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Effects of Bisphenol A and its Derivatives on the Response of $GABA_A$ Receptors Expressed in *Xenopus* Oocytes

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To study the effects of bisphenol-A (BPA) known to have estrogenic actions, and its derivatives, 3,5-dimethylphenol (DMP) and *p-t*-butylphenol (TBP), on ionotropic γ-aminobutyric acid (GABA) receptors, $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting both poly(A)$^+$RNA prepared from rat whole brain and cRNAs synthesized from cloned cDNAs of α_1 and β_1 subunit of the bovine receptors, and their electrical responses were measured by the voltage clamping method. BPA caused the potentiation and inhibition of the former receptor-responses, while it caused only inhibition of the latter ones. In the presence of low concentrations of GABA, DMP and TBP potentiated the responses of both receptors. DMP and TBP also increased the rate of decay of the response, possibly by desensitization of the receptors when GABA solution was continuously bath-applied. Diethyl terephthalate (DTP), which is also known to have estrogenic actions, had little effect on both the responses and the decay of both receptors.

Key words: bisphenol-A; 3,5-dimethylphenol; $GABA_A$ receptor; *p-t*-butylphenol; *Xenopus* oocyte

Recently, chemical pollution has been one of the serious problems for the environment of the earth. One such compound is bisphenol-A (2,2-bis(4-hydroxyphenyl)-propane; BPA) which is used as a monomer for producing polycarbonate plastics. BPA has two phenol rings which have structural homology with a ring of β-estradiol and is suspected to bind to estrogen receptors, to mimic estrogenic actions, and to have adverse effects on humans and wild animals.[1] Because of superior stability, toughness, and pliability, resins based on BPA are used in many consumer products including the inner coating of food cans, dental composites, and drug delivery systems. However, a plastic monomer, BPA, was found as a contaminant not only in the liquid of the preserved vegetables but also in water autoclaved in the cans,[2] since it was released from polycarbonate plastics. It was also identified in saliva collected after treatment with composite dental sealants.[3] Thus, BPA and its derivatives, 3,5-dimethylphenol (DMP) and *p-t*-butylphenol (TBP), may be present in unexpected places.[4] Diethyl terephthalate (DTP) is also known to have estrogenic actions and is used widely. However their hormonal activities are 10000–100000 times weaker than that of β-estradiol.[5] So it is necessary to examine their other effects on biological systems. If these compounds are absorbed into the blood through the stomach, small intestine, or lungs, they may affect neurotransmission in a brain since they are lipophilic, cross the blood-brain barrier easily, and accumulate in the brain.

The ionotropic neurotransmitter receptors are known to have been evolved from the same ancestor gene[6] and to have commonly a noncompetitive inhibition site for hydrophobic compounds, which are numerous and lipid-dependent, probably at the interface of the receptors with membrane lipids,[7] though their inhibition is not strong.[8] Additionally the $GABA_A$ receptors have a complex pharmacology, with binding sites for direct GABA agonists and antagonists together with multiple allosteric sites for the benzodiazepine tranquillizers, for the barbiturate central nervous system depressants, for both synthetic and endogenous steroids, for general anaesthetics, and for ethanol.[9] These structurally diverse compounds increase the response of $GABA_A$ receptors in the presence of low concentrations of GABA. In previous papers,[10,11] we expressed $GABA_A$ receptors in *Xenopus* oocytes by injecting rat whole brain mRNA, and measured the potentiation of their responses caused by various compounds such as lipid

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Abbreviations: BPA, bisphenol-A (2,2-bis(4-hydroxyphenyl)-propane); DMP, 3,5-dimethylphenol; DTP, diethyl terephthalate; GABA, γ-aminobutyric acid; TBP, *p-t*-butylphenol

hydroperoxide or fragrant compounds. Since *Xenopus* oocytes which are globular, with a diameter of more than 1 mm, are more stable, larger, and simpler in shape than neurons, electrophysiological measurements of the receptors expressed in the oocyte can be made easily and repetitively for a long period.

In this paper, we expressed the $GABA_A$ receptors by injecting poly(A)$^+$RNA prepared from rat whole brain or cRNAs synthesized from cloned cDNA of α_1 and β_1 subunits of the bovine $GABA_A$ receptors. Effects of BPA, DMP, TBP, and DTP on the responses of both types of $GABA_A$ receptors were examined electrophysiologically. BPA, DMP, and TBP even at a very low concentration of about 10 μM significantly potentiated the 1 μM GABA-elicited response of $GABA_A$ receptors expressed by injecting rat brain poly(A)$^+$RNA. However BPA at high concentrations inhibited the $GABA_A$ receptor-mediated response, and the extent of the inhibition increased with its incubation time. DMP and TBP potentiated the responses of $GABA_A$ receptors composed of only α_1 and β_1 subunits of the bovine receptors in the presence of low concentrations of GABA, while BPA caused only noncompetitive inhibition of the responses. DMP and TBP accelerated the decay of the GABA-response of both types of the receptors, possibly by desensitization of $GABA_A$ receptors when they were added to the GABA solution. DTP induced only a small effect on both the response and the decay of the response, desensitization, of both types of $GABA_A$ receptors.

Materials and Methods

Materials. γ-Aminobutyric acid (GABA) was purchased from Nacalai Tesque, Kyoto. Bisphenol-A (2,2-bis(4-hydroxyphenyl)-propane: BPA), 3,5-dimethylphenol (DMP) and *p-t*-butylphenol (TBP) were purchased from Wako Pure Chemical Ind., Osaka. Diethyl terephthalate (DTP) was purchased from Kanto Chemical Co., Inc. Tokyo. All chemicals were of guaranteed reagent quality.

Preparation of poly(A)$^+$RNA, cRNA and Xenopus oocytes. The whole brains were obtained from male adult Wistar rats (weighing about 100 g) after they were anesthetized with diethyl ether. Poly-(A)$^+$RNA was prepared from rat brains by the procedure described by Maniatis *et al.*[12] The cRNAs of α_1 and β_1 subunits of $GABA_A$ receptors were synthesized from cloned cDNAs of bovine brain by RNA polymerase according to the general procedure. These cloned cDNAs were a gift from Prof. Eric A. Barnard in the MRC Center, UK.

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 by the procedure of Kusano *et al.*[13] The oocytes were microinjected with poly(A)$^+$RNA 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 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 (about 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 -60 mV. The oocyte placed on the net was continuously perfused from the bottom with a normal frog Ringer solution (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 about 2 ml/min.[14]

Measurement of the receptor response. GABA was dissolved in a normal frog Ringer solution. To examine the effects of phenol derivatives on the GABA-elicited response, each was dissolved in ethanol and added to the solutions. The final concentration of ethanol was less than 0.1%, which did not affect the response of the receptors. 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 is 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. In some cases, the compound was added 1 min before co-application with GABA when desensitization of the receptors was significantly induced before attaining equilibrium of the compound binding.[15] The measurement was repeated several times with the same oocyte, and control values were obtained after every two or three measurements. Values of data were usually means of four experiments. To eliminate desensitization of the receptors, the oocyte was washed for more than 10 min in a normal frog Ringer solution before the next measurement, since desensitization of the $GABA_A$ receptors is a reversible process and the receptors usually recover after about 10 min of washing.[16]

When the effect of the compound on the rate of response-decay was examined, it was applied to the oocytes 30 sec before the mixture of GABA and the

compound was applied continuously. Then GABA solution with and without the compound was applied continuously for several minutes. Both the flow rate of the bath application and the chart speed of the recorder were increased.

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

Results

Figure 1 shows some examples of the electrical GABA-elicited responses of GABA receptors expressed in *Xenopus* oocytes by injecting poly(A)$^+$RNA prepared from rat whole brains, which will be composed of similar combinations of various subunits in a rat brain. These responses are thought to be induced by ionotropic GABA receptors ($GABA_A$ receptors), since co-expression of orphan receptors is necessary for the functional expression of the metabotropic GABA receptors ($GABA_B$ receptors) in *Xenopus* oocytes[17] and the responses were inhibited completely by addition of 10 μM picrotoxin (data not shown), which binds to the ion channel domain of the ionotropic receptors.[6] Addition of 0.5 mM DMP and TBP potentiated the response of $GABA_A$ receptors in the presence of 10 μM GABA. As shown in Fig. 2, their potentiation of 10 μM GABA-elicited response increased with their concentration, reached the saturation level, and was suppressed slightly at higher concentrations of DMP and TBP. The apparent dissociation constant (K_p) and the saturated amount of potentiation (V_m) in the presence of 10 μM GABA were estimated to be 0.11 mM and 252% for DMP, and 0.15 mM and 264% for TBP, respectively.

Addition of BPA showed complex effects on the response elicited by 10 μM GABA. At lower BPA concentrations, it increased the potentiation with BPA concentrations, then decreased the potentiation, and inhibited the response of $GABA_A$ receptors elicited by 10 μM GABA when 1 mM BPA was present (Figs. 1 and 2). Pre-incubation of BPA for 5 min increased their inhibition extent (Fig. 2). On the other hand, 0.5 mM DTP showed a slight potentiation of the response elicited by 10 μM GABA, but preincubation of DTP for 5 min did not cause a clear difference (simultaneous application of 1 mM DTP; 131.0± 16.7%, 5 min-preincubation of 1 mM DTP; 117.9± 7%, $p=0.20$).

Effects of 0.1 mM DMP, TBP, and BPA on the $GABA_A$ receptor-mediated response was examined at various GABA concentrations (Fig. 3). DMP and TBP had little effect on the response elicited by 1 mM GABA. Then potentiation of the response by DMP and TBP increased with the decrease of GABA concentration. BPA slightly inhibited the response elicited by 1 mM GABA. Then it induced potentiation of the response with the decrease of GABA concentration.

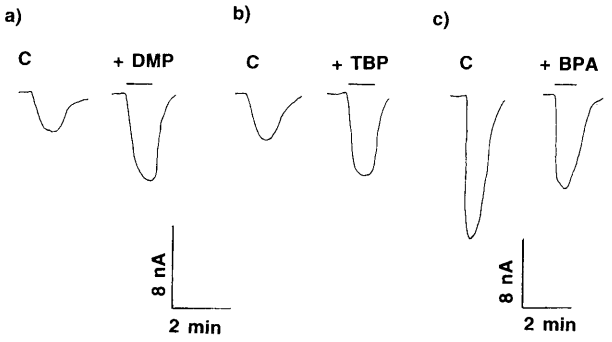

Fig. 1. Examples of the Potentiation or Inhibition of GABA-mediated Current of $GABA_A$ Receptors by DMP, TBP, or BPA.

$GABA_A$ receptors were expressed in *Xenopus* oocytes by injection of rat whole brain poly(A)$^+$RNA. All traces were obtained with a voltage clamp at -60 mV. An inward current is shown as a downward curve. Ten μM GABA with and without the phenol derivative was applied for about 1 min. The lines above the traces show when the phenol derivative was present. Each of the two responses was obtained from the same injected oocyte, but the responses in a), b) and c) were from different one. C: Control (only GABA). a) 0.5 mM DMP; b) 0.5 mM TBP; c) 1 mM BPA.

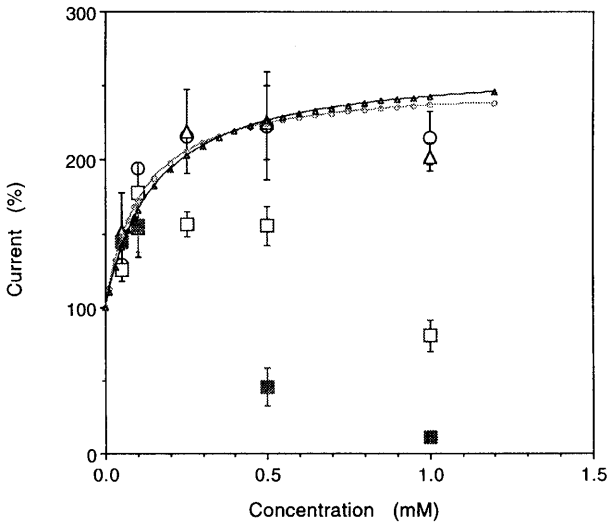

Fig. 2. Dose-response Relationship of DMP, TBP, or BPA in the Presence of 10 μM GABA.

$GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting rat whole brain poly(A)$^+$RNA. DMP (○) and TBP (△) were applied simultaneously with 10 μM GABA. Data are mean ±SD (bars) values from four experiments. The theoretical curves in the presence of DMP (-·-·-·-) and TBP (-·-·-·-) were drawn by use of a apparent dissociation constant, K_p, and maximum potentiation, V_m, of 0.11 mM and 252% for DMP and 0.15 mM and 264% for TBP. The maximum potentiation is the estimated response when all the potentiation site of the receptors is occupied by DMP or TBP. BPA was applied simultaneously with 10 μM GABA (□), or it was applied 5 min before coapplication of 10 μM GABA (■). $p<0.003$ by Student's t test.

Since the effects of phenol derivatives on $GABA_A$ receptor-elicited response increased with the decrease

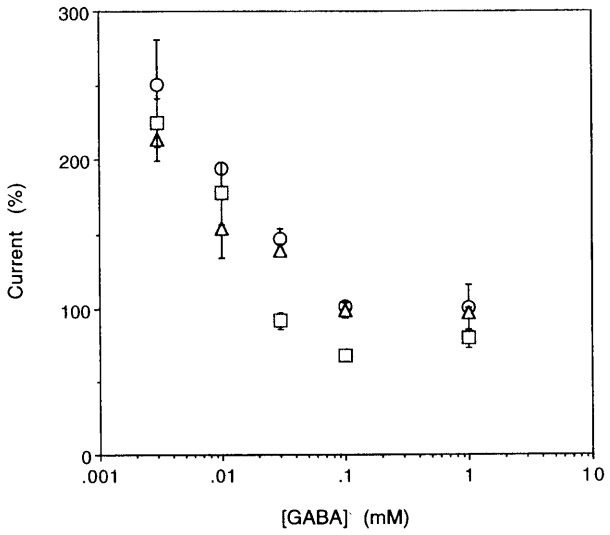

Fig. 3. Effects of GABA Concentrations on the Current of $GABA_A$ Receptors in the Presence of 0.1 mM DMP (○), TBP (△), or BPA (□).

The phenol derivatives were applied 1 min before co-application with GABA when high concentrations (30–1000 μM) of GABA were applied, since GABA induced fast desensitization of the receptors before attainment to the equilibrium of phenol derivative binding. But they were applied simultaneously with 3 and 10 μM GABA, since the rate of desensitization was slow and it takes about one minute to reach the peak current. Data are mean ± SD (bars) values from four experiments. $p<0.002$ by Student's t test except the value of TBP in the presence of 1 mM GABA.

of GABA concentration, we examined the effect of 10 μM phenol derivatives on the response elicited by 1 μM GABA (Table 1). Even 10 μM phenol derivatives induced the significant potentiation of the response.

Figure 4a shows the effect of 0.5 mM DMP on the decay of $GABA_A$ receptor response caused by 10 μM GABA, when 10 μM GABA solution with and without DMP was continuously applied. DMP accelerated the decay of the response of $GABA_A$ receptors as shown in Fig. 4b where the logarithm of the response was plotted against bath-application time. Assuming first order kinetics in the decay, the rate constant of the decay caused by 10 μM GABA in the presence of 0.5 mM DMP was 2.5 times faster than that in the absence of DMP ($p<0.0001$ by Student's t test). Addition of 0.5 mM TBP also accelerated the rate of decay twice as fast as the control ($p<0.0001$ by Student's t test). DTP showed no notable effect on the rate of the response-decay.

Since injection of rat whole brain poly(A)$^+$RNA into a *Xenopus* oocyte will express an enormous number of combinations of pentamers of $GABA_A$ receptors, which will show different and complex pharmacology,[18] and also many other proteins which may act on the receptors indirectly after the phenol derivative binds to these proteins, we expressed the $GABA_A$ receptors composed of only α_1 and β_1 subunits by injecting their cRNAs synthesized from

Table 1. Effects of BPA and its Derivatives on $GABA_A$ Receptor-mediated Response Elicited by 1 μM GABA

The response elicited by 1 μM GABA without any compound was taken as a control, 100%. n, number of experiments.

Compounds	Response (%)	n	Significance
Control	100		
11.4 μM BPA	123.0 ± 2.1	4	$p<0.001$
10 μM TBP	115.8 ± 4.0	4	$p<0.002$
10 μM DMP	113.6 ± 7.3	4	$p<0.02$

cloned cDNA of α_1 and β_1 subunits of bovine $GABA_A$ receptors to examine whether these compounds act directly on $GABA_A$ receptors. Figure 5 shows the concentration dependence of the phenol derivatives on the responses elicited by 10 μM GABA. DMP and TBP potentiated the responses similarly and their dissociation constant (K_p) and the saturated amount of potentiation (V_m) were estimated to be 0.53 mM and 348% for DMP, and 0.41 mM and 289% for TBP, respectively. These constants, K_p and V_m of $GABA_A$ receptors composed of α_1 and β_1 subunits were larger than those of GABA receptors expressed by injecting rat brain poly(A)$^+$RNA. As shown in Fig. 6, DMP- and TBP-potentiation of the response of $GABA_A$ receptors composed of α_1 and β_1 subunits increased with decreasing GABA concentration, which are similar characteristics to the potentiation by anesthetics and alcohols.[11,19] DMP and TBP also accelerated the decay of 10 μM GABA-response of $GABA_A$ receptors composed of α_1 and β_1 subunits (data not shown).

Addition of BPA caused a slight inhibition of the response, but no potentiation of the response of $GABA_A$ receptors composed of α_1 and β_1 subunits (Fig. 5). Inhibition of the $GABA_A$ receptors by BPA was independent of the GABA concentration (Fig. 6), indicating that BPA binds to the noncompetitive inhibition site of the receptor. The responses of $GABA_A$ receptors by 10 μM GABA were inhibited with the increase of BPA concentration (Fig. 5), from which the noncompetitive inhibition constant was estimated to be about 1.5 mM. Furthermore the responses of $GABA_A$ receptors by 10 μM GABA were inhibited more strongly with the increase of preincubation time as shown in Fig. 7. The inhibition constant was estimated apparently to be 0.48 mM when BPA was added 5 min before co-application with GABA.

The effect of DTP on the response of $GABA_A$ receptors was also examined. Only a little noncompetitive inhibition was observed, and preincubation of DTP for 5 min did not increase the inhibition of the receptors (simultaneous application of 0.4 mM DTP; 84.0 ± 8.7%, 5 min-preincubation of 0.4 mM DTP; 85.0 ± 10.0%, $p=0.89$). DTP induced no notable effect on the rate of decay of $GABA_A$ receptor-response.

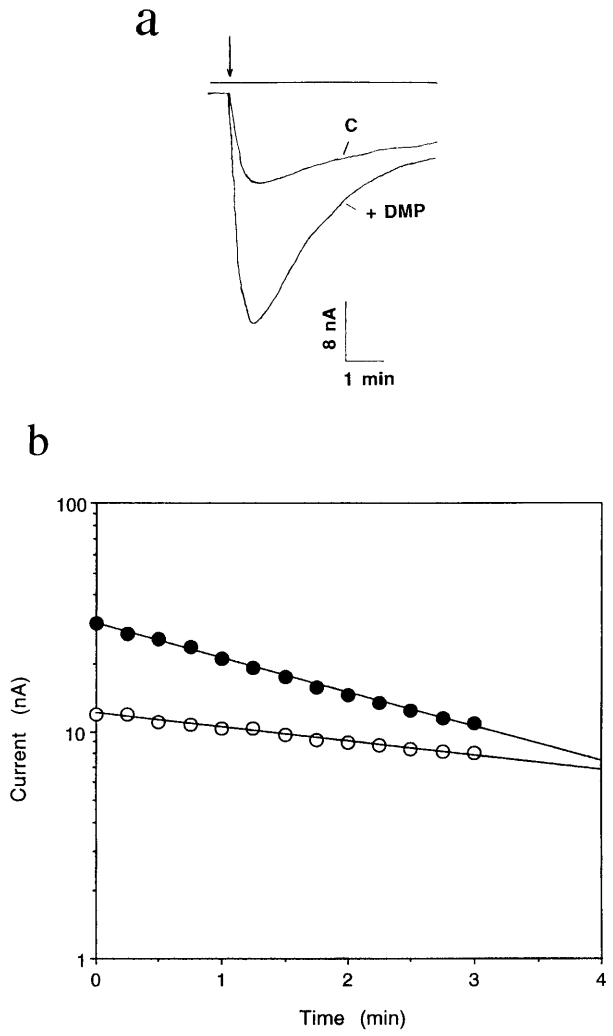

Fig. 4. a) Example of the Acceleration of the Response-decay of $GABA_A$ Receptors Expressed by Injecting Rat Whole Brain mRNA When 0.5 mM DMP was Added.

To examine the effect of DMP on the decay of the response, possibly because of desensitization, 0.5 mM DMP was applied for 30 sec, and then a mixture of 10 μM GABA and 0.5 mM DMP was applied continuously to the oocyte where $GABA_A$ receptors were expressed. The arrow shows the start of application of 10 μM GABA with or without 0.5 mM DMP. The line above the trace shows when DMP was present.

b) Evaluation of the Rate Constant of the Response-decay of $GABA_A$ Receptors in the Absence (○) and the Presence (●) of 0.5 mM DMP.

The current expressed in logarithmic scale was plotted against incubation time. The data started just after the peak current. The rate constants of the response-decay of $GABA_A$ receptors caused by 10 μM GABA in the absence and presence of 0.5 mM DMP were estimated to be 0.16 min^{-1} and 0.38 min^{-1}, respectively.

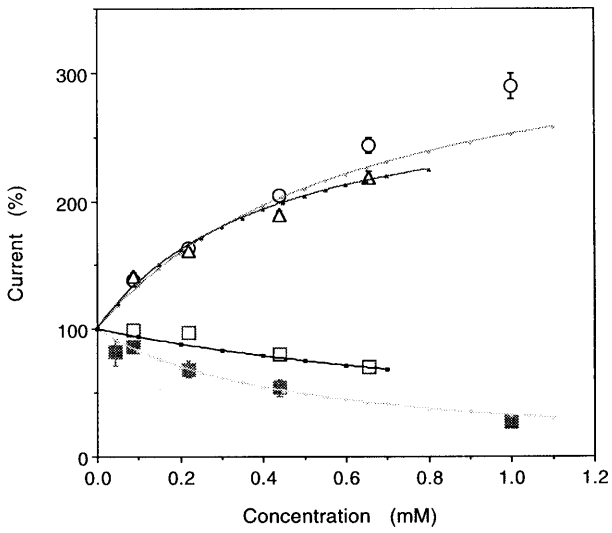

Fig. 5. Dose-potentiation or -inhibition Relationship of DMP, TBP, or BPA in the Presence of 10 μM GABA.

$GABA_A$ receptors were expressed by injecting cRNAs of α_1 and β_1 subunits. DMP (○) and TBP (△) were applied simultaneously with 10 μM GABA. Data are mean ± SD (bars) values from four experiments. The theoretical curves in the presence of DMP (-○-○-○-) and TBP (-△-△-△-) were drawn by use of a dissociation constant, K_p, and maximum potentiation, V_m, of 0.53 mM and 348% for DMP and 0.41 mM and 289% for TBP. The maximum potentiation is the estimated response when all the potentiation site of the receptors is occupied by DMP or TBP. From these V_m values, the dissociation constant between the receptor and GABA was calculated to change from 59 μM to 20 μM for DMP and to 25 μM for TBP when the potentiation site of the receptors was occupied with DMP or TBP on the basis of a minimal model proposed before.[16] BPA was applied simultaneously with 10 μM GABA (□), or it was applied 5 min before co-application of 10 μM GABA (■). The theoretical curve when BPA was simultaneously applied with GABA (-□-□-□-) was drawn by use of an inhibition constant, K_i, of 1.5 mM with the assumption of a simple noncompetitive inhibition of $GABA_A$ receptors, while the theoretical curve when BPA was applied 5 min before co-application with GABA (-■-■-■-) was drawn by use of a noncompetitive inhibition constant of 0.48 mM. $p<0.0001$ by Student's t test for the values of both DMP and TBP. $p<0.03$ by Student's t test for the values of BPA except simultaneous application of 0.088 mM and 0.219 mM BPA.

Discussion

BPA,[1] known to bind to estrogen receptors and to mimic estrogenic actions, is lipophilic and easily accumulates in the brain. It is also known that polyphenol in foods and drinks such as vegetables or tea works as an antioxidant when it was absorbed into our body. So it is necessary to examine the effects of phenol derivatives on the receptors or channels which carry out neurotransmission in the brain. $GABA_A$ receptors are known to be potentiated by higher alcohols[20] and neuroactive steroids such as pregnenolone and dehydroepiandrosterone.[21] So effects of phenol derivatives on $GABA_A$ receptors expressed in *Xenopus* oocyte were examined electrophysiologically.

All examined phenol derivatives potentiated the response of $GABA_A$ receptors expressed in the oocyte by injecting rat whole brain mRNA, when both concentrations of GABA and phenol derivatives were low. However BPA had complex effects on $GABA_A$ receptor-mediated responses, that is, low concentrations of BPA potentiated the responses in the presence of low concentrations of GABA, but high

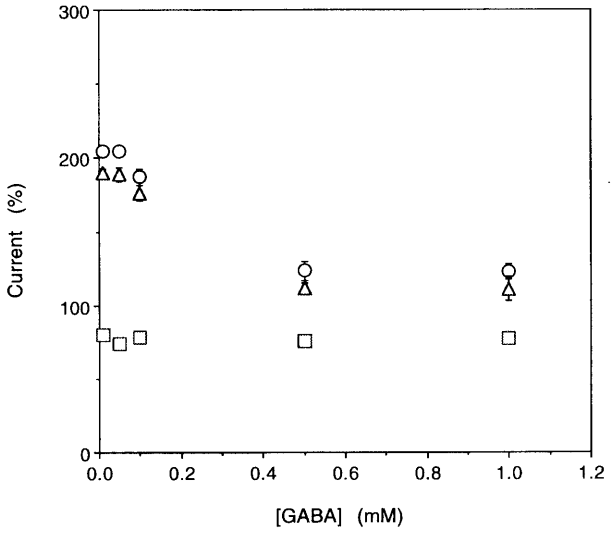

Fig. 6. Effects of GABA Concentrations on the DMP (○)- or TBP (△)-potentiation and the BPA (□)-inhibition of the Responses of $GABA_A$ Receptors Composed of α_1 and β_1 Subunits.

The concentrations of phenol derivatives were 0.438 mM and they were usually applied 1 min before co-application with GABA, since GABA induced fast desensitization of the receptors before attainment to the equilibrium of phenol derivative binding. But they were applied simultaneously with 10 μM GABA, since the rate of desensitization was slow and it takes about one minute to reach the peak current. Data are mean ± SD (bars) values from four experiments. $p<0.002$ by Student's t test except the value of TBP in the presence of 1 mM GABA.

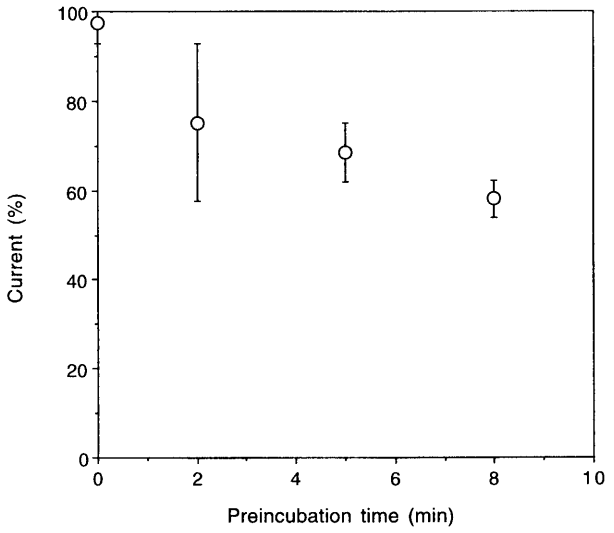

Fig. 7. Effects of BPA-preincubation Time on the Response of $GABA_A$ Receptors Composed of α_1 and β_1 Subunits.

The response of $GABA_A$ receptors caused by the mixture of 10 μM GABA and 0.219 mM BPA was measured after preapplication of 0.219 mM BPA for various time. The response caused by 10 μM GABA without BPA was taken to be 100%. Data are mean ± SD (bars) values from four experiments. $p<0.04$ by Student's t test except the value of simultaneous application of BPA (time 0).

concentrations of BPA inhibited them. The extent of this inhibition increased with the pre-incubation time. Since ionotropic receptors are known to have commonly noncompetitive inhibition site for hydrophobic compounds,[7] BPA possibly binds to this inhibition site more weakly than to the potentiation site. This inhibition site is probably at the interface of the receptor with membrane lipids and prevents the channel opening. It takes possibly some time for BPA to reach the binding site, which may be present in the lipid membrane or inside the oocyte cells. The similar potentiation and inhibition of $GABA_A$ receptor-mediated response by lipid hydroperoxide were observed before.[10] DMP and TBP with only one hydroxyl group potentiated the response of $GABA_A$ receptors caused by low concentrations of GABA. Since very slight suppression of the potentiation of $GABA_A$ receptors was observed in the presence of 1 mM DMP and TBP (Fig. 2), these compounds at high concentrations may also bind to the noncompetitive inhibition site much more weakly than BPA. Pre-application of DMP and TBP did not induce a clear increase of the inhibition.

Since BPA had complex effects on $GABA_A$ receptors, we used the $GABA_A$ receptors composed of only the α_1 and β_1 subunit combination. Unexpectedly, BPA with two hydroxyl residues caused only noncompetitive inhibition of the response of the $GABA_A$ receptors composed of only α_1 and β_1 subunits. The extent of this inhibition increased with the preincubation time, that is, the inhibition constant, K_i, was apparently about 1.5 mM when BPA was applied simultaneously with GABA, but decreased to 0.48 mM apparently when BPA was applied 5 min before application of the mixture of BPA and GABA solution. At present, we cannot explain the reason of this different effect of BPA on two types of $GABA_A$ receptors. A plausible explanation is that this difference comes from the different subunit combinations of the receptor, since $GABA_A$ receptors expressed by injecting whole brain mRNAs will express many isoforms of the receptors.[6] It is known that $GABA_A$ receptor isoforms composed of different subunit combinations show different pharmacological characteristics.[18] For example, $\gamma 2$ subunit is essential for the potentiation of $GABA_A$ receptors by benzodiazepine, suggesting that benzodiazepine binding site is located on both the α and $\gamma 2$ subunit.[22] Since we measured $GABA_A$ receptors from different species, the rat and the bovine, it cannot be denied that different effect of BPA on $GABA_A$ receptors comes from different pharmacology of the receptors of different species. Another slight possibility is that some unknown protein may contribute to the potentiation indirectly when whole brain mRNAs were injected.

On the contrary, DMP and TBP with only one hydroxyl group potentiated the response of $GABA_A$ receptors composed of only α_1 and β_1 subunits in the presence of low concentrations of GABA, while they

induced little effect on the response caused by high concentrations of GABA. The effects of these compounds on $GABA_A$ receptors composed of only α_1 and β_1 subunits are similar to those expressed by rat whole brain mRNA injection. So these compounds must bind to the potentiation site composed of only α and β subunits where ethanol and general anesthetics bind[23] and increase the affinity of GABA molecules for the receptors,[19] that is, the dissociation constant (59 μM) between the receptor and GABA may decrease 2–3 times when the potentiation site of the receptor is occupied by these compounds.

DTP, which is known to have estrogenic actions, had little effect on the response of both types of the $GABA_A$ receptors. Thus modulation of $GABA_A$ receptors was strongly dependent on little differences of the compound structure and also possibly on the subunit combination of the receptors.

When GABA solution with and without phenol derivatives was continuously applied to the receptors, all phenol derivatives accelerated the rate of the response-decay of $GABA_A$ receptors (Fig. 4). Since DMP and TBP bind the potentiation site of the receptors and increase the fraction of the receptors bound by one or two GABA molecules, which will not only potentiate the response, but also accelerate the rate of desensitization.[16] BPA also accelerated the decay of $GABA_A$ receptor-mediated response more than DMP and TBP. However this is possibly in part because BPA increases the inhibition of the response of $GABA_A$ receptors with incubation time.

It is well known that ethanol affects our human mind by modulating $GABA_A$ receptors,[24] NMDA receptors[25] and G-protein-coupled inwardly rectifying potassium channels,[26] though the modulations were induced by ethanol above around 100 mM. It is unlikely that BPA inhibits the response of $GABA_A$ receptors under physiological conditions, since its concentrations required for the inhibition were high. However the potentiation of the $GABA_A$ receptor-response was induced significantly by about 10 μM phenol derivatives in the presence of 1 μM GABA (Table 1). To consider the effects of phenol derivatives on the neurotransmission *in vivo* under physiological conditions, we have to know both how high a concentration they will reach in the brain and what effects will be induced in the brain by 10–20% potentiation of the $GABA_A$ receptor-response in future. We have to be careful about the risk that some compounds may modulate some types of $GABA_A$ receptors critically during their catabolism in the brain and modify the neural transmission, since the modulation of the receptors strongly depends on both the functional group or its number of compounds,[11] and the subunit combination of the receptors.[18] Moreover these compounds are lipophilic and are likely to accumulate in fatty organs such as a brain.

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