Comparison of the Effects of Convulsant and Depressant Barbiturate Stereoisomers on AMPA-type Glutamate Receptors

Yoshinori Kamiya, M.D.*, Tomio Andoh, M.D., Ph.D., † Ryosuke Furuya, M.D., ‡ Satoshi Hattori, M.D., Ph.D., ‡ Itaru Watanabe, M.D., ‡ Tosio Sasaki, M.D., ‡ Hideki Ito, M.D., ‡ Fukuichiro Okumura, M.D., Ph.D.§

Background: α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors mediate fast excitatory synaptic transmission in the central nervous system. Although barbiturates have been shown to suppress the AMPA receptor-mediated responses, it is unclear whether this effect contributes to the anesthetic action of barbiturates. The authors compared the effects of depressant [R(−)] and convulsant [S(+)] stereoisomers of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) on the AMPA and γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor-mediated currents to determine if the inhibitory effects on AMPA receptors correlate to the in vivo effects of the isomers.

Method: The authors measured whole-cell currents in the rat cultured cortical neuron at holding potential of −60 mV. Kainate 500 μM was applied as the agonist for AMPA receptors. Thiopental (3–300 μM), R(−)-MPPB or S(+)MPPB (100–1,000 μM) was coapplied with kainate under the condition in which the GABA<sub>A</sub> receptor-mediated current was blocked. Effects of MPPB isomers on the current elicited by GABA 1 μM were studied in the separate experiments.

Results: Thiopental inhibited the kainate-induced current reversibly and in a dose-dependent manner, with a concentration for 50% inhibition of 49.3 μM. Both R(−)-MPPB and S(+)MPPB inhibited the kainate-induced current with a little stereoselectivity. R(−)-MPPB was slightly but significantly more potent than S(+)MPPB. In contrast, R(−)-MPPB enhanced but S(+)MPPB reduced the GABA-induced current.

Conclusions: Both convulsant and depressant stereoisomers of the barbiturate inhibited the AMPA receptor-mediated current despite of their opposite effects on the central nervous system in vivo. Although thiopental exhibited a considerable inhibition of AMPA receptors, the results suggest that the inhibition of AMPA receptors contributes little to the hypnotic action of the barbiturates. (Key words: Anesthetic mechanism; cortical neurons; ion channels; patch clamp.)

BARBITURATES have been known to potentiate the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor-mediated current, and this effect has been considered to play an important role in anticonvulsive and hypnotic effects of barbiturates. 1,2 Several studies have shown that barbiturates also affect a variety of other ligand-gated and voltage-dependent ion channels in the nervous system. 3–7 For example, barbiturates inhibit the currents mediated by neuronal nicotinic acetylcholine receptors. 5,8 α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors, 7,9,10 voltage-dependent sodium and calcium channels. 5,4 It is not necessary that the enhancement of GABA<sub>A</sub> receptor function is the sole mechanism for barbiturate anesthesia. However, it has not been clarified whether the effects on other ion channels contribute to the anesthetic action of barbiturates.

Because AMPA-type glutamate receptors mediate fast excitatory synaptic transmission in a large part of the central nervous system (CNS), suppression of the AMPA receptor-mediated response may be a potential mechanism for producing anesthesia. In fact, it has been reported that 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline, a competitive AMPA receptor antagonist, reduces minimal alveolar concentration of halothane and causes loss of righting reflex at high doses in the rat. 11,12 This finding is consistent with the idea that AMPA receptor antagonism is one of the mechanisms responsible for anesthesia. It has been shown that pentobarbital inhibits the AMPA receptor-mediated current at clinically relevant concentrations in cultured rat cortical neurons and an expression

* Postgraduate Student.
† Assistant Professor.
‡ Lecturer.
§ Professor and Chair.

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Address reprint requests to Dr. Andoh, Department of Anesthesiology, Yokohama City University School of Medicine, 3-9 Fukaura, Kanaawaku, Yokohama 245-0004, Japan. Address electronic mail to: tandoh@med.yokohama-cu.ac.jp

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system using *Xenopus* oocyte.\textsuperscript{7,9} Thiopental also has been shown to depress the AMPA-induced depolarization in mouse cortex.\textsuperscript{13} We aimed to clarify whether inhibitory effects of barbiturates on AMPA receptors play significant roles in the anesthetic action of barbiturates.

Many barbiturates are optically active, and the optical isomers show different potencies in their hypnotic and anticonvulsive effects.\textsuperscript{14} Among these, the stereoisomers of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPP) exhibit the opposite effects on the CNS: i.e., although R(−) isomer depresses CNS function and results in loss of righting reflex, S(+) isomer exerts the pure stimulating effects including convulsion.\textsuperscript{15} It is noteworthy that S(+) isomer of MPPP shows no sedative action at any doses. It has been shown that R(−) MPPP potentiates but S(+) MPPP reduces GABA-stimulated Cl flux in mouse brain membranes, supporting the view that GABA\textsubscript{A} receptors are responsible for clinical effects of barbiturates.\textsuperscript{16} We have used these stereoisomers to explore the significance of AMPA receptor inhibition by barbiturates. If the inhibitory effects of MPPP isomers on AMPA receptors correlate with their in vitro effects on the CNS, it would be likely that AMPA receptor inhibition contributes to barbiturate anesthesia in addition to GABA\textsubscript{A} receptor potentiation. We have compared the effects of the optical isomers of MPPP on the AMPA receptor-mediated current in cultured cortical neurons and examined whether thiopental, a barbiturate widely used for induction of anesthesia, inhibits AMPA receptors at clinically relevant concentrations. We found that although thiopental depressed the AMPA receptor-mediated currents significantly at clinically relevant concentrations, both the convulsant and depressant isomers of MPPP reduced the currents, with a small difference in their potencies. These results indicate that AMPA receptor inhibition is not important for the hypnotic action of barbiturates.

### Materials and Methods

#### Tissue Culture

This study was approved by our institutional animal care and use committee. Cortex neurons were prepared using methods similar to those described elsewhere.\textsuperscript{17} Wistar rats (of 17–18 days’ gestation) were anesthetized with diethylether and killed by cutting of aorta. The cerebral hemispheres were removed from 8 to 12 fetuses and placed in cold Leibovitz L-15 medium. After removal of the meninges, the hippocampi were dissected out, and the cortices were minced and incubated in PBS(−) with 0.25% trypsin (Life Technologies, Rockville, MD) for 12 min at 37°C. The cortical tissue was rinsed once and dissociated in 1:1 mixture of Dulbecco modified Eagle’s and Ham’s F-12 media supplemented with 5% fetal calf serum. A heat-polished Pasteur pipette was used to disperse the neurons. The dissociated neurons were plated at a density of approximately 10,000/cm\textsuperscript{2} on polyornithine-coated cover slips in multiwell culture plates. The cultures were maintained in a humid atmosphere of 95% air and 5% CO\textsubscript{2} at 37°C. Twenty-four hours after plating, the medium was changed with a serum-free medium (Dulbecco modified Eagle’s/Ham’s F-12 media supplemented with transferrin, insulin, progesterone, and selenite). Thereafter medium change was performed every 7 days. For the present study, neurons cultured for 6–21 days were used.

### Electrophysiology

Membrane currents were measured by conventional whole-cell voltage-clamp method.\textsuperscript{18} Cells on the cover slips were placed in a recording bath with an approximate volume of 1.5 ml and continuously perfused at the rate of 1–2 ml/min with an external solution containing NaCl 145 mm, KCl 5 mm, CaCl\textsubscript{2} 2.4 mm, MgCl\textsubscript{2} 1 mm, N-2-hydroxy-ethylpipperazine-N’-2-ethanesulphonic acid (HEPES) 10 mm, and D-glucose 10 mm (pH was adjusted to 7.4 with NaOH). Tetrodotoxin (0.5 μm) and bicuculline methobromide (10 μm) were included in the external solution to prevent voltage-dependent sodium channel-induced action potential firing and GABA\textsubscript{A} receptor-mediated Cl current. Heat-polished patch pipettes had tip resistance of 2–10 MΩ. In the experiments studying AMPA receptor-mediated currents, an internal solution contained CsF 140 mm, CsCl 10 mm, HEPES 10 mm, and ethylene glycol-bis-(β-aminoethyl ether)tetraacetic acid 5 mm (pH was adjusted to 7.3 with CsOH), which brought the reversal potential for Cl close to the holding potential. Cells were voltage-clamped at −60 mV with a patch clamp amplifier (CEZ 2400, Nihon Koden, Tokyo, Japan). Effects of MPPP isomers on the GABA-induced currents were studied in the separate experiments, in which bicuculline was omitted and the reversal potential for CI was close to 0 mV with an internal solution containing CsCl 150 mm, HEPES 10 mm, ethylene glycol-bis-(β-aminoethyl ether) tetraacetic acid 5 mm, and adenosine 5’-triphosphate magnesium salt 2 mm (pH was adjusted to 7.3 with CsOH). Whole-cell currents were filtered at 0.5 kHz with a Bessel filter and digitized at 1
kHz. The currents were stored on a computer using pClamp software (Axon Instrument, Foster City, CA) and analyzed using Axograph 3.0 software (Axon Instrument, Foster City, CA). All experiments were performed at room temperature (22–26°C).

**Drug Application**

A Y-tube method described elsewhere was used for delivery of the agonists and the barbiturates. Kainate, 500 μM, was used as the AMPA receptor agonist because it induces the non-desensitizing current through AMPA receptors and the elicited responses were almost saturated at 500 μM (data not shown). The saturating concentration of the agonist was chosen, because glutamate, the endogenous agonist, reaches the saturating concentrations at the synaptic cleft in vivo. Thiopental (3-300 μM), R(+)–MPPB or S(+)–MPPB (100–1,000 μM) in the external solution were coapplied with kainate. The agonist with or without the barbiturates was applied for 5 s, and each application was separated by 4 min. For the experiments using 6-cyano-7-nitroquinolxaline-2,3-dione (CNQX), cells were pretreated with CNQX 10 μM for 5 min and then CNQX was coapplied with kainate. In the experiments studying GABA<sub>δ</sub> receptors, GABA 1 μM with or without the barbiturates was applied in the same manner as described for kainate.

We applied both MPPB isomers to a given cell to compare the effects of the isomers on the kainate- or GABA-induced current in the same population of cortical neurons. One half of the recorded cells received R(+) isomer first and the other half received S(+) isomer first to eliminate time-dependent bias.

**Drugs**

Drugs used in this study included kainate, adenosine 5′-triphosphate magnesium salt, GABA, CNQX (Sigma, St. Louis, MO), thiopental sodium (Tanabe, Osaka, Japan), tetrodotoxin, dimethyl sulfoxide (Wako, Osaka, Japan), and bicuculline methobromide (Research Biochemicals, Natick, MA). S(+)–MPPB and R(+)–MPPB were kindly provided by Prof. J Knabe (Saalander University, Germany). The enantiomers are chemically pure, and their optical purity is about 96%. Thiopental sodium was dissolved in distilled water to make a stock solution of 100 mM and stored at −80°C. S(+) and R(+)–MPPB were dissolved in 0.1 N NaOH to make a 30-mM solution just before the experiments. They were diluted with the external solution to the designated concentration. The solutions containing kainate and/or the barbiturates were checked for their pH. They were at pH 7.35–7.45, except for the ones containing 1,000 μM of MPPB isomers. We added 50 μl of 1-M HCl in 50 ml of the external solution containing 1,000 μM of MPPB isomer to adjust pH to between 7.3 to 7.45. The increase in Cl concentration by this procedure was estimated to be only 2 mM. CNQX was dissolved in DMSO to make 20-mM stock solution, stored at −80°C, and diluted with the external solution just before the experiments.

**Data Analysis**

We measured the steady-state current, which was defined as the average amplitude from 4.95 to 5 s during agonist application. Because the kainate-induced currents declined slightly with each application of kainate, the responses in the presence of the barbiturates were compared with the average amplitude of the elicited currents before and after the barbiturate application. On the other hand, the GABA-induced currents did not decline over several repeated applications, the responses in the presence of the barbiturates compared with the control amplitude before the barbiturates application. Concentration-inhibition curves were fitted to the empirical Hill equation by a least-squares fit,

\[ I = I_0 - C^n/(C^n + IC_{50}^n), \]

where I is the relative current normalized to the control currents, C is the concentration of the barbiturates, n is the Hill coefficient, and IC<sub>50</sub> is the concentration for 50% inhibition.

**Statistical Analysis**

The data were expressed as means ± SEM. The significance of differences were analyzed using one-way analysis of variance followed by Dunnnett test for comparison among different doses of MPPB. A paired t test was performed to analyze differences between R(−) and S(+) isomers. A P value less than 0.05 was considered to be significant.

**Results**

**Effects of Thiopental on the Kainate-induced Current**

Kainate, 500 μM, elicited inward currents exhibiting no desensitization in rat cultured cortical neurons at −60 mV, and these currents were strongly depressed by CNQX 10 μM, a selective AMPA receptor antagonist (fig. 1). These observations were consistent with the AMPA receptor-mediated current. Thiopental alone induced no
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Fig. 1. Inhibition of the current elicited by kainate 500 μM by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) 10 μM in the cultured rat cortical neuron. The neuron was held at ~60 mV, and kainate 500 μM with or without CNQX 10 μM was applied during the period indicated by the horizontal bar. The neuron was preincubated with CNQX 10 μM for 5 min before CNQX was coapplied with kainate. The kainate-induced current was recorded again after CNQX was washed out by plain external solution for 5 min (wash). CNQX 10 μM almost completely abolished the kainate-induced current. This effect was partially reversible in 5-min interval.

current responses at 100 μM or 300 μM in tested cells (n = 4 for each dose). A concentration of 500 μM is about 10 times higher than the reported EC50 for general anesthesia and the maximum concentration used in the present study. This finding confirmed that the direct activation of GABA_A receptors by thiopental was effectively blocked in the present condition. Thiopental, 3–300 μM, coapplied with kainate, inhibited the kainate-induced current and caused the current decay (fig. 2). The inhibition was evaluated by measuring the amplitudes of the steady-state current at the end of drug application. The inhibition was reversible and dose-dependent, with IC50 being 49.3 ± 5.6 μM and Hill’s coefficient being 1.17 ± 0.14 (fig. 3). These results showed that thiopental inhibits the AMPA receptor-mediated current significantly at clinically relevant concentrations.

Effects of MPPB Isomers on the Kainate-induced Current

At 1,000 μM, MPPB isomers evoked no current when they were applied alone (n = 5 for each). Both R(-)-MPPB and S(+)-MPPB depressed the kainate-induced current.

Fig. 2. Inhibition of the kainate-induced current by thiopental 100 μM. Kainate 500 μM was applied with or without thiopental 100 μM during the period indicated by the horizontal bar. Thiopental was applied without preincubation. Thiopental 100 μM inhibited the kainate-induced current and caused the decay of the current. These effects were reversible.
current reversibly in a dose-dependent manner (fig. 4). R(−)-MPPB was slightly but significantly more potent as an AMPA receptor inhibitor than S(+)-MPPB at three doses tested (fig. 5). R(−)-MPPB and S(+)-MPPB reduced the kainate-induced current to 53 ± 3% and 72 ± 5% of control at 1,000 μM. These results demonstrated that although the effects of MPPB isomers on the CNS were completely opposite in vitro, both isomers inhibited AMPA receptors expressed in rat cultured cortical neurons with a little stereoselectivity.

Effects of MPPB Isomers and Thiopental on the GABA<sub>A</sub> Receptor-mediated Current

The slowly decaying inward currents were evoked by GABA 1 μM at −60 mV in the separate experiments, in which bicuculline was omitted and the reversal potential for Cl was close to 0 mV, so that the GABA<sub>A</sub> receptor activation should result in an inward current at the negative membrane potential. R(−)-MPPB 300 μM, the depressant, enhanced but S(+)-MPPB 300 μM, the convulsant, reduced the GABA<sub>A</sub> receptor-mediated current to 580 ± 90% and 64 ± 10% of control (figs. 6, 7). R(−)-MPPB alone induced inward currents that were inhibited by 10 μM of bicuculline (fig. 7). The experiments showed that R(−)-MPPB directly activated the GABA<sub>A</sub> receptor as did the other barbiturates and that R(−)-MPPB 300 μM was almost equally potent to GABA 1 μM. On the other hand, S(+)-MPPB 300 μM elicited no inward current when applied alone. It induced small outward currents, which were not characterized in this study. These results confirmed that MPPB isomers have the opposite effects on GABA<sub>A</sub> receptors and these effects correlate to their in vivo effects on the CNS.

For the comparison, effects of thiopental on the GABA-induced currents were also studied. Thiopental enhanced the current elicited by GABA 1 μM to 248 ± 50% (n = 6) and 721 ± 157% (n = 5) of control at 10 and 50 μM. The magnitude of enhancement by 50 μM of thiopental was not statistically different from that by 300 μM of R(−)-MPPB, according to the unpaired t test.

Fig. 3. The concentration–inhibition curve of thiopental for the kainate-induced current. The steady-state currents in the presence of thiopental were normalized to the average of the control currents before and after thiopental and plotted against the concentration of thiopental. A least-squares fit was performed using the equation described in materials and methods. The fitting procedure gave a concentration for 50% inhibition (IC<sub>50</sub>) of 49.3 ± 5.6 μM and Hill's coefficient of 1.17 ± 0.14. Each point represents the mean of five to seven experiments, and the error bar indicates SEM when it is larger than the symbol.

Fig. 4. The effects of 1,000 μM of S(+)-1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) and R(−)-MPPB on the kainate-induced current in a single neuron. MPPB isomers were coapplied with kainate 500 μM in the same way as in the procedure reflected in figure 2. Both isomers inhibited the kainate-induced current reversibly. Inhibition by R(−)-MPPB was greater than that by S(+)-MPPB.
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Fig. 5. The effects of the increasing concentrations of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) isomers on the kainate-induced currents. Each bar represents the mean ± SEM of the relative current, which is the normalized steady-state current in the presence of MPPB isomers relative to the average of control before and after MPPB. Dotted bars = S(+)MPPB; hatched bars = R(-)MPPB. Both isomers inhibited the kainate-induced current in a dose-dependent manner, and inhibition was statistically significant at the concentrations of 300 and 1,000 μM for both isomers. Inhibition by R(-)MPPB is significantly greater than that by S(+)MPPB at each dose. The relative currents in the presence of S(+)MPPB and R(-)MPPB were as follows: at 100 μM (n = 7), 0.39 ± 0.01 versus 0.93 ± 0.02; at 300 μM (n = 9), 0.83 ± 0.03 versus 0.76 ± 0.03; and at 1,000 μM (n = 14), 0.72 ± 0.05 versus 0.53 ± 0.03.

Discussion

The results of the present study demonstrate that both depressant [R(-)] and convulsant [S(+)] isomers of MPPB suppress the AMPA receptor-mediated current with little stereoselectivity in rat cultured cortical neurons. Although the inhibition by R(-) isomer was significantly stronger than that by S(+) isomer, the changes in the kainate-induced current caused by these stereoisomers were the same in their directions despite their opposite effects on the CNS in vivo. On the other hand, the depressant isomer potentiated but the convulsant isomer suppressed the GABA<sub>A</sub> receptor-mediated current, reflecting their in vivo effects on the CNS. Although thiopental inhibited the AMPA receptor-mediated current at clinically relevant concentrations, these results suggest that the inhibition of AMPA receptors contributes little to the hypnotic ef-

Fig. 6. The contrasting effects of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) isomers on the y-aminobutyric acid (GABA)-elicited currents. A single neuron was exposed to GABA 1 μM, GABA with S(+)-MPPB 300 μM, and GABA with R(-)-MPPB 300 μM sequentially with 4-min intervals. Although R(-)-MPPB 300 μM strongly potentiated the current elicited by GABA 1 μM, S(+)-MPPB 300 μM slightly decreased it.
Fig. 7. The effects of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) isomers on the γ-aminobutyric acid (GABA)-elicited current and the direct actions of the stereoisomers. Each bar depicts the mean ± SEM of the steady-state current normalized to the control current recorded before application of the barbiturates. The relative currents elicited by coapplication of R(-)-MPPB and S(+)-MPPB 300 µM with GABA 1 µM were 5.8 ± 0.9 (n = 8) and 0.6 ± 0.1 (n = 8), respectively. When 300 µM of each of the MPPB isomers was applied alone, R(-) isomer induced inward currents sensitive to bicuculline 10 µM, but S(+)-isomer elicited no inward current. The relative currents were 1.1 ± 0.16 for R(-)-MPPB 300 µM alone (n = 6) and 0.06 ± 0.05 for R(-)-MPPB plus bicuculline 10 µM (n = 6).

Effect of barbiturates, one of the important elements of the anesthetic actions.

Limitations of this Study

Temperature. In this study, all experiments were performed at room temperature. An experiment at 37°C may have different results. However, the effects of pentobarbital on the GABA_A receptor-mediated current have been shown to be qualitatively similar at room temperature and at 36°C in dorsal root ganglion neurons. Moreover, the effects of MPPB isomers on GABA_A receptor function were qualitatively the same in a flux study performed at 30°C as those in our electrophysiological study at room temperature. Maksay et al. reported that temperature did not significantly affect the displacing potencies of MPPB enantiomers on [35S]t-butylycyclopentasilane binding to GABA_A receptors from 19 to 37°C. These observations suggest that effects of the barbiturates on GABA_A receptors are qualitatively similar at different temperatures. We do not have any data for temperature dependence or independence of barbiturates’ effects on AMPA receptors. However, it is unlikely that the changes in temperature affect the inhibitory effects of MPPB isomers on the AMPA receptor-mediated current in opposite directions.

Different Sensitivities of AMPA Receptors to Different Barbiturates. We assumed that the hypnotic effects of R(-)-MPPB share the same properties as those of other barbiturates, so we concluded that AMPA receptor inhibition is of little importance for the hypnotic effect of R(-)-MPPB as well as for other barbiturates such as thiopental and pentobarbital. Our conclusion is based upon the unproved assumption that the anesthetic barbiturates induce anesthesia through the common mechanisms. The assumption seems rational, because many anesthetic barbiturates exhibit similar effects on the CNS in vivo and in vitro. For example, pentobarbital and thiopental similarly modulate GABA_A receptors, neuronal nicotinic receptors, voltage-dependent calcium channels, and so on. They inhibit AMPA receptor-mediated response similarly at concentrations equal to or slightly higher than the clinically relevant concentrations.

However, AMPA receptors were less sensitive to R(-)-MPPB compared with pentobarbital and thiopental. R(-)-MPPB 1000 µM inhibited the kainate-induced current by only 47% at 300 µM of thiopental inhibited it by 80%. There seem to be no available data for the free aqueous concentration of MPPB corresponding to EC50 for the anesthetic effects. Therefore, potency of R(-)-MPPB as an anesthetic is unknown. It has been reported that an intraperitoneal injection of 90 mg/kg of R(-)-MPPB has almost the same potency as 40 mg/kg of pentobarbital in inducing the loss of the righting reflex in rats. Assuming that MPPB and pentobarbital behave similarly in terms of pharmacokinetics and plasma protein binding, MPPB seems about 0.4 times less potent than pentobarbital; i.e., free aqueous concentration of R(-)-MPPB would be about 2.5 times higher than pentobarbital at the equianesthetic dose, because the molecular weights of both compounds are about the same. In our experiments, 300 µM of R(-)-MPPB was equally effective as 30 µM of thiopental in augmentation of the GABA-induced current. R(-)-MPPB 300 µM and 30 µM of thiopental inhibited the kainate-induced current by about 24% and 37%, respectively. Therefore, it seems that AMPA receptors are slightly less sensitive to R(-)-MPPB than thiopental, compared at the concentrations producing the same enhancing effects on the GABA-induced current. However, if 300 µM is considered to be the relevant concentration based upon the effects on
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GABA_A receptor, R(−)-MPPB exhibited a significant inhibition of AMPA receptors at the relevant concentration, indicating that AMPA receptor inhibition is the common property of anesthetic barbiturates.

Preincubation with the Barbiturates. Cortical neurons were not pretreated with the barbiturates before the coapplication of the barbiturates with kainate. This is mainly because of the limited amount of available MPPB isomers. For thiopental, preincubation was not performed to compare its effects with those of MPPB isomers in the same protocol. When neurons were pretreated with thiopental and then thiopental and kainate were coapplied, the current decay observed in this study was negligible, and the inhibitions of the steady-state current were the same regardless of the presence or absence of pretreatment in the ongoing experiments, indicating that the current decay mainly reflect the delayed onset of blockade (unpublished data). We evaluated the inhibition by comparing the steady-state current measured at the end of agonist application, and the currents in the presence of the barbiturates seemed to reach the steady level during drug application. Therefore, the inhibitions by the barbiturates were appropriately evaluated at the steady state condition in this study.

Cultured Cortical Neurons. Although an earlier study reported that cultured rat cortical neurons express GluR 1–3 but not GluR 4 subunit, all subunit compositions or splice variants expressed in this preparation have not been identified to our knowledge. Agonist and antagonist pharmacology of AMPA receptors in cultured cortical neurons appears to be roughly comparable with that of native receptors from mature rats, because it has been reported that EC50 for kainate and glutamate as well as IC50 for CNQX and GYKI 52666, a noncompetitive antagonist, are similar in the receptors expressed in cultured cortical neurons and those in mature rat brain. However, it is possible that some population of AMPA receptors from adult animals or humans may have different sensitivities to barbiturates, because heterogeneous receptors distribute differentially and sensitivities to barbiturates vary depending on subunit compositions.

Significance of Differences in AMPA Receptor Inhibition by MPPB Isomers

We found that the blockade of the kainate-induced current by R(−) isomer was slightly but significantly larger than that by S(+) isomer. This difference indicates that the putative binding sites on which MPPB isomers act discriminate the stereoisomers not strongly but weakly. It is likely that MPPB isomers blocked the current through binding to the targets with the site remote from the chiral center, but not through the mechanisms based on their physicochemical properties. The small stereoselective differences also have been demonstrated for the binding of MPPB isomers to albumin, a soluble protein. 33

Effects of MPPB Isoomers on GABA_A receptors

The depressant isomer [R(−)] of MPPB potentiated but the convulsant isomer [S(+)] of MPPB reduced the GABA-induced current. The results of present study agree with those obtained by CI flux measurements using mouse brain membranes, 16 and support the concept that modulation of GABA_A receptor function is one of the responsible mechanisms for anesthetic effects of barbiturates. The stereoselectivity in GABA_A receptor modulation by barbiturate enantiomers also has been reported for pentobarbital, 56 suggesting that the binding sites on GABA_A receptors for barbiturates discriminate the chiral structure of the barbiturates.

Significance of AMPA Receptor Inhibition by Barbiturates

It has been clearly demonstrated that thiopental and pentobarbital suppress the AMPA receptor-mediated current at concentrations equal to or slightly higher than those clinically used in the present and previous studies. 9 These effects do not seem to be important for their hypnotic or anticonvulsant effects, because of a lack of correlation between AMPA receptor inhibition by MPPB isomers and their in vivo effects. However, it is possible that AMPA receptor inhibition contributes to other elements of the anesthetic state such as loss of memory, analgesia, attenuation of autonomic response to surgical stimuli, and so on. It is also possible that the inhibition of AMPA receptors is important for other actions on the CNS by barbiturates such as neuronal protection, because AMPA receptor antagonists have been shown to exert neuroprotective actions in vitro and in vivo. 35, 36 Pentobarbital and thiopental have been shown to be effective in reducing neuronal damage in the animal models of brain ischemia. 37–39 Further studies are needed to address if AMPA receptor inhibition is associated with these actions of barbiturates.

In conclusion, we demonstrated that both depressant and convulsant isomers of MPPB inhibited the AMPA receptor-mediated current in rat cortical neurons with a little stereoselectivity, despite their opposite effects on the CNS in vivo. These results suggest that AMPA recep-

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tor inhibition is not important for hypnosis induced by barbiturates. However, it is possible that inhibition of AMPA receptor may contribute to other actions on the CNS by barbiturates, because thionpental inhibited AMPA receptors at the relevant concentrations.

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