Does Propofol Enhance GABA-mediated Inhibition?

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The effects of propofol on synaptic transmission were characterized and compared with pentobarbital in the rat hippocampal slice preparation. Hippocampal CA1 population spike after stimulation of Schaffer collaterals indicated that the postsynaptic response was primarily mediated by non-N-methyl-D-aspartate class glutamate receptors since it was abolished by the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX). Propofol and pentobarbital depressed CA1 population spike amplitude in a dose dependent fashion. Dose-response curves for population spike amplitudes were determined for propofol and pentobarbital and the concentrations producing a half-maximum response (ED_{50}) were 110 μM and 160 μM for propofol and pentobarbital, respectively. By contrast, when $GABA_A$-mediated inhibition was blocked by addition of 100 μM pictotoxin, propofol, in concentrations up to 400 μM had no significant effect on population spike amplitudes. These results suggest that propofol attenuates synaptic transmission in the central nervous system in part by enhancing $GABA_A$-mediated inhibition and not by depressing glutamate-mediated excitation, as occurs with pentobarbital. (Key words: propofol, hippocampus, evoked potential, rat brain slice)

(Otsuka H, Yamamura T, Hanaoka Y, et al: Does propofol enhance GABA-mediated inhibition? J Anesth 6: 305–311, 1992)

There have been few studies that investigate the effects of propofol on synaptic events in the central nervous system (CNS). It has only been suggested that propofol could potentiate inhibition or attenuate excitation of evoked potentials in the cat spinal cord[1]. Collins[2] reported that propofol potentiated r-aminobutyric acid (GABA)-mediated transmission of the rat olfactory cortex slice, suggesting that propofol anesthesia is closely associated with the enhancement of GABA-mediated inhibition. However, general anesthetics are known to exert multiple effects on neural elements at different concentrations and/or in different preparations[3]. For example, barbiturates, that manifest anesthetic effects similar to propofol, are known to exert a dual action on synaptic events, potentiating GABA-mediated inhibition and depressing glutamate-mediated excitation[4–6].

Thus the following questions still remain unanswered: a) Is synaptic transmission depressed in the presence of propofol; b) Are the effects of propofol on synaptic transmission

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dose-dependent; c) Do the effects of propofol on synaptic transmission occur at clinically relevant concentrations? And d) Is there a difference between the synaptic effects of propofol and barbiturates?

In this study, we shall report the effects of propofol and pentobarbital on synaptic events by analyzing their dose-response relationships. For this, we recorded evoked field potentials from the cell body region of the CA1 area of the rat hippocampal slice while stimulating the Schaffer collaterals. The population spike amplitude reflects postsynaptic responses in CA1 pyramidal cells, where the primary excitatory transmitter has been shown to be glutamate[7–9]. The pyramidal cells also receive both feed-forward and feed-back recurrent collateral inhibition where the transmitter is mainly GABA[10,11]. The postsynaptic evoked responses were also examined after application of either picrotoxin (PTX), 2-amino-5-phosphonovaleric acid (APV) or 6,7 dinitroquinoxaline 2,3-dione (DNQX) which specifically antagonized the action of $GABA_A$, N-methyl-D-aspartate (NMDA) receptor agonists and non-NMDA glutamate receptor agonists, respectively.

Methods

The study protocol was approved by the Animal Ethical Committee of Hokkaido University School of Medicine. Male Wister rats (body weight, 80–115g) were anesthetized with halothane. After craniotomy, the rats were decapitated, the left hemisphere of the brain was rapidly removed and placed in cold, oxygenated artificial cerebrospinal fluid (ACSF). Slices of hippocampus, 300 μM thick, were cut perpendicular to the septotemporal axis using a vibrating slicer (Dosaka EM, DTK-1000, Japan) and incubated in ACSF for 2 to 3 hours at 30°C. The ACSF had the following composition (mM): NaCl, 125; KCl, 4; NaH_2PO_4, 1.25; $MgSO_4$, 1.5; $CaCl_2$, 2; $NaHCO_3$, 26; Glucose, 10; and was maintained at pH 7.3–7.4 when ACSF was continuously bubbled with 95% O_2 and 5% CO_2. Following incubation, a hippocampal slice was gently placed in a bathing chamber (capacity, 1.5 ml) were it was continuously perfused with ACSF at a rate of 2 to 3 $ml \cdot min^{-1}$. The temperature was kept at 30°C with a thermoelectric heating device.

A bipolar stimulating electrode made of a pair of teflon-coated platinum-iridium wires (a diameter, 25 μM and tip separation, 0.2 to 0.3 mm) was placed on Schaffer-collaterals of the stratum radiatum and 0.1 ms square wave pulses were delivered at 0.2 Hz. Stimulus intensity was adjusted to obtain the maximum amplitude of population spikes and maintained unchanged. Extracellular glass recording electrodes were pulled with a programmable micropipette puller (Sutter, P-87, USA). The electrodes were filled with 3M NaCl and had a resistance of 2 to 5 Mohm. The electrode was connected to a unity-gain high impedance electrometer (WPI, Duo-773, USA) and positioned in the cell body region of the CA1 area to record evoked field potentials. The distance between the stimulating and the recording electrodes was 1.2 to 1.7 mm. Population spike amplitudes were measured from threshold to the peak negativity.

After control response was obtained with normal ACSF, dose-response relationship was studied by perfusing a slice with ACSF which contained different concentrations of test anesthetics in the absence and the presence of 10 μM PTX. A sufficient period of time (10 to 15 min) elapsed before a plateau effect was achieved. Propofol (10 $mg \cdot ml^{-1}$; Diprivan) was supplied by ICI pharmaceuticals (England). An ultrasonic agitator was used to achieve

stable dilution of propofol emulsion in ACSF. The effect of the vehicle was examined with fat emulsion (Intralipid). All drugs and compounds were from Wako (Japan), except for APV (Sigma, USA), and DNQX (Tocris Neuramine, England) Each slice was used for one dose-response study and then discarded.

Signals were collected and processed by using a Hewlett-Packard 340CH computer system linked with a 14-bit, high speed A/D converter (TEAC, PS-9351, Japan). Values in the text are expressed as mean ± SD. Comparisons with control values were performed using analysis of variance (one way ANOVA) followed by a paired Student's t-test, and $P < 0.05$ was deemed statistically significant. The ED_{50} of anesthetics was determined by regression analysis.

Results

While bathed with normal ACSF, maximum population spike amplitudes in CA1 was obtained at stimulus intensities from 2.5 to 4 times of the threshold response. They ranged from 3.2 to 5.6 mV (4.4 ± 0.59 mV, n=36) and the peak latency was from 6.8 to 13.3 msec (10.2 ± 2.3 msec, n=36). Typical recordings are shown in figure 1. The population spike was completely abolished by the addition of 5 μM DNQX but was little affected with APV. This confirmed that the population spike reflected the postsynaptic response which is mainly mediated by non-NMDA glutamate receptors. The vehicle, soy bean oil up to 10 mg·ml^{-1}, had no effects on the population spikes.

Effects of propofol and pentobarbital on population spike are shown in figure 2. Population spike amplitude was reduced in the presence of both anesthetics. The effects were dose-dependent. The addition of 100 μM PTX almost reversed the effect of 200 μM propofol on the early population spike and it usually produced another late potential (fig. 2A). The reduction of population spike amplitude by pentobarbital was also partially reversed by PTX. The effects of both anesthetics and PTX were completely reversed after washing with normal ACSF. Since these findings suggested that the anesthetics might affect both on glutamatergic and GABAergic systems, we next analyzed the effects of propofol and pentobarbital on the population spike amplitude in the absence

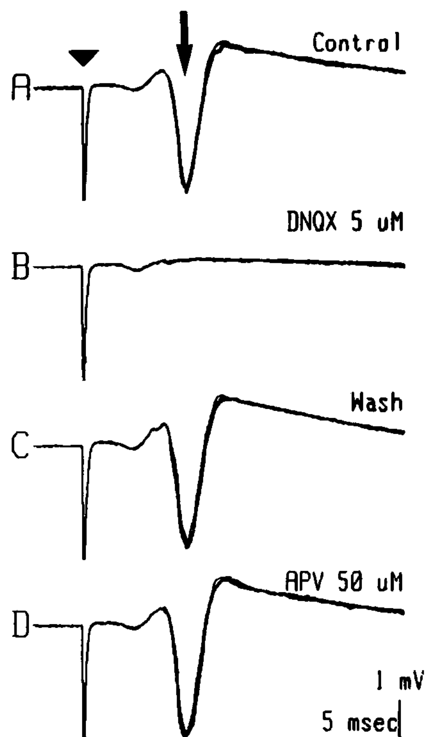

Fig. 1. Recordings of CA1 field potentials after stimulation of Schaffer collaterals. The stimulus artifact is marked with a triangle and a population spike is indicated by an arrow. Four consecutive traces are superimposed in each set. The population spike was completely abolished in the presence of 5 μM DNQX (B) and the effect was reversed by washing out of DNQX (C). APV had no significant effects on the field potentials (D).

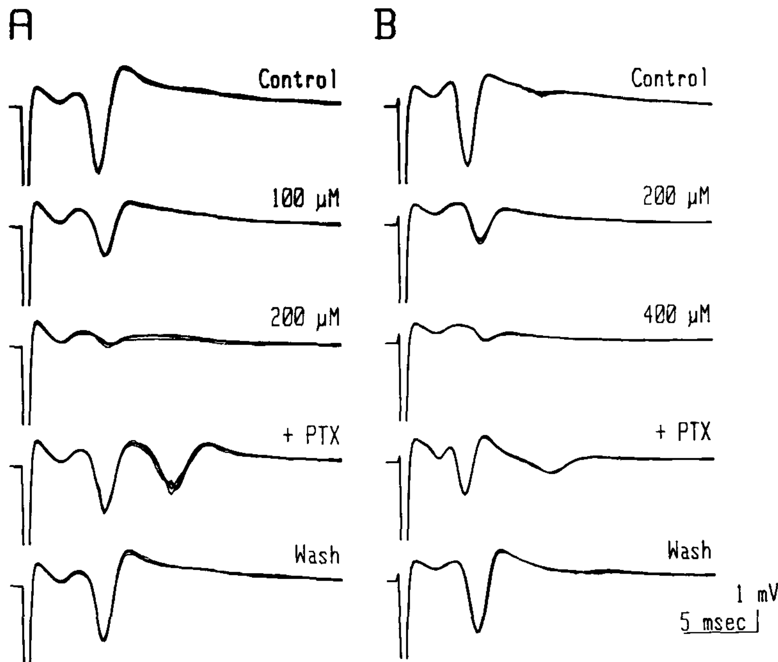

Fig. 2. Effects of propofol (A) and pentobarbital (B) on CA1 evoked field potentials. Four traces are superimposed in each set. The concentration of propofol or pentobarbital is labeled at right top. The depressant effect of 200 μM propofol was reversed by the addition of 100 μM picrotoxin. Note that picrotoxin produced another late potential.

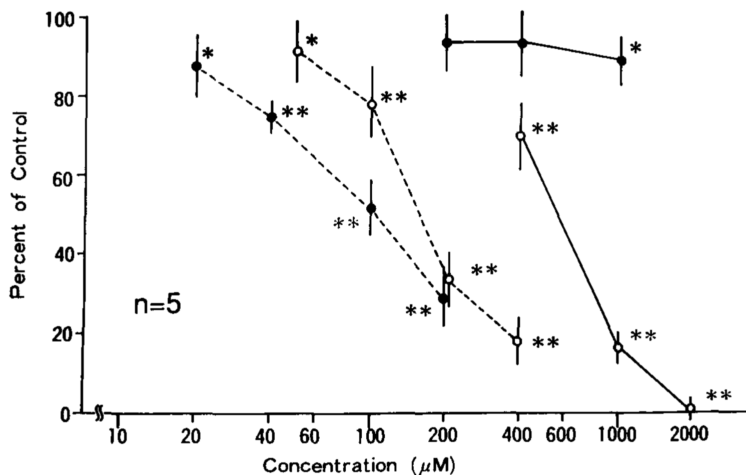

Fig. 3. Dose-response curves for propofol (filled circles) and pentobarbital (open circles) in the absence (dashed lines) and the presence (solid lines) of 100 μM picrotoxin. Data obtained from five slices were normalized to percent of the control values. Bars indicate SD of the mean. $*P < 0.05$, $**P < 0.01$ versus control values.

and the presence of picrotoxin and plotted dose-response curve (fig. 3). The population spike amplitudes were normalized to percent of the control value.

In the absence of picrotoxin, the concentration which produced a half-maximum response of the population spike amplitude (ED_{50}) was 110 μM for propofol and 160 μM for pentobarbital. The addition of 100 μM PTX shifted the dose-response curves to the right. The ED_{50} of pentobarbital became 560 μM which was 3.5-fold of the original value. The depressant effect of propofol on population spike amplitudes markedly decreased in the presence of picrotoxin. Population spike amplitude remained 89.7% of the control at 1000 μM propofol and ED_{50} was no longer obtained.

Discussion

The three major postsynaptic re-

ceptor subtypes for excitatory amino acids (EAAs) have been classified according to agonists for which they have high affinity, NMDA, kainate and quisqualate[8]. The latter are often grouped together as the 'non-NMDA' system and is generally considered to mediate fast synaptic transmission at glutamatergic synapses[12]. A series of studies in the *in vitro* hippocampal slice has demonstrated that fast excitatory postsynaptic potentials (EPSPs) appear to be mediated primarily by the non-NMDA receptor subtypes[13,14]. EPSPs mediated by NMDA receptors were revealed in specific experimental conditions. When both non-NMDA glutamatergic excitation and GABAergic inhibition were blocked a late field potential with multiple spiking became apparent with a latency from 20 msec to 100 msec at higher stimulus intensities[9,15]. Elimination of Mg^{2+} from the perfusate also promotes this response. In our experimental condition, the population spike is considered to primarily reflect responses mediated by non-NMDA receptors since it was abolished by DNQX. Late potentials which appeared after addition of PTX might reflect potentials mediated by NMDA receptors. However, evoked field potentials with large multiple spiking were rarely observed. In the present study, possibly due to the presence of Mg^{2+} (1.5 mM) and the lower bathing temperature.

Collins, using rat olfactory cortex slices, observed that field potentials which reflect GABA-mediated transmission were enhanced in the presence of propofol[2]. He also observed that propofol potentiated the response to bath-applied GABA and GABA agonists. The results obtained in this study further elucidate the synaptic effents of propofol and distinguish the action of propofol from that of pentobarbital. Since PTX only partially reversed the population spike amplitude which was reduced by pentobarbital this anesthetic seemed to attenuate population spike amplitudes by at least two separate mechanisms; depression of non-NMDA class glutamate-mediated excitation and enhancement of GABA-mediated inhibition. Which of the mechanisms are more sensitive to pentobarbital may depend upon the density and distribution of the inhibitory and the excitatory synapses[4–6].

By contrast, the population spike amplitude remained at 89.7% of control value in the presence of PTX with propofol concentrations as high as 1 mM. The results indicate that propofol attenuates population spike amplitudes by enhancing feed forward and feedback $GABA_A$-mediated inhibitory system with slight depression of non-NMDA-mediated excitation. Although it remains to be established whether propofol affects the other excitatory synaptic transmission systems, enhancement of $GABA_A$-mediated inhibition may be one of the most important mechanisms involved in the anesthetic action of propofol.

Anesthetic plasma levels of propofol are reported to be from 20 to 40 μM in human subjects[16]. The range corresponds to the approximate ED_{25} obtained in this study. However, it is well known that clinical levels of anesthesia are usually associated with only partial impairment of normal function of the CNS[3] because higher nervous function is composed of polysynaptic circuits where spatial and temporal summations are continually taking place. Therefore, it seems reasonable that the ED_{50} which was obtained based on monosynaptic responses might be much higher than that obtained based on total behavioral response. The potency ratio of pentobarbital to propofol obtained in this study was 2.4 and 1.5 at ED_{25} and ED_{50}, respectively. These values are consistent with intravenous

anesthetic doses in humans (2.0)[16], human anesthetic plasma levels (2.0)[16–18] and plasma levels in animals (1.8)[19].

During preparation of this manuscript, a paper appeared which indicates propofol augmented GABAA-mediated inhibition of the evoked potentials in the hippocampal dentate gyrus of rats anesthetized with urethane[20]. Our study would give evidences for their hypothesis.

Acknowledgment: This study was supported by grants from the Japanese Ministry of Education, Science and Culture to TY (No. 03454369).

(Received Nov. 18, 1991, accepted for publication Jan. 8, 1992)

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