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Potentiation of the Ionotropic GABA Receptor Response by Whiskey Fragrance

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It is well-known that the target of most mood-defining compounds is an ionotropic γ-aminobutyric acid receptor ($GABA_A$ receptor). The potentiation of the response of these inhibitory neurotransmitter receptors induces anxiolytic, sedative, and anesthetic activity in the human brain. To study the effects of whiskey fragrance on the $GABA_A$ receptor-mediated response, $GABA_A$ receptors were expressed in *Xenopus* oocyte by injecting rat whole brain mRNA or cRNA prepared from the cloned cDNA for the α_1 and β_1 subunits of the bovine receptors. Most whiskey components such as phenol, ethoxy, and lactone derivatives potentiated the electrical responses of $GABA_A$ receptors, especially ethyl phenylpropanoate (EPP), which strongly potentiated the response. When this compound was applied to mice through respiration, the convulsions induced by pentetrazole were delayed, suggesting that EPP was absorbed by the brain, where it could potentiate the $GABA_A$ receptor responses. The extract of other alcoholic drinks such as wine, sake, brandy, and shochu also potentiated the responses to varying degrees. Although these fragrant components are present in alcoholic drinks at low concentrations (extremely small quantities compared with ethanol), they may also modulate the mood or consciousness of the human through the potentiation of the $GABA_A$ receptor response after absorption into the brain, because these hydrophobic fragrant compounds are easily absorbed into the brain through the blood−brain barrier and are several thousands times as potent as ethanol in the potentiation of the $GABA_A$ receptor-mediated response.

KEYWORDS: Ethyl phenylpropanoate; $GABA_A$ receptor; potentiation; whiskey fragrance; *Xenopus* oocyte

INTRODUCTION

It has been reported that some components of foods or drinks can act on receptors, channels, and enzymes in the brain to modulate human consciousness (*1*). For example, nicotine in tobacco binds to nicotinic acetylcholine receptors in the brain and modulates human consciousness. Capsaicin in hot chilli peppers opens the heat-activated ion channel (warm receptors) (*2*), whereas menthol in spearmints opens cold receptors (*3*). Caffeine in tea or coffee is known to work as a central nervous stimulant (*1*), acting on cellular phosphodiesterase, ryanodine receptors, and purine receptors (*4*). Ethanol in liquors potentiates the response of ionotropic γ-aminobutyric acid receptors ($GABA_A$ recptors) (*5*), whereas it inhibits that of *N*-methyl-D-aspartate (NMDA) receptors (*6*). It also opens G-protein-coupled inwardly rectifying K^+ channels (*7*, *8*). Moreover, it has been reported that the fragrance in whiskey (*9*) and wine (*10*) has effects on human brain function, altering moods and relaxing consciousness. Thus, it is important to know whether the fragrance in liquors can really affect neural transmission via a modulation of receptor, channel, or enzyme function in brain to change people's moods or consciousness.

It is known that many mood-defining drugs target ionotropic GABA receptors ($GABA_A$ receptors) in the brain. The $GABA_A$ receptors have a complex pharmacology (*11*, *12*), with binding sites for direct GABA agonists and antagonists, as well as a noncompetitive inhibition site that interacts with various lipid-dependent hydrophobic compounds (*13*) together with multiple allosteric sites for benzodiazepine tranquilizers, barbiturate central nervous system depressants, both synthetic and endogenous steroids (*14*), general anaesthetics (*15*), and ethanol (*5*). These structurally diverse compounds can inhibit or enhance the response of $GABA_A$ receptors. Because $GABA_A$ receptors are the main inhibitory receptors in the brain, potentiation of the $GABA_A$ receptor response induces anxiolytic, anticonvulsant, and sedative activity in the human brain. In our previous papers (*16*−*19*), we reported on $GABA_A$ receptors that were expressed in *Xenopus* oocytes by injecting rat whole-brain mRNA or cRNA prepared from cDNA of the bovine $GABA_A$ receptor subunits and showed that the responses of these receptors were inhibited or potentiated by various compounds

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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 previously proposed (*20*). *Xenopus* oocytes, which are round and have a diameter of >1 mm, are larger, more stable, and simpler in shape than neurons, so electrophysiological measurements of the responses of the receptors expressed in oocytes can be taken easily and repeatedly over a long period.

GABA is a major inhibitory neurotransmitter in the brain and is essential for the overall balance between neuronal excitation and inhibition. $GABA_A$ receptors are ligand gated ion channels with subunits having amino acid sequences similar to those of ionotropic nicotinic acetylcholine, serotonin (type 3), and glycine receptors (*11*). They are thought to be hetero-pentamers made up of subunits likely to have been derived from a common ancestor. To date, at least 15 human $GABA_A$ receptor proteins have been described. There are six α-subunits, four β-subunits with two splice variants, three γ-subunits with two splice variants, one δ-subunit and one ϵ-subunit (*12*). Thus, rat whole-brain mRNA will express an enormous number of pentamer combinations for $GABA_A$ receptors, which will all show a different and complex pharmacology (*11*).

In the present study, we expressed $GABA_A$ receptors in *Xenopus* oocytes by injecting them with rat whole-brain mRNA or cRNA prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors and examined the effects of various fragrant compounds in whiskey (*21*) on the electrical response of these receptors. Most fragrant compounds potentiated the response of the $GABA_A$ receptors, although with differences in efficiency. The data were analyzed quantitatively using a simple model (*20*). Because ethyl phenylpropanoate (EPP) strongly potentiated the response, its effect on the convulsions of the mice induced by pentetrazole (*22*) was examined to show a direct effect on $GABA_A$ receptors in the brain.

MATERIALS AND METHODS

Materials. All components of whiskey were supplied from Suntory Ltd., Osaka, Japan. The fragrant components in 500 mL of whiskey, wine, shochu, brandy, and sake were prepared by extraction with 3 L of pentane, and the pentane was removed by evaporation. The residue was very slight. Therefore, each extract was diluted to usually 200 μL by dimethyl sulfoxide with a microcylinder and adjusted to obtain 5 times dilution from original liquor in the final concentration of this assay system, when the difference of the potency in the potentiation of $GABA_A$ receptor-elicited responses by different liquors was examined.

Preparation of Poly(A)$^+$RNA, cRNA, and *Xenopus* Oocytes. Whole brains were obtained from male adult Wistar rats (weighing ~100 g) that had been anesthetized with diethyl ether. Poly(A)$^+$RNA was prepared from rat brains according to the procedure described by Maniatis et al. (*23*). The cDNAs of $GABA_A$ receptors cloned from bovine brain were gifts from Prof. Eric A. Barnard (MCR Center, U.K.). The cRNAs of $GABA_A$ receptors were synthesized from these cloned cDNAs by RNA polymerase according to standard procedures.

Adult female frogs (*Xenopus laevis*) were purchased from Hamamatsu Seibutsu Kyozai (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 using the procedure of Kusano et al. (*24*). The oocytes were microinjected with ~50 ng of poly(A)$^+$RNA or cRNAs in sterilized water and then incubated in a modified Barth 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 the electrophysiological measurements.

Electrophysiological Measurements. The membrane current of the receptors evoked by GABA was measured by the voltage clamping method using a voltage clamp amplifier (CEZ-1100; Nihon Kohden Kogyo, Tokyo, Japan). An oocyte was placed on a net in 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 a current for clamping the membrane potential, usually at −40 mV. The oocyte placed on the net was continuously perfused from the bottom with normal frog Ringer solutions (115 mM NaCl, 1 mM KCl, and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) by a gravity feed system, usually at a flow rate of ~2 mL/min (*25*).

Measurement of the Receptor Response. GABA was dissolved in normal frog Ringer solution. To examine the effect of compounds on the GABA-elicited response, each compound was added to the Ringer solution and GABA solution. The solutions were then shaken vigorously for 1 min. One or the other of the solutions was selected by switching a cock in the flow system. The control response was obtained by perfusing the GABA solution without any added compound and this was taken as 100%. The effect of the compound on the response of the receptors was measured by using a mixture of GABA and the compound. Because some compounds such as lactone derivatives caused some nonspecific current even in noninjected oocyte at high concentrations, we measured the response caused by the compound dissolved in normal frog Ringer solution and confirmed that nonspecific current was negligible. We also confirmed that the solvent, dimethyl sulfoxide, induced no effect on the response when the potentiation of the $GABA_A$ receptor-elicited response by the extract of various liquors was measured. In some cases, when a significant desensitization of the receptors was induced before the binding equilibrium of the compound was attained in the presence of high concentrations of GABA, the compound was applied 1 min before the coapplication with GABA. The measurement was repeated several times with the same oocyte, and control values were obtained after every two or three measurements. To reverse the desensitization of the receptors, the oocyte was washed for >10 min in a normal frog Ringer solution before the next measurement, because desensitization of the $GABA_A$ receptors is a reversible process and the receptors usually recover after ~10 min of washing (*26*).

The values given were usually the mean of four experiments. A Student's *t* test was used to evaluate the significance of the mean values, in comparison with the control.

Determination of the Latency in Mouse Convulsions Induced by Pentetrazole. The inhalation apparatus was a lidded cage (L 31.5 × W 21.0 × H 21.0 cm). Filter paper was soaked in 1.0 mL of each sample of EPP or 1,1-diethoxyheptane in the cage. After equilibration of the cage with the vapor, the mice were introduced and exposed to each sample for 15 min. Then, pentetrazole (80 mg/kg) was administered intraperitoneally, and the behavior of the mice was observed under inhalation of each sample (*22*). The time between the injection of pentetrazole and the development of convulsions in the mice was determined as the latency.

RESULTS

Potentiation of the $GABA_A$ Receptor Response by Whiskey Extract and Its Fragrant Components. Figure 1 shows some examples of the electrical responses of the $GABA_A$ receptors expressed in *Xenopus* oocytes by the injection of poly(A)$^+$RNA prepared from rat whole brains. The receptors expressed will be composed of similar combinations of the various subunits found in rat brain. These responses are thought to be induced by ionotropic GABA receptors ($GABA_A$ receptors), because coexpression of orphan receptors is necessary for the functional expression of the metabotropic GABA receptors ($GABA_B$ receptors) in *Xenopus* oocytes (*27*, *28*) and the responses were completely inhibited by the addition of picrotoxin (data not shown), which binds to the ion channel domain of $GABA_A$ receptors (*11*). Addition of 0.2 μL/mL whiskey extract to a 10 μM GABA solution potentiated the response of the $GABA_A$ receptors (**Figure 1a**). Addition of EPP (b; 0.2 μL/mL) or 1,1-diethoxy-3-methylbutane (c; DEMB, 0.2 μL/mL),

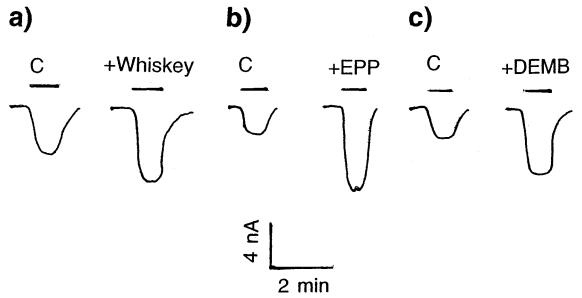

Figure 1. Effects of whiskey extract (0.2 μL/mL; a), EPP [0.2 μL/mL (1.2 mM); b], or 1,1-diethoxy-3-methylbutane [DEMB, 0.2 mL/μL (1.0 mM); c] on the 10 μM GABA-mediated current of $GABA_A$ receptors expressed in *Xenopus* oocytes by injecting mRNA prepared from rat whole brains. All traces were obtained with a voltage clamp usually 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.

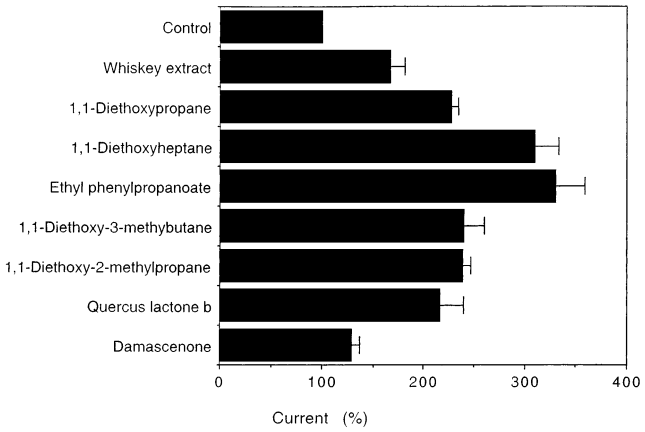

Figure 2. Comparison of the potentiation of $GABA_A$ receptor-mediated response by whiskey extract and its components. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting rat brain mRNAs. The compound (0.2 μL/mL) was applied simultaneously with 10 μM GABA. The control response was obtained by perfusing the GABA solution without any added compound and was taken as 100%. Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test.

one of the fragrant components of whiskey, to 10 μM GABA solution also potentiated the response of the $GABA_A$ receptors, even more efficiently than the addition of whiskey extract. This potentiation was reversible; we obtained a response that was almost the same as the control after washing the oocytes with normal frog Ringer solution for several minutes. Although these whiskey fragrances clearly induced potentiation of the $GABA_A$ receptor-elicited response, the potentiation showed much variation, possibly because of the difficulty in solubilizing these compounds in an aqueous solution. So the solution with a compound was shaken vigorously for ∼30 s just before every perfusion of the solution to the oocyte, which reduced the variation of the data. We then examined the effect of various typical components of whiskey fragrance on the response of $GABA_A$ receptors in the presence of 10 μM GABA (**Figure 2**). Each of these components is usually present at concentrations from 0.1 to a few parts per million in whiskey. We examined the effects of various concentrations of both the compound and GABA on the potentiation of the $GABA_A$ receptor response. As expected, the response elicited by 10 μM GABA was potentiated by these compounds in a concentration-dependent manner, increasing with their concentration until the saturation

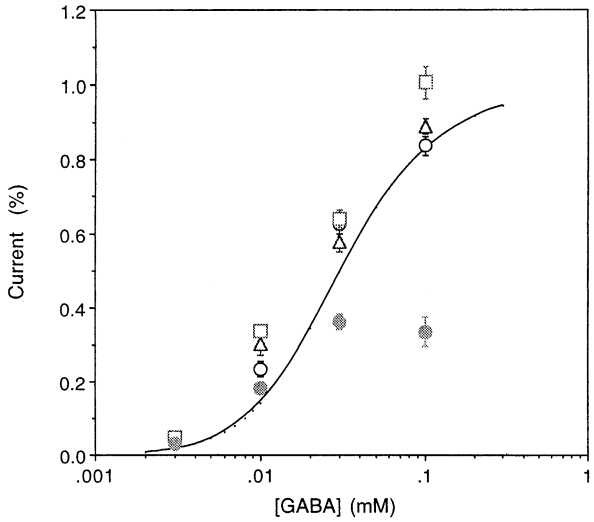

Figure 3. Effects of whiskey extract (○), quercus lactone b (△), 1,1-diethoxy-2-methylpropane (□), and β-damascenone (●) on the GABA dose–response curve. The concentration of the compounds was 0.2 μL/mL. The theoretical GABA dose–response curve without any compound was drawn using a dissociation constant (K_1) for the GABA–receptor complex of 59 μM on the basis of the minimal model reported previously. The maximum response elicited by a high concentration of GABA without any compound was taken as 1.

level was reached (data not shown). On the other hand, the potentiation of the response by these compounds decreased with an increase in the GABA concentration, indicating that the addition of most compounds except damascenone shifted the GABA dose−response curve to a lower concentration (**Figure 3**) (*29*). Damascenone potentiated the response in the presence of lower concentrations of GABA but inhibited the response in the presence of higher concentrations of GABA.

Potentiation of the $GABA_A$ Receptor Response by Lactone and Phenol Derivatives. The $GABA_A$ receptors expressed in *Xenopus* oocytes by injection of poly(A)$^+$RNA prepared from rat whole brains will express an enormous number of pentamer combinations for $GABA_A$ receptors, which will all show a different and complex pharmacology (*11*, *12*). However, because the potentiation induced in these receptors by the compounds was similar to those expressed by injecting the cRNA of bovine receptors, we next used simple $GABA_A$ receptors composed of only α_1 and β_1 subunits to examine the effects of the compounds on the responses in detail. Lactone and phenol derivatives are also present in whiskey fragrance, so their effects on the response of bovine $GABA_A$ receptors composed of only α_1 and β_1 subunits were examined in **Figures 4** and **5**. All lactone and phenol derivatives potentiated the response of the $GABA_A$ receptors, although to various levels.

Dose-Potentiation Curve of Some Compounds. Figure 6 shows the dose dependency of the potentiation in the $GABA_A$ receptor-elicited response by some components present in whiskey fragrant. From these data, the dissociation constant (K_p) and the maximum potentiation of the receptors (V_m) when all potentiation sites of the receptors were occupied by the compound were estimated with the assumption of a simple equilibrium between the compound and the receptor (**Table 1**). The dissociation constant of GABA (K_{1p}) when the potentiation site of the receptor is fully occupied with the compound was also estimated from these data on the basis of the simple kinetic model previously proposed (*20*). To examine the competition between EPP and ethanol, we measured the potentiation of the response by the addition of a mixture of these two compounds,

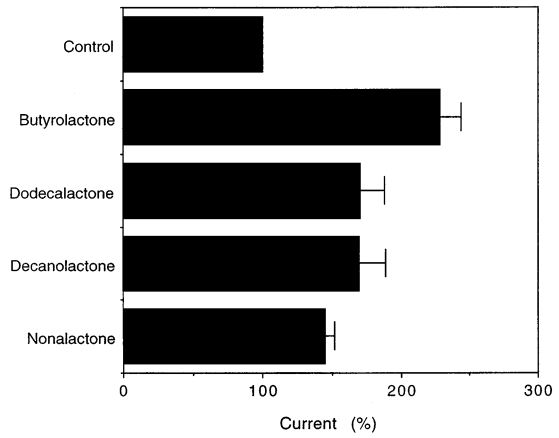

Figure 4. Comparison of the potentiation in $GABA_A$ receptor-mediated responses by lactone derivatives. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors. The concentrations of GABA and the compound were 0.25 μM and 0.02 μL/mL, respectively. The control response was obtained by perfusing the GABA solution without any added compound and was taken as 100%. Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test.

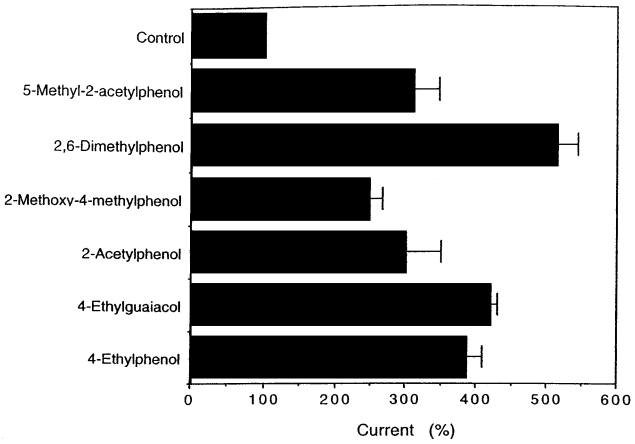

Figure 5. Comparison of the potentiation in $GABA_A$ receptor-mediated responses by phenol derivatives. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors. The concentrations of GABA and the compound were 1 μM and 0.5 mM, respectively. The control response was obtained by perfusing the GABA solution without any added compound and was taken as 100%. Data are mean ± SD (bars) values from four experiments. $p < 0.05$ by Student's t test.

which showed different maximum potentiations (V_m), and compared it with the potentiation on the addition of each compound alone. In the presence of 0.25 μM GABA, the potentiation (219 ± 42%) by a mixture of 0.62 mM EPP and 100 mM ethanol was greater than that (146 ± 15%) by 100 mM ethanol but less than that (343 ± 28%) by 0.62 mM EPP itself, which suggests there is competitive binding between these compounds for the potentiation site.

Potentiation of the $GABA_A$ Receptor-Elicited Response by Various Liquor Extracts. In **Figure 7**, we compared the potentiation of the $GABA_A$ receptor-elicited response by various liquor extracts, the concentrations of which were adjusted to the same dilution. All extracts of liquors potentiated the response but to different degrees. Extracts of wine and whiskey potentiated the response better than those of other liquors.

Effect of EPP on the Latency of the Convulsions Induced by Pentetrazole in Mice. EPP and 1,1-diethoxyheptane induced

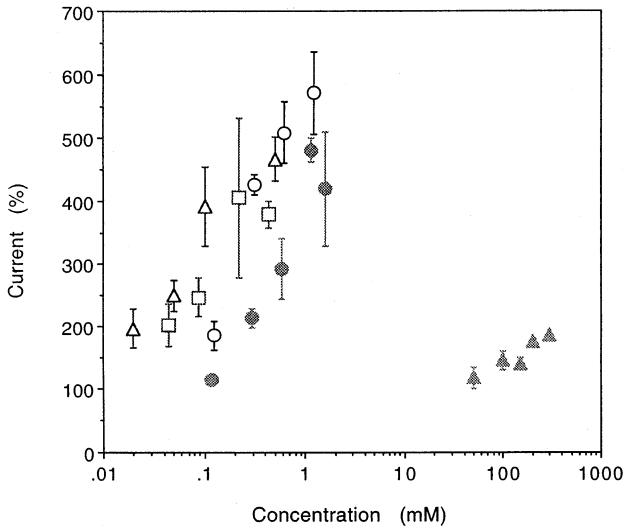

Figure 6. Dose–response relationship of whiskey components in the presence of 0.25 μM GABA: EPP (○); 4-ethylguaiacol (△); 1,1-diethoxyheptane (□); quercus lactone b (●); ethanol (▲). $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors. The dissociation constant (K_p) and the maximum potentiation (V_m) were estimated from these data and are shown in **Table 1**. Data are mean ± SD (bars) values from four experiments.

Figure 7. Comparison of the potentiation in the $GABA_A$ receptor-mediated response by various liquor extracts. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNAs prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors. GABA concentration was 0.25 μM GABA. The extracts were diluted 5 times from original liquor. $p < 0.01$ by Student's t test.

Table 1. Estimated K_p, V_m, and K_{1p} of Various Compounds

compound	K_p (mM)	V_m (%)	K_{1p} (μM)
control			59
ethanol	248	259	37
EPP	0.24	662	23
4-ethylguaiacol	0.076	524	26
1,1-diethoxyheptane	0.20	605	24
Quercus lactone b	0.56	500	27

the most potent activation of the $GABA_A$ receptor-elicited response. Therefore, we investigated the effect of these two compounds on the convulsions of mice induced by pentetrazole. Pentetrazole is thought to inhibit the $GABA_A$ receptor-elicited response in the brain and induce convulsions (*22*). **Figure 8** shows the latency of the convulsions in mice treated with EPP

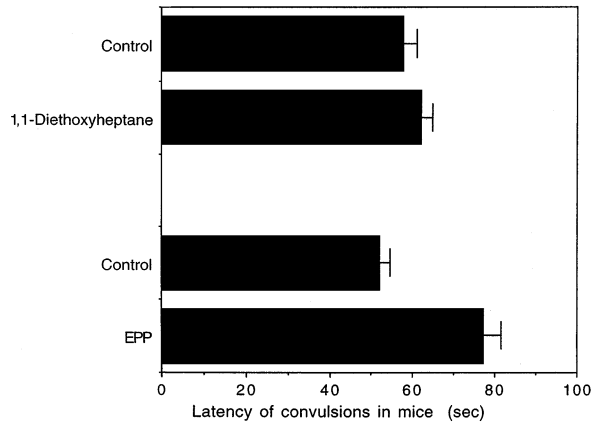

Figure 8. Effect of EPP and 1,1-diethoxyheptane on the mouse convulsions induced by pentetrazole. The latency of the convulsions in mice was measured with and without the inhalation of EPP or 1,1-diethoxyheptane. Each bar represents the mean ± SD obtained from 10 mice (see Materials and Methods for more details). $p < 0.01$ by Student's *t* test.

or 1,1-diethoxyheptane prior to the administration of pentetrazole. Because the experiments for these two compounds were carried out by using different mouse groups on different days, the control values for these two experiments showed some difference. Inhalation of these compounds significantly delayed the development of convulsions induced by pentetrazole, suggesting that they were absorbed into the brain and potentiated the $GABA_A$ receptor-elicited responses.

DISCUSSION

A number of structurally diverse compounds enhance the action of GABA on $GABA_A$ receptors; these compounds include benzodiazepine, barbiturates, pregnane steroids, general anesthetics, and ethanol (*11*, *12*). In animal models, these compounds exhibit anxiolytic, anticonvulsant, and sedative activities (*30*, *31*). In our previous papers (*16*−*19*), we also found that many fragrant compounds potentiated the response of $GABA_A$ receptors expressed in *Xenopus* oocytes.

Whiskies have many aromatic compounds (*21*), which determine the sensory characteristics of whiskey. Therefore, we examined the effects of the various components of whiskey fragrance and have found that the most fragrant compounds in whiskey potentiated the response of $GABA_A$ receptors, which were expressed in *Xenopus* oocytes by injecting poly(A)$^+$RNA prepared from rat whole brains or cRNA for the α_1 and β_1 subunits of the bovine receptors. Because both types of receptor were potentiated in a similar manner by the fragrant compounds in whiskey, the potentiation site for these compounds must be present in $GABA_A$ receptors that are composed of only α_1 and β_1 subunits (*32*). In contrast, the γ subunit is essential for the potentiation of the $GABA_A$ receptor-mediated response by benzodiazepine (*33*, *34*). The potentiation site is likely to be located at some specific site on the α_1 and β_1 subunits in the receptor. It was reported that a mutation of two specific amino acid residues in the receptor composed of only α and β subunits eliminated the potentiation by alcohols and general anesthetics, but not the response elicited by GABA (*35*). This site was also identified by using an alkanethiol anesthetic to covalently label its binding site and by mutating selected amino acids to cysteine (*36*). It is likely that EPP and ethanol bind to the same potentiation site in the receptors, because the potentiation by these compounds was competitive rather than additive. However, the specificity of the site was not so high for the potentiation by ethoxy, phenol, and lactone derivatives. Therefore, the potentiation site for whiskey fragrances is possibly the same as the reported ethanol and anesthetic binding site, which is a region of 45 amino acid residues within the TM2 and TM3 domains of the subunit (*35*, *36*).

EPP and ethoxy derivatives strongly potentiated the $GABA_A$ receptor-elicited responses. Phenol and lactone derivatives also potentiated the response to some extent (*38*). The potentiation by these compounds must be due to an increase in the GABA-binding affinity to the receptor as is found with general anesthetics (*29*). The dissociation constant (K_1) of the complex between GABA and the receptor decreased to less than half of the control value in the presence of saturating amounts of these compounds (**Table 1**). Only damascenone inhibited the responses in the presence of higher GABA concentrations despite the potentiation seen in the presence of lower GABA concentrations. This is possibly because damascenone can bind to both an inhibition and a potentiation site. A similar potentiation and inhibition of $GABA_A$ receptor-mediated response was observed when lipid hydroperoxide (*16*) or bisphenol A (*19*) was added to the GABA solution. This inhibition site is probably at the interface of the receptor with membrane lipid and prevents the channel opening (*13*). In biological experiments with mice, the respiratory application of EPP and 1,1-diethoxyheptane delayed the convulsions induced by pentetrazole, which is thought to inhibit the $GABA_A$ receptor-elicited response in the brain and induce the convulsions (*22*). Thus, these compounds may potentiate the $GABA_A$ receptor-elicited response when they were absorbed into the brain. Although ethanol is possibly the main liquor component that modulates the moods and consciousness of humans through acting on $GABA_A$ receptors (*5*), NMDA receptors (*6*), and K^+ channels (*7*, *8*), liquor fragrances such as EPP, lactone, and ethoxy derivatives may also in part modulate the mood and consciousness, because these compounds are several thousand times more potent than ethanol (**Figure 6**) in potentiating the $GABA_A$ receptor-elicited responses and in addition, because of their hydrophobicity, they are easily absorbed into the brain. The direct effect of fragrant compounds on $GABA_A$ receptors was suggested by a study showing that inhaling chamomile and lemon oil vapor decreased restriction-stress-induced increases in the plasma adrenocorticotropic hormone (ACTH) level of ovariectomized rats, as did diazepam, a benzodiazepine derivative (*39*). It was also reported that rose oil and its components showed anticonflict effects in a mouse behavior test (*40*, *41*). Moreover, the accumulation of essential oil components in the mouse brain was found when they were given by means of percutaneous or vapor exposure absorption (*42*, *43*). Using whiskey extract and its fragrant components, further experiments such as a psychological test or the anti-conflict test (*40*,*41*) using mice or rats will be necessary to test this hypothesis in the future. Our experiments were carried out by using the components at higher concentrations than the physiological ones to observe their effect on the response clearly, so it is also necessary to clarify how high of a concentration of these whiskey components reaches the synapses of the brain under physiological conditions and how much modulation of the $GABA_A$ receptor-elicited responses causes the effect on human mood or consciousness.

The different liquors have their different fragrant components. Although green tea extract inhibited $GABA_A$ receptor-elicited responses (*44*), the extract from each liquor potentiated the response in a different potency. Both ethanol and higher alcohols potentiate the $GABA_A$ receptor-elicited responses, but only

ethanol opens G-protein-coupled inwardly rectifying K^+ channels (*7*, *8*). Thus, a small amount of fragrant components in liquors may contribute to the taste of each liquor through not only the stimulation of the olfactory system but also the potentiation of $GABA_A$ receptor-elicited responses. It is known that the amount of EPP and lactone derivatives in whiskey increases with aging in a wooden barrel, and this might induce more tranquility in the human mind through the potentiation of $GABA_A$ receptor-elicited responses. In fact, a preliminary experiment showed that the extract of whiskey with aging in a wooden barrel for 30 years potentiated the response twice as potently as that of whiskey aged for 8.5 years. A potency in the potentiation of $GABA_A$ receptor-elicited responses may depend on not only the type of liquors but also each of the commercial products. Therefore, it is safe to think that the results in **Figure 7** showed a different potency of liquors but did not show the general order of the potency in the potentiation of $GABA_A$ receptor-elicited responses among liquor types.

ABBREVIATIONS USED

EPP, ethyl phenylpropanoate; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate

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