H. Aging of whiskey increases the potentiation of $GABA_A$ receptor response. *J. Agric. Food Chem.* **2003**, *51*, 5238−5244.

(3) Nykanen, L.; Nykanen, I. Distilled beverages. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 547−580.

(4) Aoshima, H.; Hossain, S. J.; Tanaka, Y.; Wen, R. Effects of Chinese medicinal liquor on both PC12h cell death by oxidizers and the ionotropic γ-aminobutyric acid receptor. *Alcohol Res.* **2004**, *9*, 7−11.

(5) Angelino, S. A. G. F. Beer. In *Volatile Compounds in Food and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 581−616.

(6) 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.

(7) Gurevoch, V. V.; Pokrovskaya, I. D.; Obukhova, T. A.; Zozulya, S. A. Preparative *in vitro* mRNA synthesis using SP6 and T7 RNA polymerases. *Anal. Biochem.* **1991**, *195*, 207−213.

(8) Kusano, K.; Miledi, R.; Stinnarkre, J. Cholinergic and catecholaminergic receptors in the *Xenopus* oocytes membrane. *J. Physiol.* **1982**, *328*, 143−170.

(9) Aoshima, H.; Hamamoto, K. Potentiation of $GABA_A$ receptors expressed in *Xenopus* oocytes by perfume and phytoncid. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 743−748.

(10) Hossain, S. J.; Hamamoto, K.; Aoshima, H.; Hara, Y. Effects of tea components on the response of $GABA_A$ receptors expressed in *Xenopus* oocytes. *J. Agric. Food Chem.* **2002**, *50*, 3954−3960.

(11) Matsumoto, K.; Satoh, T.; Bing, L. H.; Ohta, H.; Watanabe, H. Effects of forced shaking stress at low temperature on pentobarbital-induced sleeping in mice. *Gen. Pharmacol.* **1991**, *22*, 729−733.

(12) 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 $\alpha_1$ and $\beta_1$ subunits. *J. Biochem.* **2001**, *130*, 703−709.

(13) Kishimoto, T.; Wanikawa, A.; Kagami, N.; Kawatsura, K. Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agric. Food Chem.* **2005**, *53*, 4701−4707.

(14) Hossain, S. J.; Aoshima, H.; Koda, H.; Kiso, Y. Effects of coffee components on the response of $GABA_A$ receptors expressed in *Xenopus* oocytes. *J. Agric. Food Chem.* **2003**, *51*, 7568−7575.

(15) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in French and dried hop cones (*Humulus lupulus* L. variety spalter select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776−1783.
(16) Lermusieau, G.; Bulens, M.; Collin, S. Use of GC-olfactometry to identify the hop aromatic compounds in beer. *J. Agric. Food Chem.* **2001**, *49*, 3867−3874.
(17) Saikusa, T.; Horino, T.; Mori, Y. Distribution of free amino acids in the rice kernel and kernel fractions and the effect of water soaking on the distribution. *J. Agric. Food Chem.* **1994**, *42*, 1122−1125.
(18) Omori, M.; Yano, T.; Okamoto, J.; Tsushida, T.; Murai, T.; Higuchi, M. Effect of anaerobically treated tea (gabaron tea) on blood pressure of spontaneously hypersensitive rats. *Nippon Nogeikagaku Kaishi* **1987**, *61*, 1449−1451 (in Japanese).
(19) Hayakawa, K.; Kimura, M.; Kamata, K. Mechanism underlying $\gamma$-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats. *Eur. J. Pharmacol.* **2002**, *438*, 107−113.
(20) Zanoli, P.; Rivasi, M.; Zavatti, M.; Brusiani, F.; Baraldi, M. New insight in the neuropharmacological activity of Humulus lupulus L. *J. Ethnopharmacol.* **2005**, *102*, 102−106.
(21) Schellenberg, R.; Sauer, S.; Abourashed, E. A.; Koetter, U.; Brattstrom, A. The fixed combination of valerian and hops (Ze91019) acts via a central adenosine mechanism. *Planta Med.* **2004**, *70*, 594−597.
(22) Hossain, S. J.; Aoshima, H.; Koda, H.; Kiso, Y. Fragrances in oolong tea that enhance the response of $GABA_A$ receptors. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 1842−1848.
(23) 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.
(24) Roberto, M.; Madamba, S. G.; Moore, S. C.; Tallent, M. K.; Siggins, G. R. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 2053−2058.
(25) Lovinger, D. M.; White, D.; Weight, F. F. Ethanol inhibits NMDA-activated ion current in hipocampal neurons. *Science* **1989**, *243*, 1721−1724.
(26) 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.
(27) 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.
(28) Yamada, K.; Miura, T.; Mimaki, Y.; Sashida, Y. Effect of inhalation of chamomile oil vapor on plasma ACTH level on ovari-ectomized-rat under restriction stress. *Biol. Pharm. Bull.* **1996**, *19*, 1244−1246.
(29) Yamada, K. Effects of inhaling of lavender oil vapor or linalool vapor on ether-induced plasma ACTH, catecholamine and gonadotropin levels in experimental menopausal female rat. *Aroma Res.* **2004**, *5*, 371−375 (in Japanese).
(30) Umezu, T. Anti-conflict effects of plant-derived essential oils. *Pharmacol., Biochem. Behav.* **1999**, *64*, 35−40.
(31) Umezu, T. Behavioral effects of plant-derived essential oils in the Geller type conflict test in mice. *Jpn. J. Pharmacol.* **2000**, *83*, 150−153.
(32) Inoue, S.; Oshihara, H.; Uchida, K.; Yamaguchi, H. Preferential percutaneous absorption of monoterpene hydrocarbons and ester of essential oils in mice placed in aroma bath and alteration of compositions of essential oils. *Aroma Res.* **2000**, *1* (2), 75−83 (in Japanese).
(33) Inoue, S.; Yamaguchi, H. Systematic absorption and metabolism of essential oils in rats by holistic vapor exposure. *Aroma Res.* **2000**, *1* (4), 77−81 (in Japanese).
(34) Shutara, Y.; Koga, Y.; Nagata, K.; Kanno, I.; Hujita, H.; Nakagawa, T.; Nagai, H.; Takemasa, K. The effect of odor of whiskey on the human brain function: the study by using ERP and Pet. *Rinsyonouha* **1994**, *36*, 161−167 (in Japanese).
(35) Nagai, H.; Koga, Y.; Hirayasu, Y.; Nakamura, Y.; Tanahashi, H. Relaxational effects by wine aroma. *Aroma Res.* **2000**, *1* (4), 48−52 (in Japanese).

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