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The α and γ subunit-dependent effects of local anesthetics on recombinant $GABA_A$ receptors

Masahiro Sugimoto, Ichiro Uchida*, Sakae Fukami, Makoto Takenoshita, Takashi Mashimo, Ikuto Yoshiya

Department of Anesthesiology, Osaka University Medical School, 2-2, Yamadao-ka, Suita, Osaka 565-0871, Japan

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Abstract

Although convulsions due to local anesthetic systemic toxicity are thought to be due to inhibition of $GABA_A$ receptor-linked currents in the central nervous system, the mechanism of action remains unclear. We therefore examined the effects of local anesthetics on γ-aminobutyric acid (GABA)-induced currents using recombinant $GABA_A$ receptors with specific combinations of subunits. Murine $GABA_A$ receptors were expressed by injection of cRNAs encoding each subunit into *Xenopus* oocytes. The effects of local anesthetics (lidocaine, bupivacaine, procaine and tetracaine) on GABA-induced currents of receptors expressing different subunit combinations (α1β2, α1β2γ2s, α4β2γ2s and β2) were examined via the two electrode voltage clamp method. At α1β2, α1β2γ2s and α4β2γ2s $GABA_A$ receptors, all local anesthetics inhibited GABA-induced currents in a dose-dependent manner. The presence of the γ2s subunit resulted in a greater inhibition by all local anesthetics, but the presence of the α4 subunit resulted in less inhibition. At β2 homomeric receptors, local anesthetics directly induced an outward current similar to that of picrotoxin. These data indicated that (1) the α and γ subunits of $GABA_A$ receptors modulated the inhibitory effects of local anesthetics on $GABA_A$ function, and (2) local anesthetics can activate the β2 subunit and may block the $GABA_A$ receptor channel pore.

Keywords: Convulsion; Central nervous system; Systemic toxicity

1. Introduction

Local anesthetics exert their primary effects of regional anesthesia and anti-arrhythmic therapy by actions on voltage-gated Na^+ channels. They also act on a wide range of other ion channels and receptors in cardiac muscle, smooth muscle, and in the peripheral and central nervous system (Fan et al., 1995; Hara et al., 1995; Ushijima et al., 1998). Local anesthetics readily cross the blood–brain barrier when administered systemically. At lower concentrations, they may produce analgesia, particularly for certain neuropathic pain conditions. Higher brain concentrations can produce sedation or restlessness, tremulousness, dysphoria, convulsions, and, ultimately, coma. The latter actions are referred to collectively as central nervous system toxicity (Steen and Michenfelder, 1979). Especially local anesthetic-induced convulsions could be due to the depression of inhibitory circuits. Tanaka and Yamasaki (1966) reported that a lidocaine selectively blocked inhibitory synapses between cortical neurons of unanesthetized rabbits. Hara et al. (1995) examined the post-synaptic effects of the local anesthetics, lidocaine, benzocaine and lidocaine *N*-ethyl bromide (QX314), on excitatory and inhibitory amino acid-induced currents in rat hippocampal neurons and found that these local anesthetics decreased γ-aminobutyric acid (GABA)- and glycine-induced chloride currents. Recently, Ye et al. (1997) examined the effects of cocaine, which shares major properties with local anesthetics, on $GABA_A$ receptor currents of rat hippocampal neurons and suggested that the suppression of GABA-induced chloride currents might contribute to cocaine-induced convulsion. Thus, the depression of inhibitory circuits in the central nervous system, especially via depression of $GABA_A$ receptors, may be involved in the mechanism of local anesthetic-induced convulsions. Consistent with this hypothesis is the observation that positive modulators (agonists) of $GABA_A$ receptors, benzodiazepines (De Jong and Heavner, 1974), barbiturates

* Corresponding author. Tel.: +81-6-6879-3133; fax: +81-6-6879-3139.

E-mail address: iuchida@anes.med.osaka-u.ac.jp (I. Uchida).

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(Steen and Michenfelder, 1979), and propofol (Bishop and Johnstone, 1993; Lee et al., 1998), can prevent or treat local anesthetic-induced convulsions.

The $GABA_A$ receptor is considered to be a hetero-pentameric receptor and 19 genes encoding the subunits, divided into seven classes, have been identified by molecular cloning techniques (Barnard et al., 1998). Different combinations of $GABA_A$ receptor subunits have shown variable sensitivity to allosteric modulators including anticonvulsants, sedatives and general anesthetics (Sieghart, 1995; Barnard et al., 1998). However, the subunits regulating the modulatory actions of local anesthetics in recombinant $GABA_A$ receptors have not been reported. To explore the modulatory subunits of $GABA_A$ receptors for the actions of local anesthetics, we first focused on the subunits, which determine the pharmacological properties of benzodiazepines since local anesthetic induced convulsion in vivo is effectively prevented and treated with benzodiazepines (De Jong and Heavner, 1974). The α subunit variants have been shown to exhibit major effects on the affinity and efficacy of benzodiazepines (Hadingham et al., 1993a; Wafford et al., 1996). The α4 subunit, as well as the α6 subunit, confers insensitivity to the classic benzodiazepines such as diazepam and flunitrazepam (Wafford et al., 1996). The γ subunit is required for benzodiazepine affinity and influences the benzodiazepine pharmacology [(Sieghart, 1995)]. Among γ subunits, the γ2 subunit is the most abundant isoform in the brain (Quirk et al., 1994). Secondly, we investigated the β subunits of the $GABA_A$ receptor since they forms the functional homomeric receptors (Cestari et al., 1996; Hadingham et al., 1993b). The homomeric receptor is an attractive system, which dissects out the functional domains responsible for physiological and pharmacological properties. The β2 subunit can form a functional homomeric receptor, which is sensitive to general anesthetics and picrotoxin while the expression of the agonistic action of GABA requires the presence of the α subunit (Cestari et al., 1996). The β2 subunit homomeric receptors may be useful to further define the molecular sites and modes of action of drugs at the $GABA_A$ receptors.

Thus, we examined the effects of local anesthetics on GABA-induced currents of recombinant $GABA_A$ receptors expressing α1β2, α1β2γ2s and α4β2γ2s subunit combinations and the actions of local anesthetic themselves on β2 homomeric receptors in *Xenopus* oocytes.

2. Materials and methods

2.1. Preparation of cRNAs for $GABA_A$ receptor subunits

Mouse cDNAs encoding α1, 4, β2 and γ2s $GABA_A$ receptor subunits were subcloned into the transcription vector, modified pBluescript (pBluescriptMXT). The multiple cloning site of the pBluescriptMXT was flanked by β-globulin of *Xenopus laevis* in order to facilitate stable mRNA expression in oocytes. Plasmid cDNAs were purified using the Qiagen plasmid kit (Qiagen, Chatworth, CA) and resuspended in sterile water. The cloned DNAs were confirmed to be coding for their respective subunits by the pattern of fragments with restriction enzymes. Each cDNA template was linearized by restriction enzymes (Wako, Osaka, Japan) (*Bgl*I for α4 and β2, *Pvu*II for α1 and γ2s). Capped cRNA was synthesized by the T3 RNA message machine kit (Ambion, Austin, TX) following the manufacturer's recommended protocol. Each cRNA stock in RNAse-free water was stored at −80°C until use.

2.2. Oocyte expression

By the study protocol approved by the Animal Research Committee of Osaka University Medical School, female frogs (*X. laevis*) were anesthetized with 1% tricaine (3-aminobenzoic acid ethyl ester) and operated on, on ice, under sterile conditions. Oocytes were harvested through a 5-mm laparotomy incision. Frogs were returned to the main tank after recovering for 2 days in isolation. The oocytes were manually defolliculated with forceps and treated with 1.5 mg/ml collagenase type 1A (Sigma, St. Louis, MO) for 30 min at room temperature in Ca^{2+}-free ND96 (in mM: NaCl 96, KCl 2, HEPES 5, $MgCl_2$ 1). Healthy oocytes in stages 3 and 4 were selected and thoroughly rinsed with ND96. The desired combination of subunit cRNAs (1 mg/ml) was mixed in equal ratios and 50–100 nl was injected into oocytes using a Nanoject injector (Drummond Scientific, Broomall, PA). Prior to electrophysiological experiments, oocytes were incubated for 48–72 h at 20°C in ND96 containing 1.8 mM $CaCl_2$, 2.5 mM sodium pyruvate, 5000 U/dl penicillin and 5 mg/dl streptomycin.

2.3. Electrophysiology and drug application

Forty-eight to 72 h after cRNA injection, oocytes were placed in a small well and continuously perfused with frog Ringer's solution (in mM: NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 10, pH 7.4) at a rate of 10 ml/min. Polyethylene tubing was used in the perfusion system. The oocyte was impaled with two 2–5 MΩ glass electrodes filled with 3 M KCl and voltage-clamped at −80 mV using a two-electrode voltage amplifier (Nihon Khoden, Tokyo, Japan). Drug solutions were applied by switching three-way stopcocks from the frog Ringer's solution to an otherwise identical solution containing the test drug at the desired concentration. Drug-containing solutions were administered for at least 20 s to obtain a peak current. Applications of GABA were separated by intervals of a few minutes; at high concentrations, 5- to 10-min intervals were used to permit resolution of receptor desensitization. Also by confirming the same response induced by a low concentration of GABA during an experiment with one oocyte, the

cumulative desensitization of the GABA receptor was excluded. The currents were digitally recorded with AxoScope software (Axon Instruments, Burlingame, CA), running on an IBM personal computer. All electrophysiological experiments were performed at room temperature.

2.4. Data analysis

Peak amplitude of the current elicited by GABA was measured directly from the digital recordings stored in AxoScope. To obtain concentration–response curves for GABA-induced currents and the concentration–inhibition curves for local anesthetics, observed peak amplitudes were normalized and plotted, then best fitted with the following equation using Origin software (Microcal Software, MA):

$$I = I_{max} / \left(1 + \left(EC_{50}^{n} / [GABA]\right)^{n}\right),$$

where I is the peak current at a given concentration of GABA and I_{max} is the maximum current. EC_{50} and n denote the concentration of GABA eliciting a half-maximum response and the Hill coefficient, respectively.

The equation for the concentration–inhibition curve of local anesthetics is:

$$I = \left[1 - [\text{local anesthetic}]^{n} / \left(IC_{50}^{n} + [\text{local anesthetic}]^{n}\right)\right],$$

where I is the reduced current normalized by that of control at a given concentration of local anesthetic. IC_{50} denotes the concentration of local anesthetic at a half-maximal current. All data are expressed as means ± S.E.M. Statistical analysis was performed using the two-sided *t*-test, with $P < 0.05$ indicating significance.

2.5. Chemicals

Drugs used in the present study were lidocaine, bupivacaine, procaine, tetracaine (Sigma), GABA, picrotoxin, bicuculline and diazepam (Wako). All drugs except diazepam were directly dissolved in frog Ringer's solution on the day of the experiment. Diazepam was dissolved in dimethylsulfoxamide (DMSO) and then diluted with frog Ringer's solution to the desired concentration. The final concentration of DMSO did not exceed 0.05% in any experiment. The DMSO solution (< 0.05%) alone induced no currents via recombinant $GABA_A$ receptors. After local anesthetics were dissolved in frog Ringer's solution, the pH was re-adjusted with 1 N HCl or NaOH, as needed, to pH 7.4, unless otherwise specified in the pH variation experiments.

3. Results

The expression of functional receptors containing $\alpha1\beta2$, $\alpha1\beta2\gamma2s$ and $\alpha4\beta2\gamma2s$ subunits receptors was confirmed by the presence of a GABA-induced current, which was blocked by 10^{-5} M bicuculline and 10^{-5} M picrotoxin (data not shown). The homomeric expression of β2 subunit was confirmed by the outward current induced by 10^{-5} M picrotoxin and the absence of the inward current induced by GABA [(Cestari et al., 1996)]. The expression of the γ2s subunit was determined by the potentiation by 10^{-6} M diazepam of the GABA-induced current of the $\alpha1\beta2\gamma2s$ subunit receptor (data not shown) [(Verdoorn et al., 1990)]. The concentrations of GABA for the control response corresponded approximately to EC_{20} values determined from concentration–response curves of GABA for $\alpha1\beta2$, $\alpha1\beta2\gamma2s$ and $\alpha4\beta2\gamma2s$ subunit receptors. In the $\alpha1\beta2$, $\alpha1\beta2\gamma2s$ or $\alpha4\beta2\gamma2s$ subunit $GABA_A$ receptor, the stable large inward current, which showed the least effect of the cumulative desensitization was elicited by EC_{20} of GABA. Shorter drug application intervals facilitated stable recording for hours without the irreversible run-down of the evoked current.

3.1. Effects of four local anesthetics on GABA-induced currents of recombinant GABAA receptors

Local anesthetics were divided into the two groups, the amide-type (lidocaine and bupivacaine) and the ester-type (procaine and tetracaine) by the linkage between the hydrophilic and hydrophobic domains in their structures. Both types of local anesthetics inhibited GABA (EC_{20})-in-

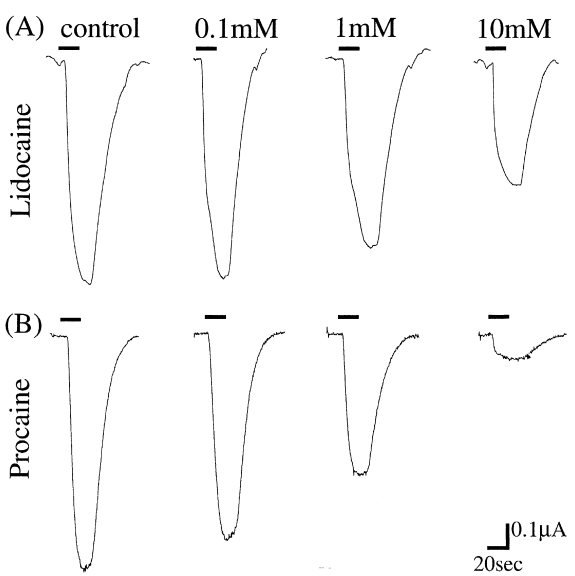

Fig. 1. The effect of lidocaine (A) and procaine (B) on GABA-induced currents of $\alpha1\beta2\gamma2s$ $GABA_A$ receptors. Co-application of GABA (EC_{20}: 5 μM) and lidocaine or procaine (0.01, 0.1, 1 and 10 mM) resulted in a reduction of current amplitude for the control response in a dose-dependent manner. The EC_{20} value of GABA was calculated from the dose–response curve constructed using the equation given in Materials and methods. Bars over current traces indicate the application of GABA and local anesthetics.

duced currents of the $\alpha1\beta2$, $\alpha1\beta2\gamma2s$ and $\alpha4\beta2\gamma2s$ subunit $GABA_A$ receptors in a concentration-dependent manner. The application of local anesthetics alone to the oocytes expressing $GABA_A$ receptors (i.e., in the absence of GABA) did not generate any measurable current over the entire range of concentrations used in these experiments. Fig. 1 shows inhibition of GABA-induced currents of the GABA-induced current of the $\alpha1\beta2\gamma2s$ subunit receptor by increasing does of lidocaine and procaine. Fig. 2 shows the effect of the γ subunit on the concentration–inhibition curves of lidocaine (A) and procaine (B) on GABA-induced currents of the $\alpha1\beta2$ and $\alpha1\beta2\gamma2s$ subunit receptors. Fig. 3 depicts the α subunit dependence of lidocaine (A) and procaine (B) on GABA-induced currents of the $\alpha4\beta2\gamma2s$ and $\alpha1\beta2\gamma2s$ subunit receptors. When compared with concentration–inhibition curves for currents of the $\alpha1\beta2\gamma2s$ subunit receptor in both figures, concentration–inhibition curves for currents of the $\alpha1\beta2$ and $\alpha4\beta2\gamma2s$ subunits were shifted to the left. The half-maximal inhibitory concentrations (IC_{50}s) of four local anesthetics on GABA-induced currents in recombinant $GABA_A$ receptors are summarized in Table 1. The presence of the $\gamma2s$ subunit conferred a two- to three-fold increase in the potency (decreased IC_{50}s) of both the amide- and ester-type local anesthetics in inhibiting GABA-induced currents. In contrast to the $\gamma2$ subunit, the $\alpha4$ subunit markedly decreased the potency (increased IC_{50}s) of local anesthetics in inhibiting GABA-induced currents. For all subunit combinations of $GABA_A$ receptor, the ester-type local anesthetics procaine and tetracaine more potently inhibited GABA-induced currents than the amide-type local anesthetics lidocaine and bupivacaine.

3.2. Effect of pH on local anesthetic inhibition of GABA-induced currents

Fig. 4 shows the concentration–inhibition curves of lidocaine on GABA-induced currents of the $\alpha1\beta2$ subunit receptor at pH 7.4 (A) and 6.2 (B). Although the effective form of local anesthetics (i.e., charged or neutral) is an important issue to understand how these compounds interact with ion channels [(Hara et al., 1995)], no effect on the dose–inhibition curves was observed between pH 7.4 and 6.2 lidocaine solutions. The change of pH in solution may

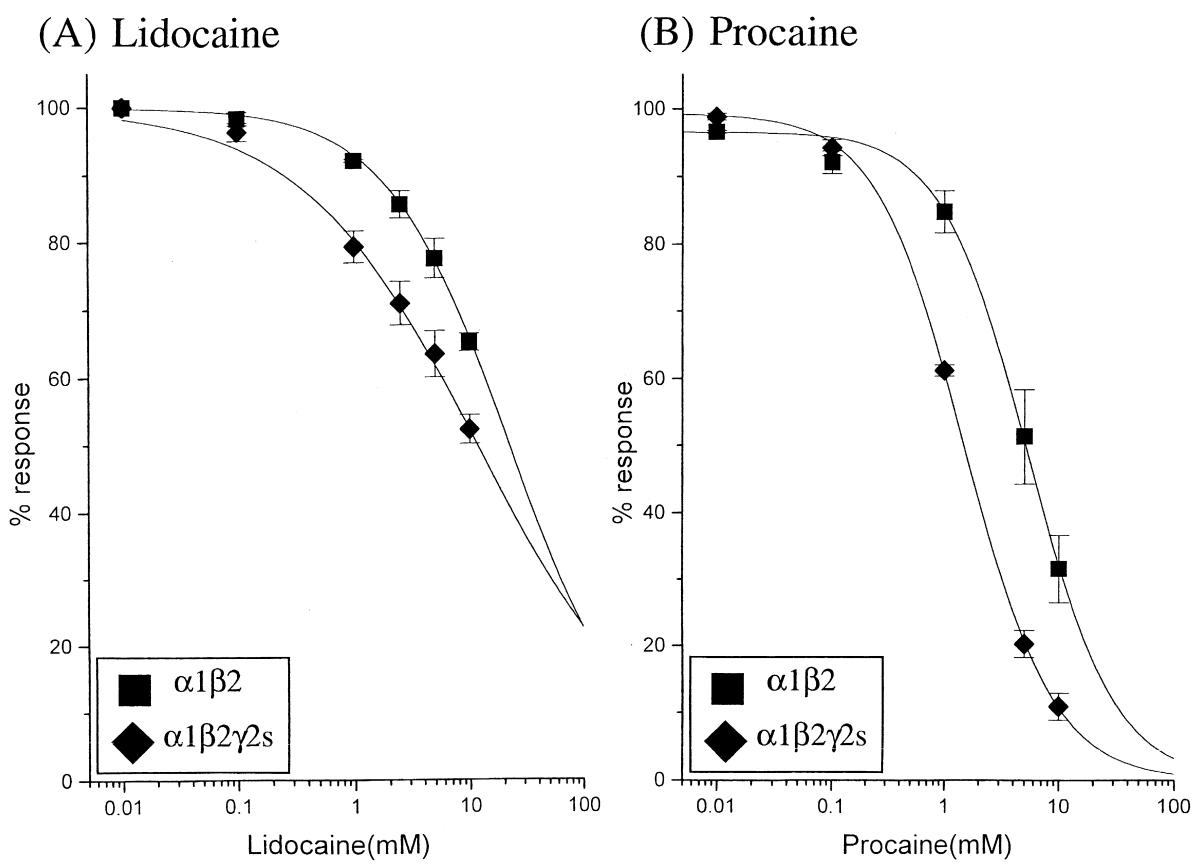

Fig. 2. The concentration–inhibition curves of local anesthetics (Lidocaine: A and Procaine: B) on GABA (EC_{20})-induced currents of $\alpha1\beta2$ and $\alpha1\beta2\gamma2s$ receptors. Each data point shows the average from four to seven oocytes and is expressed as the mean ± S.E.M. The presence of $\gamma2s$ subunit conferred a two- to three-fold increase in the sensitivity to both the amide- and ester-type local anesthetics.

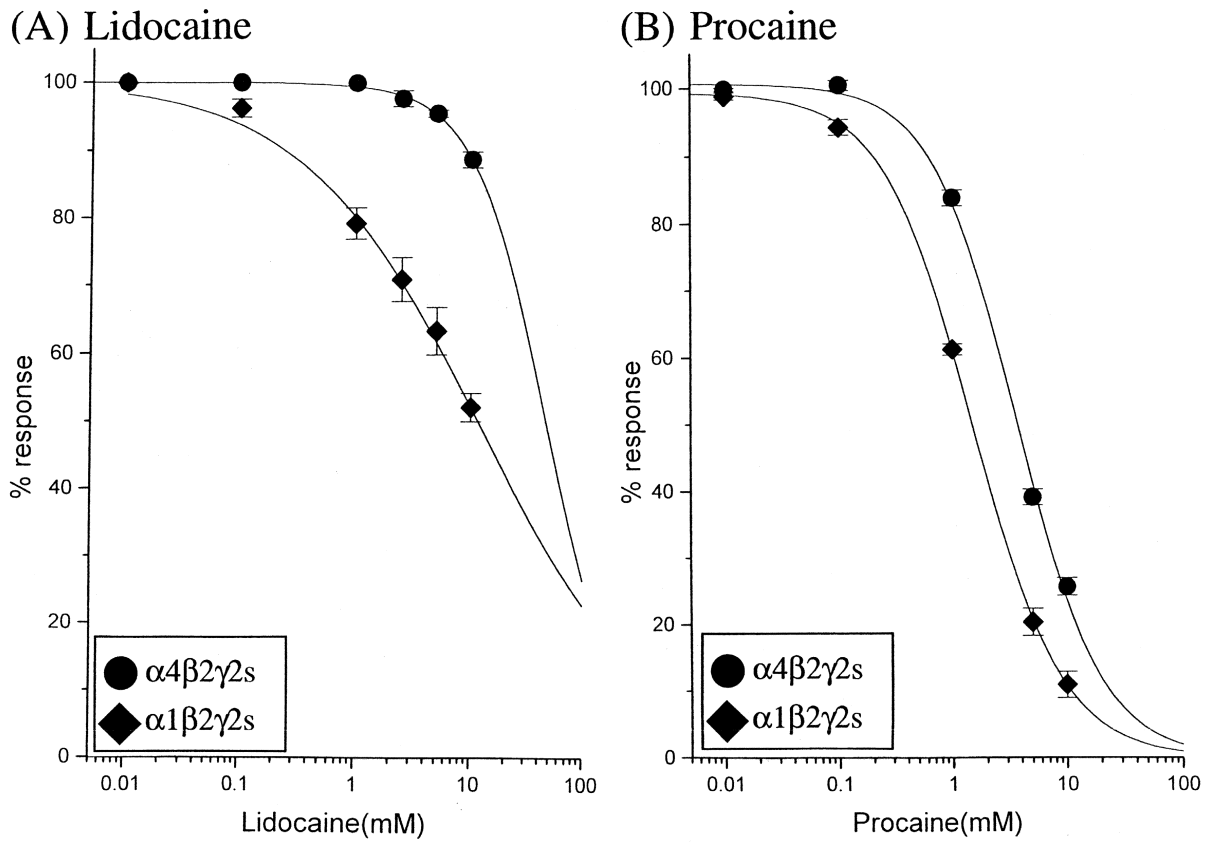

Fig. 3. The concentration–inhibition curves of local anesthetics (Lidocaine: A and Procaine: B) on GABA (EC_{20})-induced currents of α4β2γ2s and α1β2γ2s receptors. EC_{20} values of GABA for α4β2γ2s and α1β2γ2s receptors were 5 and 2 μM, respectively. Each data point shows the average from four to seven oocytes and is expressed as the mean ± S.E.M. The presence of the α4 subunit markedly decreased the sensitivity of local anesthetics at $GABA_A$ receptors.

not play a major role in the inhibitory effect of local anesthetics on recombinant $GABA_A$ receptor currents.

3.3. Effect of membrane potentials on local anesthetic inhibition

The current–voltage relation curves in the absence and presence of 10-mM lidocaine in oocytes expressing the

Table 1
The IC_{50}s and Hill coefficient (n^H) of local anesthetics in α1β2, α1β2γ2s and α4β2γ2s $GABA_A$ receptors. Values were calculated for five to eight oocytes. All data are expressed as means ± S.E.M.

Local anesthetics	Subunit combinations	IC_{50} (mM)	n^H
Lidocaine	α1β2	22.1 ± 1.2	0.82
	α1β2γ2s	11.5 ± 1.2	0.57
	α4β2γ2s	46.3 ± 6.1	1.34
Bupivacaine	α1β2	39.1 ± 10.0	0.88
	α1β2γ2s	14.5 ± 1.1	0.73
Procaine	α1β2	5.4 ± 0.4	1.15
	α1β2γ2s	1.5 ± 0.1	1.11
	α4β2γ2s	3.6 ± 0.1	1.18
Tetracaine	α1β2	3.5 ± 0.2	1.37
	α1β2γ2s	0.8 ± 0.8	1.10

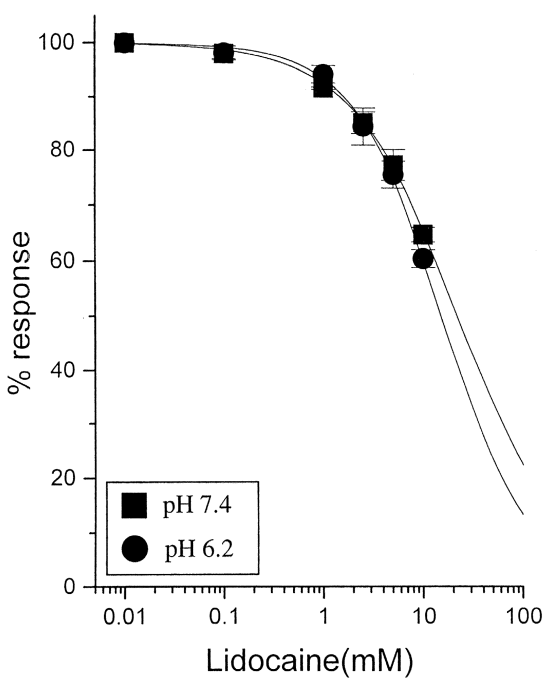

Fig. 4. The concentration–inhibition curves of lidocaine on GABA-induced current at pH 7.4 (A) and 6.2 (B) of the α1β2 receptor. A change in pH had no influence on the inhibition of GABA (EC_{20})-induced current by lidocaine. All data are expressed as means ± S.E.M.

α1β2γ2s subunit receptor are shown in Fig. 5A. Quantitative data for the effect of lidocaine at different membrane potentials examined (−80 mV to +20 mV) demonstrated that lidocaine inhibited the GABA-induced current in a voltage-dependent manner, the depression being particularly pronounced at negative membrane potentials [(Fig. 5B)].

3.4. Effects of local anesthetics at the β2 homomeric receptor

The expression of homomeric β2 receptors resulted in the formation of functional channels that were sensitive to picrotoxin, lidocaine, procaine and bicuculline [(Fig. 6)]. Local anesthetics and picrotoxin differed in the duration of

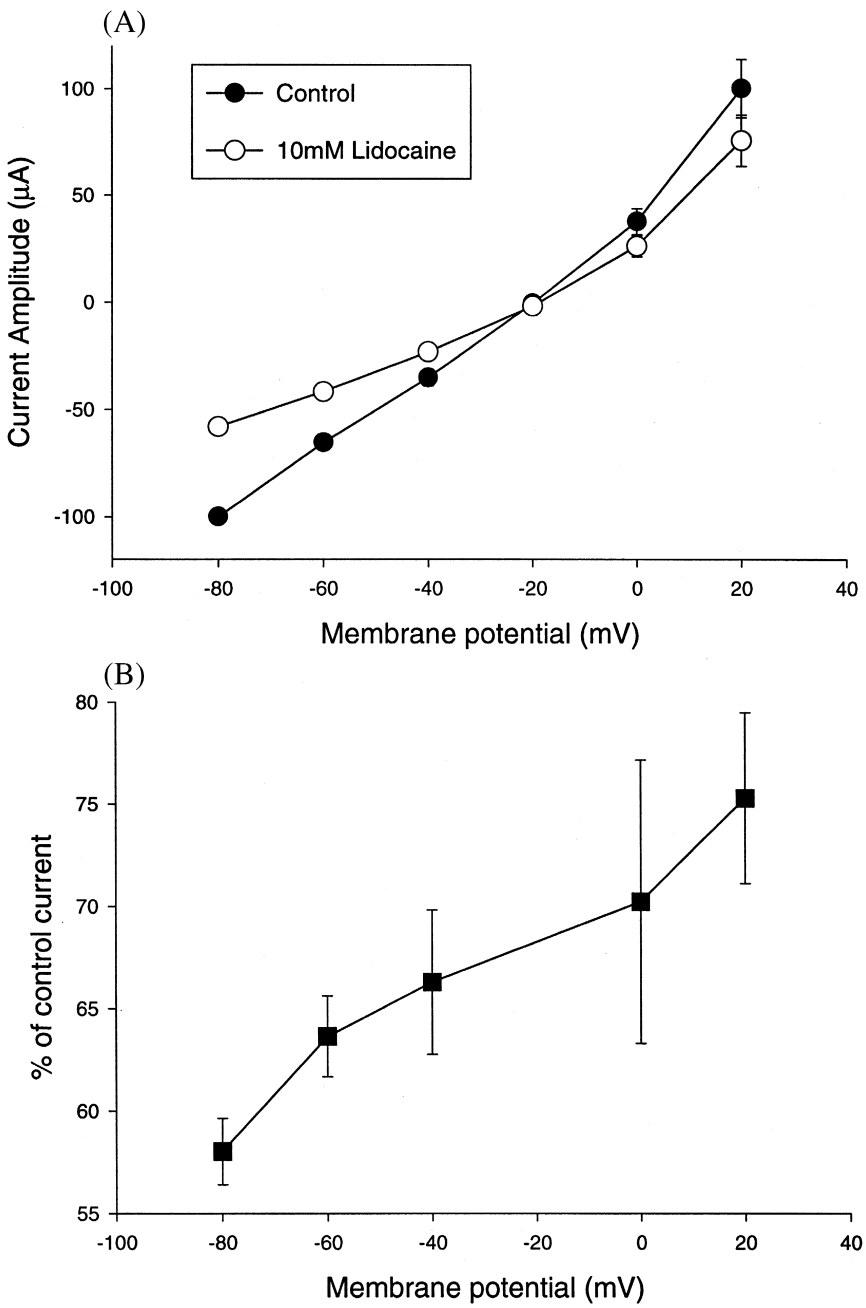

Fig. 5. Effect of lidocaine at different membrane potentials. (A) The current–voltage relation curves in the absence and presence of 10-mM lidocaine. All currents were induced by 5-μM GABA in oocytes expressing α1β2γ2s receptors. (B) The percentages of the control currents were plotted at different membrane potentials (−80 to +20 mV). The inhibition by lidocaine was strong at more negative potentials, showing the voltage-dependence. The data from four oocytes are expressed as means ± S.E.M.

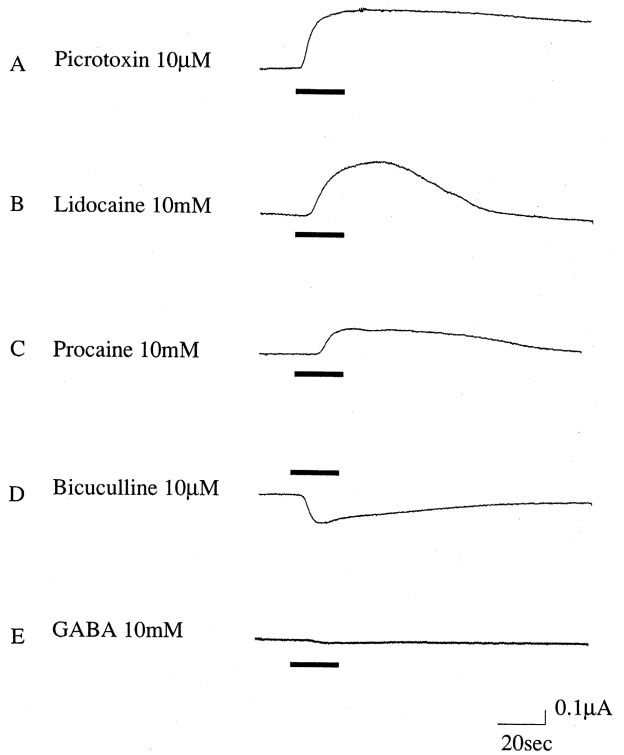

Fig. 6. The currents induced by picrotoxin, local anesthetics and bicuculline mediated by β2 homomeric receptors. Current traces for application of 10-μM picrotoxin (A), 10-mM lidocaine (B), 10-mM procaine (C), 10-μM bicuculline (D) and 10 mM GABA (E). Bars over current traces indicate the duration of applications of these drugs.

activation of receptors while both induced the same direction of currents. The outward current induced by local anesthetics did not last longer than that induced by picrotoxin. Bicuculline elicited small inward currents, corresponding to those mentioned in a previous report (Cestari et al., 1996). However, the homomeric β2 receptor was almost insensitive to GABA in very high concentrations ($> 10^{-3}$ M) (Fig. 6).

4. Discussion

$GABA_A$ receptors are the major receptors for inhibitory neurotransmission in the mammalian central nervous system. The $GABA_A$ receptors are known to be the sites of action for a large number of clinically important drugs such as benzodiazepines, barbiturates, ethanol and general anesthetics (Sieghart, 1995; Franks and Lieb, 1994; Yang and Uchida, 1996). The $GABA_A$ antagonists, bicuculline and picrotoxin, are potent convulsants, whereas central nervous system depressants, such as benzodiazepines and barbiturates, increase the chloride current induced by GABA and act as anticonvulsants (Sieghart, 1995; Barnard et al., 1998). Our present findings show that the local anesthetics, lidocaine, bupivacaine, procaine and tetracaine, can inhibit the GABA-induced currents of recombinant $GABA_A$ receptors. This results in suppression of inhibitory circuits, and thereby makes the central nervous system more excitable, which results in convulsions when the concentration of local anesthetics reaches a sufficient level in the brain. Other local anesthetics, such as benzocaine, QX314 (Hara et al., 1995) and cocaine (Ye et al., 1997), were recently shown to inhibit the GABA-induced chloride current of the $GABA_A$ receptor. Taken together, it is suggested that the inhibition of GABA-induced currents by local anesthetics may be a primary mechanism underlying local anesthetic-induced convulsions.

A concentration-dependent inhibition by each local anesthetic of GABA-induced currents was observed. The relative inhibitory potency was in the order of tetracaine > procaine > lidocaine > bupivacaine. Contrary to the expectation from clinical data (Foldes et al., 1960; Steen and Michenfelder, 1979), procaine was more potent than lidocaine and bupivacaine in terms of the inhibition of the GABA-induced current. In all subunit combinations, the ester-type local anesthetics were more potent than the amide-type ones. Considering the lack of pH dependence of the action of lidocaine on the GABA-induced current, the mode of action of local anesthetics on $GABA_A$ receptors appears to be somewhat different from their main effects on Na^+ channels. This observation merits further study.

The observed IC_{50} values in this study, except those for tetracaine and procaine, were relatively higher than the clinically reported plasma concentration that causes convulsions (Munson et al., 1975; Steen and Michenfelder, 1979). There may be a considerable gradient between local anesthetic concentrations in the blood and in the brain. Tissue distribution studies of both cocaine (mice) and lidocaine (rats) showed roughly five-fold higher brain concentrations than blood concentrations (Akerman et al., 1966; Patrick et al., 1993). There are no data available on the equivalent brain concentrations of local anesthetics at which convulsions occur, as the precise time course of distribution in the brain versus the course of maximal convulsant effect after a dose need to be considered. Thus, the plasma concentrations of local anesthetic that induce convulsions in animal studies may underestimate brain concentrations and this may partially explain the higher IC_{50}s in vitro. There are other plausible explanations to account for the discrepancy of the concentrations between in vitro and in vivo. One is that $GABA_A$ receptors may be only one among many ion channels involved in local anesthetic-induced convulsions. Hara et al. (1995) reported that not only $GABA_A$ receptors but also glycine receptors were effectively suppressed by local anesthetics. They suggested that the inhibition of both glycine and $GABA_A$ receptor functions in the central nervous system contributes to local anesthetic-induced convulsions. The summation of those inhibitory restraints at a concentration

lower than the IC_{50} of local anesthetic may be enough to permit the opposing excitatory drive, leading to the neuronal excitation that contributes to the convulsion. Also, local anesthetics may induce convulsions not only by suppression of inhibitory synapses but also by facilitation of excitatory synapses, including those involving *N*-methyl-D-aspartate: (NMDA) receptors. Kasaba et al. (1998) reported that bupivacaine induced convulsions were suppressed by the NMDA receptor antagonist, MK-801. Ushijima et al. (1998) reported that dizocipine (MK-801) inhibited convulsions due to cocaine, and that this effect was reversed by a low dose of NMDA, which itself did not induce convulsions.

The α subunit of $GABA_A$ receptors, when combined with the β and γ subunits, is involved in the increases in chloride currents produced by benzodiazepines (Hadingham et al., 1993a; Wafford et al., 1996). In this study, we demonstrated that local anesthetics showed considerably lower potency at α4 subunit-containing $GABA_A$ receptors. It is interesting that α4 subunit-containing receptors also showed insensitivity to classic benzodiazepine agonists (Barnard et al., 1998). The relationship between the modulatory sites of local anesthetics and benzodiazepines remains unclear from the present data. The α6 subunit, whose deduced amino acid sequence is most homologous to that of the α4 subunit, showed a benzodiazepine pharmacological profile similar to that of the α4 subunit (Wafford et al., 1996). With regard to general anesthetic modulation of $GABA_A$ receptor-mediated currents, pentobarbital potentiated the current mediated by α4 subunit-containing and α6 subunit containing receptors to an equivalent degree, while propofol potentiated the current mediated by the α4 subunit-containing receptor significantly less than that mediated by the α6 subunit-containing receptor. It suggested that these drugs act via different sites in the $GABA_A$ receptor. The γ subunit of $GABA_A$ receptor is involved in modulation by benzodiazepines, general anesthetics and zinc. The potentiation of GABA-induced chloride currents by benzodiazepines required the presence of the γ2 subunit (Sieghart, 1995). The efficacy of the general anesthetic enflurane in potentiating GABA-induced currents was greater for $GABA_A$ receptors expressing α1β2 subunits than for those expressing α1β2γ2s subunits (Whiting et al., 1995). Interestingly, the divalent cation zinc has been shown to be an antagonist of $GABA_A$ receptors made up of the αβ subunit combinations, and the introduction of the γ subunit reduced the sensitivity to zinc blockade (Whiting et al., 1995; Sieghart, 1995). In this study, we demonstrated that γ2s subunit-containing receptors (α1β2γ2s) showed high sensitivity to local anesthetics compared with the receptor without the γ2s subunit (α1β2). Unlike benzodiazepine, local anesthetics showed their modulatory properties on the αβ subunit combination in the absence of the γ subunit. These observations suggest that the action of local anesthetics appear to be regulated by the α and γ subunits and that local anesthetics have a different pharmacological profile from general anesthetics, benzodiazepines and zinc cation.

The α4 subunit is the least abundant α subunit in the brain and is located mostly in the thalamus (Wisden et al., 1992). Although the physiological role of receptors containing the α4 subunit is unclear, it appears from the pharmacological profile of local anesthetics at GABA receptors containing the α4 subunit to local anesthetics in vitro that this subunit contributes little to local anesthetic-induced convulsion. However, the γ2 subunit is the most abundant and ubiquitous γ subunit in the brain [(Wisden et al., 1992)], which confers a pharmacology comparable to that of most native $GABA_A$ receptors when co-expressing the α and β subunits (Whiting et al., 1995). The effects of the γ2 subunit on local anesthetic pharmacology could contribute significantly to the local anesthetic-induced convulsion.

Receptors formed by a single subunit, i.e., homomeric receptors, are attractive systems for determining the functional domains responsible for physiological and pharmacological properties of ligand-gated ion channels. Despite the lack of response to GABA, β2 homomeric receptors responded to barbiturates and propofol with inward currents. The β2 homomeric receptors responded to picrotoxin with outward currents, probably due to a blockade of spontaneously opening channels (Cestari et al., 1996). In our study, β2 homomeric receptors showed the same pharmacological responses to GABA, barbiturates and picrotoxin as previously reported. Interestingly, local anesthetics elicited an outward current, as did picrotoxin. Local anesthetics induced an outward current, which did not last as long as that induced by picrotoxin, suggesting different channel kinetics in β2 homomeric receptors. These results, considered together with the voltage-dependent inhibition by lidocaine of GABA-induced currents of the α1β2γ2s subunit receptor, and the phenomenon observed with the β2 homomeric receptors, are consistent with the hypothesis that an effect of local anesthetics on $GABA_A$ receptors may result from the blockade of the chloride channel pore. Also, our results for the β2 homomeric receptor at least revealed that the β2 subunit of the $GABA_A$ receptor forms a functional chloride channel that contains sites for the direct activation effects of local anesthetics. The molecular mechanism of these actions is not understood, and the question of whether there is a specific site for these agents on the $GABA_A$ receptor remains to be determined.

In conclusion, we demonstrated that local anesthetics dose-dependently inhibited GABA-induced currents mediated by α1β2, α1β2γ2s and α4β2γ2s subunit receptors and that the α and γ subunits modulated their actions. The γ2s subunit increased but the α4 subunit decreased the sensitivity to inhibition by local anesthetics. The data for the β2 homomeric receptor indicated local anesthetics could activate the β2 subunit and block the $GABA_A$ receptor channel, like picrotoxin.

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