Anesthesiology
2000; 93:463–72

Effects of Propofol on Hippocampal Synaptic Transmission in Behaving Rats

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Background: **The action of propofol has been studied in vitro and *in vivo*, but the effects of intravenously administered propofol on synaptic transmission in freely behaving rats have not been studied before.**

Methods: **Rats were implanted with recording electrodes in the dentate gyrus and with stimulation electrodes in the medial perforant path (MPP). Paired pulses at different interpulse intervals (IPIs) were delivered to the MPP, and average evoked potentials were recorded in the dentate gyrus before and after a bolus of propofol (10 or 20 mg/kg administered intravenously) or control vehicle was injected *via* a femoral vein cannula. Because of the layered structure of the hippocampus, population excitatory postsynaptic potentials and population spikes could be distinguished and analyzed.**

Results: **Propofol has no significant effect on the population excitatory postsynaptic potentials or population spike evoked by a single MPP stimulus pulse. However, paired-pulse inhibition of the dentate population spikes was increased at IPI of 20 and 30 ms. Paired-pulse inhibition of the population spike was most prominent when tail pinch response was lost (deep and moderate anesthesia), but it persisted during light anesthesia. At 200 ms IPI, paired-pulse facilitation of population spikes was observed during moderate anesthesia in most rats.**

Conclusions: **In freely behaving rats, intravenous propofol enhanced paired-pulse inhibition at < 50 ms IPI, likely by enhancing γ-aminobutyric acid$_A$ receptor–mediated inhibition. Propofol also increased paired-pulse facilitation at 200 ms IPI through an unknown mechanism, which may contribute to the neuroexcitatory effect of propofol. (Key words: GABA$_A$ receptor; paired-pulse facilitation; paired-pulse inhibition.)**

PROPOFOL (2,6-diisopropylphenol) is a popular intravenous anesthetic drug that produces a rapid induction of anesthesia and usually uneventful recovery. Occasionally, like many other general anesthetics, the administration of propofol has been reported to be associated with abnormal movements (*e.g.*, myoclonus, seizure-like movements, and opisthotonos).[1] Electrophysiologic studies among patients with epilepsy have yielded conflicting reports concerning the effect of propofol on interictal spike activity and seizure activity.[2–4] Although the cause of these phenomena remains poorly understood, considerable interest has focused on the effects of propofol on neurotransmission.

Propofol has been suggested to act on the γaminobutyric acid$_A$ (GABA$_A$), receptor but the binding site is not known. At a high dose, propofol may directly activate the GABA$_A$ receptor–chloride channel.[5–8] At low doses within the dose range used clinically, propofol may augment inhibitory transmission by enhancing chloride flux.[5–8] Propofol has also been found to enhance glycine receptor–mediated inhibition[5,8] and to inhibit the *N*-methyl-D-aspartate but not kainate receptor–activated current.[9]

Previous studies of the effects of propofol on synaptic transmission have been conducted mainly *in vitro*,[10–12] during circumstances in which the anesthetic dose of propofol can only be inferred. Two major studies have evaluated the effects of propofol *in vivo*. In one study,[13] urethane was administered before the effects of propofol were evaluated. Thus, the effects of propofol may have been masked by urethane.[14] In the other study, Hasan and Woolley[15] reported the results of an intraperitoneal injection of propofol on behaving rats. In humans,

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Received from the Departments of Physiology, Clinical Neurological Sciences, and Anaesthesiology, University of Western Ontario, London, Canada. Submitted for publication June 28, 1999. Accepted for publication March 15, 2000. Supported by a Natural Sciences and Engineering Research Council of Canada grant (to Dr. Leung) and an internal research grant from London Health Sciences Centre, London, Canada (to Drs. Herrick and Leung). Presented in part at the 28th Annual Meeting of the Society for Neuroscience, Los Angeles, California, November 10, 1998.

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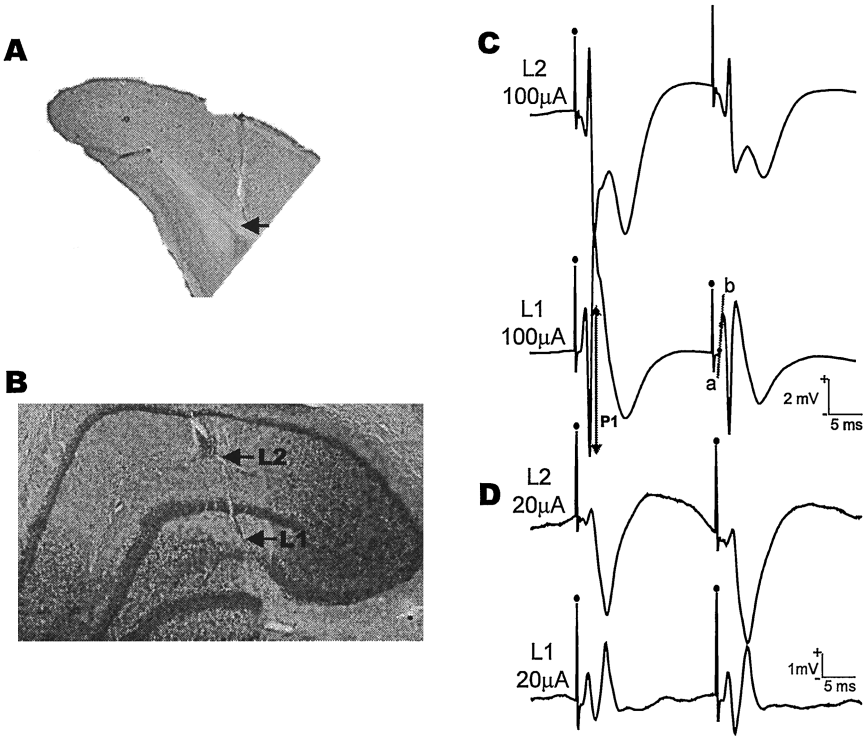

Fig. 1. Placement of electrodes and evoked field potentials in a representative rat. (*A*) Location of stimulating electrode (arrow) targeted at the medial perforant path (MPP) in the angular bundle. (*B*) Location of the shallow (L2) at near the hippocampal fissure and deep (L1) recording electrodes in the hilus of the dentate gyrus. (*C*) Average evoked potential (n = 8) recorded from the electrode L2 (*top*) and L1 (*bottom*) after 100 μA stimulation of the MPP. The slope of the line "ab" measured the increasing slope of the population excitatory postsynaptic potential (EPSP) before the onset of the population spike. The population spike amplitude (P1) was measured as the difference between the positive and negative peaks of the transient event. Shock artifacts indicted by dots. (*D*) Average evoked potential at L2 and L1 after 20 μA stimulation of the MPP, which evoked a small population spike.

5propofol is only administered intravenously. Intraperitoneal administration of propofol does not consistently yield deep anesthesia in rats.

In this study, we used freely behaving rats to assess synaptic transmission in relation to behavioral anesthesia.[16] This preparation allows changes in synaptic transmission to be correlated temporally with the depth of anesthesia (level of consciousness and response to noxious stimuli) assessed clinically. Rats were implanted with chronically indwelling electrodes to stimulate the major afferent pathway of the hippocampus, the medial perforant path (MPP).[17,18] Extracellular potentials in the dentate gyrus (DG) were recorded. Because of the parallel arrangement of granule cells in a layered structure, the evoked potential in the DG can be clearly interpreted as the population excitatory postsynaptic potential (EPSP) and the population spike, resulting from a synchronous firing of neurons in the DG.[17,19] We report that paired-pulse inhibition of the population spikes at short (< 50 ms) interpulse intervals (IPIs) and paired-pulse facilitation at 200-ms IPI were both enhanced by propofol.

Materials and Methods

Procedures were approved by the Animal Use Committee at the University of Western Ontario. Male Long Evans rats weighing 260–540 g were anesthetized with sodium pentobarbital (65 mg/kg intraperitoneally) and chronically implanted with intracranial electrodes. Each electrode was a 125-μm diameter wire, which was insulated with Teflon except at the cut ends. A stimulating electrode was placed in the MPP at posterior 8.0 mm, lateral 4.4 mm with reference to Bregma, and 3.3 mm ventral from the skull surface (fig. 1A)[18,20] according to the atlas of Paxinos and Watson.[21] A pair of recording electrodes was placed at posterior 3.8 mm, lateral 2.4 mm, with the deeper electrode at the hilus of the DG (ventral 4 mm) and the shallow electrode near the hippocampal fissure (ventral 3.7 mm; fig. 1B).[18,20] Two jeweler's screws were implanted in the skull to serve as reference electrode and stimulus anode, respectively. The electrodes were held in place using dental cement.

Rats in which the electrodes showed responses typical of those evoked by the MPP (fig. 1) were given a second operation using pentobarbital to cannulate a femoral vein. A PE 50 tubing filled with heparinized saline was secured within the vein, tunneled subcutaneously, and brought out through the area of skin behind the rat's ears. Rats were allowed to rest for 2–6 days after the second operation before recording.

During recording, the rat was habituated in a transparent plastic cage and connected to a flexible cable.

Table 1. Characteristics of the Different Anesthesia Phases Recorded

Anesthesia Phase	Ability to Move Voluntarily	Tail Pinch Reflex	Hippocampal EEG
Baseline awake immobility	Yes	Yes	Large irregular slow activity
Deep anesthesia	No	No	Flat (<5% baseline EEG power)
Moderate anesthesia	No	No	Low-amplitude irregular slow activity
Light anesthesia	No	Yes	Irregular slow activity
Recovery (awake immobility)	Yes	Yes	Large irregular slow activity

Hippocampal electroencephalogram (EEG) was only used to define the deep anesthesia phase, when the EEG was almost flat, and not used to define the other phases.

EEG = electroencephalogram.

Cathodal pulses were applied to the MPP stimulating electrode, using a skull screw as the anode. A programmable digital pulse generator (Master-8, A.M.P.I., Jerusalem, Israel) was used to trigger pulses through an S11 stimulator and PSIU-6 isolation unit (Grass Instrument Co, Quincy, MA). Evoked potentials were amplified by 7P511 amplifiers on a Grass 7D Polygraph (Grass Instrument Co.), which was also used to record electroencephalograms (EEGs). Two to three channels of potentials were then sampled at 7 KHz and averaged (n = 4–8 traces) using a custom program on a microcomputer. The threshold for evoking a detectable evoked potential in the DG after MPP stimulation ranged from 20 to 70 μA using a 0.1-ms pulse duration. The range of stimulus intensities used was 40–700 μA, or 2–10 times the response threshold. Pairs of stimulation pulses were delivered at an interval of > 8 s, with a paired-pulse IPI of 10, 20, 30, 50, 100, 150, 200, or 500 ms. To reduce the behavioral variation of the hippocampal evoked responses,[22–24] MPP stimulation was only delivered selectively during immobility in the rat. In the undrugged state, immobility was operationally defined to be the behavior when the rat made no body movement (other than respiratory and small vibrassal movements) and was clearly awake (*e.g.*, with head held up against gravity).

After at least 1 h of baseline recording, propofol (10 mg/kg or 20 mg/kg administered intravenously) or a control injection was given. The low dose of propofol (10 mg/kg administered intravenously) induced antinociception[25] and EEG burst suppression,[26] whereas 20 mg/kg administered intravenously caused burst suppression or flat EEG in animals.[27] Propofol was purchased from Zeneca Pharma (Mississauga, Ontario, Canada) or Abbott Laboratories Ltd. (Saint-Laurent, Quebec, Canada). The control vehicle injected was a 10% fat emulsion, purchased from Clintec Nutrition Company (Mississauga, Ontario, Canada) and given in the same volume as propofol. The injections were given slowly over 10–30 s. After propofol injection, tail pinch was given to the rat periodically (between evoked potentials). The middle of the tail was pinched manually by the nails of the experimenter with a force that slowly increased to approximately 2 kg and maintained for at least 2 s. Tail pinch was delivered on a different location of the tail until the rat showed some movement to the pinch. In some rats, rectal temperature was monitored before and after propofol anesthesia.

In this report, deep (surgical) anesthesia was defined as the period immediately after propofol injection in which a nearly flat EEG was observed. Power spectral analysis revealed that the hippocampal EEG power was suppressed by > 40 times across all frequencies (not shown). Because the period of flat EEG was relatively brief, complete characterization of the paired-pulse response (*i.e.*, at all IPIs) could not be achieved. Thus, we operationally defined a moderate anesthesia phase as the period after propofol during which a tail pinch induced no movement. The hippocampal EEG amplitude during the latter period was suppressed (but not flat). After the rat moved a part of the body during tail pinch, the rat was considered to have entered a light anesthesia phase. After the rat moved spontaneously, it was considered to have entered the recovery phase. Thus, excluding the deep anesthesia phase, four phases were distinguished in an experiment (table 1): (1) baseline phase before the injection of propofol; (2) moderate anesthesia phase; (3) light anesthesia phase; and (4) recovery phase. Because the control vehicle injection did not induce anesthesia, the experiment was separated into three phases: baseline before injection, postinjection phase (0–24 min after injection), and recovery (> 24 min after injection). The postinjection phase was used for comparison with the moderate and light anesthetic phases after propofol.

Most rats were used for other doses of propofol or control vehicle experiments after 2 days' rest. At the end of the experiments, rats were euthanized with a large dose of urethane (> 1.5 g/kg administered intraperitoneally) and perfused with saline followed by 4% formal-

dehyde. Coronal brain sections 40 μm thick were stained with thionin, and the positions of the electrodes were confirmed (figs. 1A and 1B).

Statistical Analysis

The population EPSP slope was defined as the increase of the potential over a time interval of 0.5–1 ms; this slope was measured on the increasing phase of the EPSP and clearly before the onset of the population spike (fig. 1C). The population spike amplitude was measured as the difference between the onset and the negative peak of the transient event (fig. 1C). The paired-pulse population spike ratio was calculated as the amplitude of the population spike evoked by the second pulse (P2) divided by that evoked by the first pulse (P1), *i.e.*, P2/P1. The paired-pulse EPSP ratio was similarly defined as the ratio of the EPSP slope evoked by the second pulse (E2) to that evoked by the first pulse (E1), *i.e.*, E2/E1.

Different experimental measures were compared using a random block two-factor (time phase × IPI) analysis of variance (ANOVA), followed by a *post hoc* Tukey protected *t* test. Two nonparametric statistical tests, the Kruskal-Wallis one-way ANOVA and Wilcoxon two group signed-rank test, were also used.

Results

Effect of Propofol on Electroencephalogram and Behavior

An intravenous bolus dose of 20 mg/kg propofol delivered in approximately 30 s had an immediate effect on the rat's behavior. Within 15 s after injection, the head dropped and spontaneous respiration slowed. Within 0.5–1 min, the hippocampal EEG amplitude was greatly diminished, showing mainly an isoelectric (flat) trace. The latter was defined as the deep anesthesia phase (table 1), which may last 2–3 min. There was no response to a tail pinch during deep anesthesia. Typically, by 4 min, the hippocampal EEG changed to a low-amplitude slow δ activity, although no tail pinch response could be elicited until approximately 8 min. The latter was defined to be the moderate anesthesia phase. After the return of the tail pinch response, a rat typically remained immobile for many minutes; this was defined as the light anesthesia phase. Spontaneous head or body movements demarcated the onset of the recovery phase, typically at 20–30 min after the 20-mg/kg intravenous dose.

A 10-mg/kg intravenous dose of propofol induced similar EEG and behaviors as compared with the higher dose, except with a shorter duration. An exception was that the hippocampal EEG was not flattened by a 10-mg/kg intravenous dose. The period of EEG burst suppression (deep anesthesia) was typically < 3 min, and it was followed by a period with slow δ EEG. Thus, the moderate anesthesia phase started at 1–3 min after injection and lasted for 4–5 min, until a tail pinch elicited a response. The rat then remained spontaneously immobile for another 5–8 min (light anesthesia phase). Spontaneous head movements started at 9–15 min after a 10-mg/kg intravenous injection.

Although some rats engaged in a period of ataxic walking during the early recovery period, the recovery from anesthesia was gradual and usually not accompanied by any signs of hyperactivity**.** No change in behavior was observed after injection of the vehicle.

Normal Hippocampal Evoked Response after Medial Perforant Path Stimulation

Evoked potentials were obtained from 26 rats. In most rats, both EPSPs and population spikes could be observed (fig. 1), although in some rats the population EPSP could be not be readily distinguished.

A low-intensity stimulus (typically < 40 μA) evoked a positive wave of approximately 10-ms duration in the hilus of the DG, accompanied by a negative wave near the molecular layer of the DG (fig. 1D). This wave was interpreted as the population EPSP generated by excitatory currents at the mid-dendrites of granule cells.[17,18] High stimulus intensity evoked a population spike which was negative at the granule cell layer and in the hilus and positive in the middle molecular layer and dorsal CA1 (fig. 1C).[18,19] With increasing stimulus intensity, the amplitude of the population spike increased and its peak latency decreased.

During baseline immobility, the P2/P1 ratio was a triphasic function of the IPI for a moderate stimulus intensity of approximately two times the threshold of P1 (usually 100–300 μA). Typically, the P2/P1 ratio was less than 1 at short (< 30 ms) and long (> 150 ms) IPI, and larger than 1 at intermediate IPIs of 50–100 ms (fig. 2).

Effect of Propofol on the Population Spikes and Paired-pulse Depression

In some rats, the relation of evoked potentials in relation to the deep anesthesia phase was studied immediately after injection of 20 mg/kg intravenous propofol. In six experiments on six rats, the first-pulse population

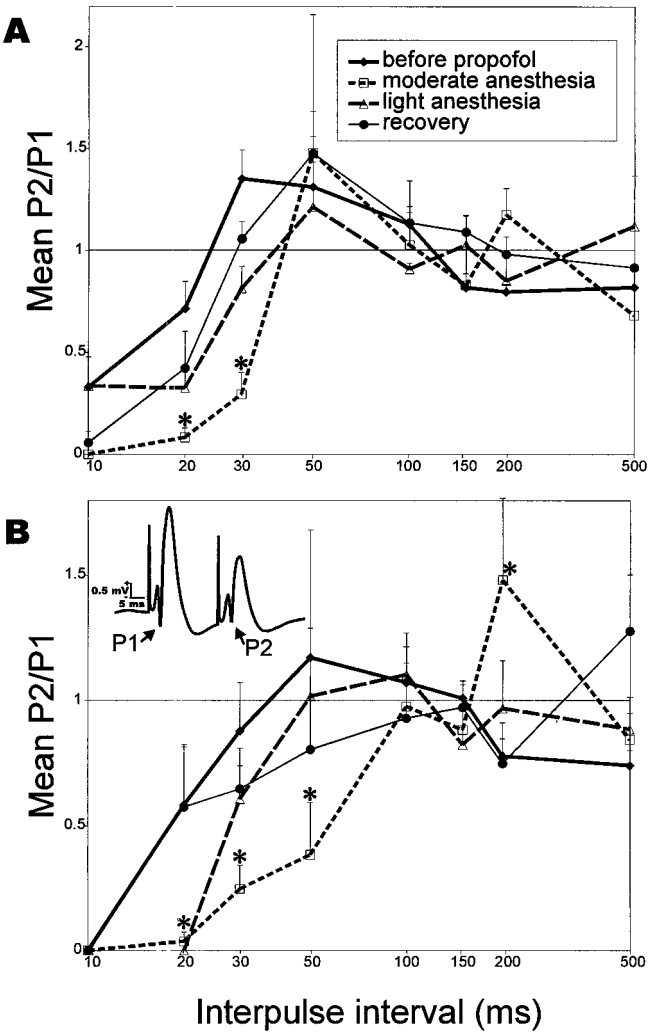

Fig. 2. The effect of propofol on paired-pulse population spike ratio (P2/P1) as a function of the interpulse interval (IPI) shown in a logarithmic scale. Stimulating intensity was approximately 2× threshold of P1. An illustration of population spike amplitudes P1 and P2 is shown in the inset of (*B*). (*A*) Group average of seven rats given 10 mg/kg intravenous propofol at four different time phases—baseline before propofol (filled diamonds), moderate anesthesia (unfilled squares), light anesthesia (unfilled triangles), and recovery (filled circle). (*B*) Group average of six rats injected with 20 mg/kg intravenous propofol, same time phases as (*A*). Error bars are 1 SEM. Asterisks indicate a significant difference ($P < 0.05$, *post hoc* test after two-factor analysis of variance) in P2/P1 values between baseline and moderate anesthesia.

spike, P1, was significantly suppressed during the time the EEG showed a flat or burst-suppression pattern (*i.e.*, < 4 min after 20 mg/kg intravenous propofol). P1 amplitude was significantly reduced to 61 ± 7% of the baseline amplitude (mean ± SEM; n = 6) during deep anesthesia. By the time the EEG regained its amplitude during the moderate anesthesia phase, there was no significant change of P1 from the baseline values. P1 amplitude was not consistently decreased within 4 min after a 10-mg/kg intravenous injection, but a detailed correlation with the EEG was not performed. Injection of the control vehicle also caused no significant change in P1.

The following results were obtained during the moderate and light anesthesia phase. Because the stimuli used to evoke the field potentials introduced considerable artifacts to the EEG record, EEG was only sampled periodically when evoked potentials were not recorded.

The amplitude of the second population spike (P2) evoked by a stimulus of moderate intensity at IPI < 30 ms showed the most robust change with anesthesia. Either a 10- or a 20-mg/kg intravenous dose of propofol significantly reduced P2 immediately (fig. 3). In some experiments, P2 at IPI < 30 ms was completely abolished during deep or moderate anesthesia (figs. 2–4) and remained suppressed during light anesthesia (6 min after 10 mg/kg intravenous propofol in fig. 3A and 17 min after 20 mg/kg intravenous propofol in fig. 3B). Thus, the P2/P1 ratio at IPI < 30 ms was suppressed during the whole duration of anesthesia (fig. 4A). P2/P1 values for all IPIs and time phases for rats given 10 mg/kg intravenous propofol were subjected to a randomized block ANOVA, which yielded a significant main effect for time phase ($F_{3,18} = 12.97$; $P < 0.0001$) and IPI ($F_{7,24} = 44.32$; $P < 0.0001$). The interaction between time phase and IPI was also significant ($F_{21,136} = 5.27$; $P < 0.0001$). Tukey protected *t* test revealed significant differences ($P < 0.01$) at 20 and 30 ms IPI for all paired comparisons between baseline, moderate anesthesia, and light anesthesia phases (fig. 2A). Similar statistical results were found for the P2/P1 values after 20 mg/kg intravenous propofol. Significant effects were found for the time phase ($F_{3,15} = 4.08$; $P = 0.027$), IPI ($F_{6,30} = 10.86$; $P < 0.0001$), and the interaction between time phase and IPI ($F_{18,90} = 5.88$; $P < 0.0001$). Tukey protected *t* test revealed a significant difference ($P < 0.01$) for all paired comparisons between baseline and moderate anesthesia for 20-, 30-, 50-, and 200- ms IPIs (fig. 2B).

Paired-pulse facilitation (PPF) was observed at 200-ms IPI after 20 mg/kg intravenous propofol (fig. 2B). Figure 4B shows the development of the paired-pulse facilitation during moderate anesthesia and the large variation of the P2/P1 ratio. A plot of P2 *versus* P1, using all the single sweep responses in one representative rat, is shown in figure 5. P2 was significantly larger during moderate (or light) anesthesia than baseline (or recov-

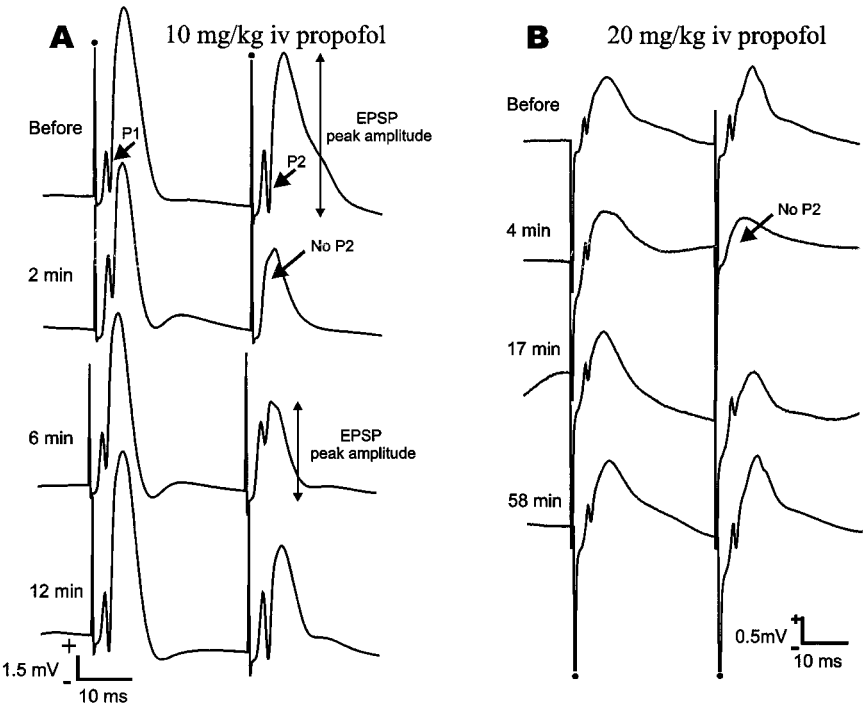

Fig. 3. The second-pulse population spike, P2, was suppressed during anesthesia induced by propofol. Perforant-path average evoked potentials (n = 8 sweeps) in the dentate gyrus were recorded in two representative rats before and after (*A*) 10 mg/kg and (*B*) 20 mg/kg intravenous propofol. The four traces for each injection are during immobility before injection, moderate anesthesia (2 min for *A*, 4 min for *B*), light anesthesia (6 min for *A*, 17 min for *B*), and recovery (12 min for *A*, 58 min for *B*). Note the complete suppression of P2 (No P2) immediately after propofol and smaller suppression of P2 during light anesthesia. The peak of the second-pulse evoked EPSP was also decreased during propofol-induced anesthesia. Shock artifacts indicated by dots.

ery) for an intermediate range of P1 (1.3–1.8 mV in fig. 5; $P < 0.05$, Kruskal-Wallis one-way ANOVA). Extensive single sweep data of P2 *versus* P1 at 200-ms IPI were available in a total of five rats given 20 mg/kg intravenous propofol. Four of the five rats yielded the same result that propofol suppressed P2 at a fixed range of P1; there was no significant change in the P2 *versus* P1 relation in the remaining rat.

For rats in the 10-mg/kg dose group given the control vehicle (n = 5), a randomized block ANOVA yielded only a significant effect of IPI ($F_{7,28} = 11.51$; $P < 0.0001$) but no significant effect of time phase ($F_{2,8} = 2.06$; $P = 0.19$) or time phase and IPI interaction ($F_{14,56} = 1.87$; $P = 0.49$). Control injections in animals in the 20-mg/kg intravenous propofol group (n = 5) yielded the same result. ANOVA revealed a significant effect for IPI ($F_{7,28} = 2.87$; $P = 0.22$) but not for time phase ($F_{2,8} = 1$; $P = 0.41$) or interaction ($F_{14,56} = 1.39$; $P = 0.19$).

Effect of Propofol or Its Vehicle on Population Excitatory Postsynaptic Potentials in the Dentate Gyrus

To study the effect of propofol on the population EPSP without contamination by the population spike, near-threshold stimulus intensity (< 60 μA) was used (which evoked no population spike). A 20-mg/kg intravenous propofol dose was administered in five experiments on four rats. No significant effect of propofol was found on the EPSP amplitude (either increasing slope or peak) during any phase of anesthesia, nor was there a detectable change in EPSP threshold after propofol.

The EPSP slope (E1) evoked by above-threshold stimulus intensities, before the onset of the population spike, also showed no significant change after either dose of intravenous propofol (10 or 20 mg/kg; data not shown). Concomitant with the increase in paired-pulse inhibition of the population spikes, a slight increase of the paired-pulse inhibition of the perforant-path EPSPs was also observed after propofol at IPIs < 30 ms. In some rats in which the EPSP in the DG appeared distinct (*e.g.*, fig. 3), the second-pulse population EPSP clearly decreased its peak amplitude and duration after propofol. However, across the group of rats given 10 or 20 mg/kg intravenous propofol, the EPSP slope ratio (E2/E1) at different IPIs and stimulus intensities showed no significant change after propofol. Vehicle injection also had no effect on EPSP slope or E2/E1 ratio.

Effect of Propofol on the Trisynaptic CA1 Response

In six rats, the shallow electrode was located in the distal dendrites of CA1 and a long-latency (10–15 ms onset) negative wave in CA1, which was mainly attributed to trisynaptic excitation, was found after MPP stimulation.[18] Paired-pulse depression of the CA1 trisynaptic

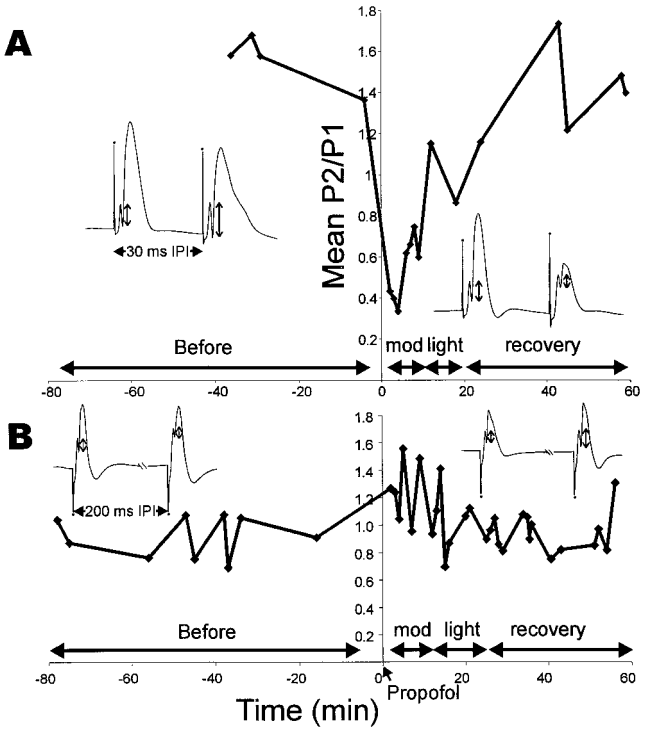

Fig. 4. Time course of action of propofol on paired-pulse population spike ratio (P2/P1) at (*A*) 30-ms interpulse interval (IPI) or (*B*) 200-ms IPI. Propofol was given at time zero. (*A*) P2/P1 at 30-ms IPI before and after 10 mg/kg intravenous propofol. Note depression of P2/P1 during the moderate (mod) and light anesthesia. (*B*) P2/P1 at 200-ms IPI before and after 20 mg/kg intravenous propofol. Note the large variability but general enhancement in P2/P1 ratio during moderate anesthesia after propofol. All measures were derived from average evoked potentials of four to eight sweeps. Responses with population spikes P1 and P2 (arrows) are shown in the inset (*left*, before propofol; *right*, after propofol).

wave at < 50-ms IPI was increased during propofol-induced anesthesia, when the same was observed for the dentate population spike. At an IPI of 150–200 ms, both the mean and the variance of the PPF of the CA1 trisynaptic wave appeared to be increased after 20 mg/kg intravenous propofol.

Discussion

Effects of Propofol on Behavior and Electroencephalogram

Induction of anesthesia was rapid and consistent among rats. In general, the recovery was also without incident. No seizure activity was observed in the EEG of rats given propofol.

The time course of behavioral anesthesia in this study is consistent with previous studies. The rat's plasma concentration of propofol would be expected to peak at approximately 30 s after a bolus injection of 10 mg/kg intravenous propofol.[28] This would coincide with the time of maximal EEG burst suppression observed in our study and in another study that administered an 8-mg/kg intravenous bolus dose of propofol.[24] The recovery from EEG burst suppression[24] and the expected decline in plasma propofol[28] roughly corresponded with the recovery of the tail pinch response and spontaneous movement in our study.

Inhibitory Action of Propofol

Propofol had a profound and robust effect in increasing paired-pulse inhibition of the MPP-evoked population spikes (*i.e.*, decreasing P2/P1 ratio at < 30-ms IPI). The amplitude of a single population spike was not significantly affected by propofol except during the deep phase of anesthesia. Population EPSP was not affected significantly by propofol at any phase of anesthesia. However, paired-pulse inhibition was greatly increased immediately after an intravenous injection of 10–20 mg/kg propofol.

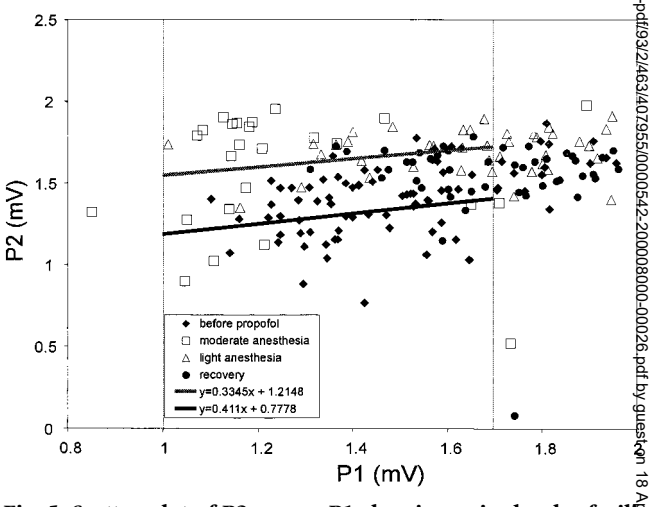

Fig. 5. Scatter plot of P2 *versus* P1 showing paired-pulse facilitation at 200-ms IPI before and after 20 mg/kg intravenous propofol. Each P2 and P1 pair was derived from a single sweep after paired-pulses of 150 μA delivered to the medial perforant path of one rat. The four different conditions are baseline before propofol (filled diamonds), moderate anesthesia (unfilled squares), light anesthesia (unfilled triangles), and recovery (filled circle). Note that for the range of P1 within the dotted lines, excluding the largest and smallest P1, the P2 amplitudes during moderate anesthesia are generally larger than those before propofol or after recovery from propofol. Lines of best fit are shown for before propofol (black line) and moderate anesthesia (gray line) for the fixed range of P1. Each line accounts for > 50% of the variance and the lines are shown for illustrative purpose only.

The increase in paired-pulse inhibition was large during deep and moderate anesthesia, but it persisted to a smaller degree during light anesthesia (fig. 4A). It may be inferred that paired-pulse inhibition is not a correlate of the lack of pain reflex or of deep anesthesia alone. Rather, it may be an indication of the depression of the hippocampal cortex (DG in particular) after the administration of propofol. There is no *a priori* reason why hippocampal synaptic depression should be causally related to a nociceptive reflex. However, hippocampal depression may interfere with memory or other hippocampus-dependent functions. The increase in paired-pulse inhibition persisted longer in rats given 20 mg/kg compared with those given 10 mg/kg intravenous propofol. During the moderate anesthesia phase, the 20-mg/kg intravenous dose of propofol also increased paired-pulse inhibition at 50-ms IPI, in addition to the increase of paired-pulse inhibition at 10–30-ms IPI observed after 10 mg/kg intravenous propofol.

The paired-pulse inhibition effect, or P2/P1 ratio, was somewhat dependent on stimulus intensity. At very low stimulus intensity, the second-pulse population spike (P2) may be observed without the first-pulse population spike (P1). During these conditions, no significant change in P2 was observed after propofol (P2/P1 ratio was indeterminate since P1 = 0). The effect of propofol on the P2/P1 ratio was most robust at a moderate stimulus intensity and < 50-ms IPI. During the latter conditions, propofol had a graded effect on the P2/P1 ratio, which became very small 1–2 min after injection and then slowly regained its baseline amplitude as the rat recovered. At supramaximal stimulus intensities, the P2 and P2/P1 ratio became zero immediately after propofol and remained zero until shortly before the rat recovered.

The increase in paired-pulse inhibition at < 50-ms IPI is consistent with the enhancement of inhibition after propofol. Inhibitory postsynpatic potentials (IPSPs) in hippocampal neurons, including dentate granule cells, lasted < 100 ms with moderate stimulus intensity. Somatic IPSPs are effective in suppressing spike firing, and dendritic IPSPs may shunt the excitatory currents, resulting in a decrease in amplitude of the EPSP. The IPSPs of < 100 ms duration were abolished by $GABA_A$ receptor antagonist bicuculline and enhanced by barbiturates and propofol.[5–8,13,29,30] Paired-pulse inhibition of population spikes in the DG *in vivo* was suppressed by $GABA_A$ receptor antagonists and enhanced by drugs that enhanced $GABA_A$ receptor currents, such as diazepam, barbiturates, and GABA uptake blockers.[31–33]

This study confirms previous studies reporting that propofol enhanced paired-pulse inhibition in CA1 of the hippocampal slice *in vitro*[10,12] and in DG of the urethane-anesthetized rat.[13] Hasan and Woolley[15] also found that paired-pulse inhibition of MPP-evoked dentate population spikes was increased by a dose of intraperitoneal propofol. Our study differs from that of Hasan and Woolley[15] in the use of intravenous propofol, which is the normal route administered to patients. In contrast to Hasan and Woolley, who used IPIs of 20 and 32 ms, our study evaluated the range of IPI from 10 to 500 ms. The paired-pulse responses of Hasan and Woolley[15] also appeared to be rather unstable, as shown by the large variability of their baseline recordings and their recovery responses 24 h after administration of propofol (fig. 7 of Hasan and Woolley[15]). This may be attributed to their high repetition rate (i.e., once every 2 s) since other hippocampal responses are labile at 0.5 Hz stimulation rate. For example, stimulation of the lateral olfactory tract at rates of 0.5/s to 2/s "produced gradually augmenting responses" in the hippocampus,[34] and monosynaptic inhibitory postsynaptic responses *in vitro* showed paired-pulse inhibition at 2-s IPI.[35] For MPP stimulations, paired-pulse inhibition of the population spikes was still found at an IPI of 2 s,[32] suggesting that a repetition interval of 2 s is too short.

Many general anesthetics have been found to increase $GABA_A$ transmission[35] and suppress spiking.[36] Our results suggest that propofol increased evoked $GABA_A$ transmission for all phases of anesthesia. However, the first-pulse population spike in the DG (evoked by MPP stimulation) was only suppressed during deep anesthesia. Although the first-pulse population spike was not significantly altered during moderate and light anesthesia, a second pulse at 20–50 ms after the first pulse evoked a smaller population spike than baseline. Thus, spike output after paired (and repetitive) inputs was suppressed after propofol.

Excitatory Action of Propofol

Propofol increased PPF of the MPP-evoked population spikes at an IPI of 200 ms. This effect was significant after 20 mg/kg intravenous propofol (fig. 2). Variability of the PPF of population spikes was fairly large (fig. 4B), but statistical analysis of single sweeps in individual rats also confirmed this result (fig. 5). Single-sweep analysis showed that in addition the PPF increase was not dependent on changes in the first-pulse response (P1). In contrast, Hirota *et al.*[37] found that intravenous anesthetics, including propofol, decreased PPF of the population spikes in CA1 *in vitro*, whereas volatile anesthetics in-

creased PPF. The difference between *in vivo* and *in vitro* preparations may explain the different results.

The mechanism of PPF of the population spike is not fully understood. It appears that PPF of the population spike at 100–200 ms is a separate mechanism independent of the $GABA_A$ receptor–mediated paired-pulse inhibition at < 50-ms IPI, because PPF persisted after blockade of $GABA_A$ receptor–mediated inhibition and paired-pulse inhibition at < 50 ms.[29] The MPP-evoked EPSPs did not facilitate with paired pulses.[18] This suggests that PPF of population spikes is not caused by enhanced excitation. It is possible that inhibition at the time of the second-pulse may be reduced. The first stimulus pulse may release GABA that acts on presynaptic $GABA_B$ receptors on GABAergic terminals, thus suppressing the (second-pulse) evoked IPSP[33] and enhancing spiking.[38] Suppression of inhibition facilitates the opening of *N*-methyl-D-aspartate receptor–mediated channels.[13]

An increase in PPF at 200-ms IPI may be caused by a depolarizing GABA-mediated response after propofol. An enhancement of $GABA_A$ receptor function by propofol may increase the participation of HCO_3^- conductance in the GABA-mediated response and shift the IPSP to a more depolarized level.[39] Propofol has been shown to enhance the depolarizing GABA-mediated postsynaptic response in hippocampal CA1 neurons *in vitro*.[40] Alternatively, enhanced GABA function may change the Cl^- gradient and induce depolarizing IPSPs at the dendrites.[39,41] There is increasing evidence that the hippocampus may drive motor activity through a part of the basal ganglia, the nucleus accumbens.[42,43] Hippocampal stimulation evoked dopamine release in the nucleus accumbens, which mediates locomotor activity.[42] Propofol may enhance the hippocampus–accumbens output, which may contribute to abnormal movements and postures. In addition, the hippocampal θ rhythm[44,45] showed power at 5 Hz (or a period of 200 ms) at a low (sedative) dose of propofol (not shown). As a rat recovers from anesthesia, the θ rhythm may further facilitate hippocampal activity at 5 Hz.

Compared with the paired-pulse depression observed at 20–50-ms IPIs, the increase in PPF of population spikes at 200-ms IPIs demonstrates that propofol can produce excitatory effects on synaptic transmission in the hippocampus. Whether such a mechanism could operate in relation to propofol-induced abnormal movements[1] is speculative. However, the hippocampus is known to influence behavior, including motor activity, *via* the hippocampal–accumbens circuit.[42,43]

The authors thank Bixia Shen, technician, Department of Physiology, University of Western Ontario, London, Ontario, Canada, for technical assistance and Lisa Tichenoff, technician, Department of Anatomy and Cell Biology, University of Western Ontario, for demonstration of the cannulation of the femoral vein.

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