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Propofol exhibits antiepileptic activity in hippocampal pyramidal neurons

Sameer A. Otoom[a,*], Zuheir Hasan[b]

[a]Department of Pharmacology, Jordan University of Science and Technology, School of Medicine, Irbid 22110, Jordan
[b]Department of Physiology, Jordan University of Science and Technology, School of Medicine, Irbid 22110, Jordan

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Abstract

Propofol was reported to exhibit an antiepileptic activity. This study was performed to investigate the effect of propofol on evoked and spontaneous seizure-like activity induced by the convulsant veratridine. Studies were performed on rat brain slices using conventional electrophysiological intracellular techniques. The alteration of sodium channel function by veratridine (0.3 μM) induced an evoked and spontaneous seizure-like activity in the hippocampal CA1 pyramidal neurons. Therapeutic concentrations of propofol (10 μM) were ineffective in inhibiting veratridine-induced seizure-like activity. However, higher concentrations (50–100 μM, $n=6$) inhibited both evoked and spontaneous bursting, induced by veratridine. The inhibitory effect of propofol (100 μM) was associated with membrane hyperpolarization [after veratridine, -66 ± 0.71 mV (mean ± S.E.M.), and after propofol, -77 ± 2.15 mV] and with an increase in input resistance [after veratridine (37.8 ± 1.2 MΩ) and after propofol (43 ± 1.3 MΩ)]. The drug also produced an increase in current threshold. Results from this study are valuable in solving critical questions regarding the antiepileptic activity of propofol and strengthen the validity of the veratridine model in testing for potential antiepileptic drugs.

Keywords: Propofol; Veratridine; Spontaneous and evoked bursting; Hippocampus; Brain slice; CA1

1. Introduction

There are several factors that increase the need for new models of epilepsy. Some of these factors include the inability of the presently available antiepileptic drugs to make all epileptic patients seizure-free and the possible future development of tolerance to these drugs (Swinyard and Woodhead, 1982). Thus, the need for developing new models of epilepsy continues to be essential. Previously, we have shown that the plant alkaloid veratridine enhances depolarizing inward rectification, which leads to the generation of seizure-like activity in hippocampal CA1 pyramidal neurons (Tian et al., 1995). We also provided evidence to support the notion that veratridine can be a model of epilepsy (Otoom et al., 1998). A small concentration of veratridine (0.3 μM) was effective in converting a single intracellularly evoked action potential to bursting discharge in the CA1 neurons of the hippocampus. The veratridine model could also generate spontaneous epileptiform activity after prolonged superfusion (Otoom et al., 1998). Based on this, the veratridine model is a unique model because a small concentration is needed to induce seizure-like activity. Moreover, the mechanism of seizure induction by veratridine at 0.3 μM is well studied and is due to the alteration of sodium channel function, which represents an important factor in the pathogenesis of epilepsy (Mousnier and Couraud, 1993; Otoom et al., 1998).

Propofol (2,6-diisopropylphenol) is now widely used for the induction and maintenance of anesthesia (Sebel and Lowdon, 1989). This drug was also used as a sedative agent during regional anesthesia or intensive care. Moreover, this drug may be used in the treatment of status epilepticus (Albertson et al., 1996). Reports indicate that this drug also exhibits an effect on seizure activity (e.g., Lowson et al., 1990). However, its effect on seizure activity is unclear, although some studies showed an antiepileptic activity for the drug (e.g., Lowson et al., 1990). Paradoxically, reports from clinical studies showed that propofol can also induce a proepileptic activity under certain conditions (Hodkinson et al., 1987; Borgeat, 1997). It was reported that propofol decreased the threshold of pentylenetetrazol below control (Lowson et al., 1991) and produced an overshoot in limbic-

* Corresponding author. Fax: +962-2-7247538.
E-mail address: sato@just.edu.jo (S.A. Otoom).

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evoked potentials in the behaving rat (Hasan and Woolley, 1999) during the early recovery of the drug. However, another study failed to demonstrate this effect (Hasan, 1997). The mechanism of the action of this drug is not yet known. Albertson et al. (1996) reviewed the mechanism of the action of propofol and found that the drug produced an effect on sodium, calcium, chloride, potassium and nicotinic cation channels. Moreover, the drug was found to increase the intensity and duration of GABA-mediated inhibition in rat hippocampal slices (Albertson et al., 1996).

This study is designed to characterize the effect of propofol on a model of epilepsy, known as the veratridine model. Different concentrations of propofol will be tested on the evoked and spontaneous bursting induced by veratridine.

2. Materials and methods

Male Sprague–Dawley rats (150–300 g) were utilized in these experiments. The animal was quickly decapitated, and the skull bone was removed with a ronjour. The brain was gently removed with a stainless steel spatula and placed on a blotting paper in a petri dish filled with cold (0 °C) oxygenated (95% O_2, 5% CO_2), artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM) NaCl, 127; $CaCl_2$, 2.5; KCl, 4.7; $MgCl_2$, 1.2; $NaHCO_3$, 22; NaH_2PO_4, 1.2; and Glucose, 11.0. The two hemispheres were separated by dividing the brain midsagittally, and a block of tissue containing the hippocampus was prepared. The block was fixed on a chunk of a vibroslice using cyanoacrylate glue. Transverse slices (approximately 500 μm thick) were cut using a vibroslice (Campden Instruments). From each hemisphere, 4–5 slices were transferred to a beaker of ACSF solution continuously bubbled with gas (95% O_2, 5% CO_2) and were kept at room temperature to recover for at least 4 h before recording. After the recovery period, a single slice was transferred to a recording chamber, where it was placed between two nylon nets and was superfused with oxygenated ACSF. The temperature of the ACSF in the recording bath was maintained at 32 ± 1 °C using a temperature-controlled water circulator (Lauda C3, model T-1). Intracellular recordings were obtained from the CA1 pyramidal neurons of the stratum pyramidal by using glass microelectrodes (1.0 mm, Kwik-fil, WPI) filled with 4 M potassium acetate (80–120 MΩ). A bipolar stimulating electrode was placed in the Schaffer collateral layer for synaptic stimulation. Electrical signals were amplified by an amplifier (Axoclamp-2A, Axon Instrument) and stored on videotapes (PCM Data Recorder, A. R. Veter, Model 200) for later analysis. Hard copies of the data were obtained using a laser jet printer (LaserJet III Printer, Hewlett Packard) from a digital oscilloscope (LeCroy, 9310).

Input resistance was determined by passing a hyperpolarizing current (0.2 nA, 200-ms duration) through the stimulating electrode and then measuring the potential changes across the membrane. The membrane input resistance was calculated using Ohm's law.

The experimental protocol and the procedures used are in compliance with the declaration of the National Institute of Health Guide for care and use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.1. Drugs and chemicals

Veratridine was obtained from Sigma and was dissolved in 0.1 mM HCl. Propofol was obtained from Research Biochemical International (RBI). Stock solutions of propofol (100 mM) were prepared in dimethyl sulfoxide (DMSO) and kept at 4 °C until use. The maximal concentration of DMSO used was less than 0.3% and was determined during control experiments to have no significant effect on veratridine bursting. Aliquots of the stock solutions were added to the standard ACSF to make the final concentrations given in the experiments.

2.2. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical analysis was performed using paired *t* test. A *P* value of $<.05$ was considered statistically significant.

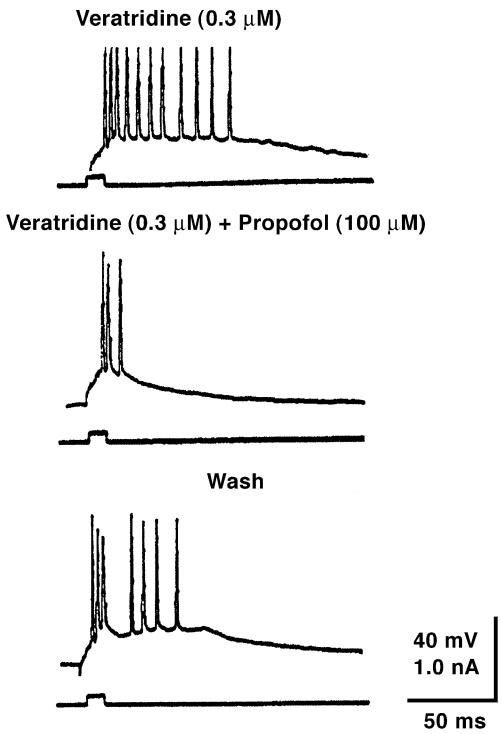

Fig. 1. The inhibition of stimulus-evoked veratridine bursting by propofol. The digitized tracings were recording from a single neuron in the CA1 area of the hippocampus (RMP* −65 mV). Treating the neuron with 0.3 μM veratridine for 25–30 min induced epileptiform discharge (Trace 1). The evoked bursting was inhibited by propofol (100 μM, $n=6$; Trace 2) in 35 min and reversed after the wash-out of the drug (Trace 3). The spikes were truncated by the chart recorder. * RMP: resting membrane potential.

3. Results

These experiments demonstrate that while therapeutic concentrations of propofol (10 μM) was ineffective in suppressing bursting induced by the veratridine model, higher concentrations (50–100 μM) produced inhibitory effects of the seizure-like activity induced by this model. Electrophysiological traces and figures represent data using 100 μM. Single-action potential was evoked by brief intracellular current pulses (20 ms, 0.2–0.5 nA). The superfusion of the brain slice with 0.3 μM veratridine for 25–30 min induced seizure-like activity. The application of propofol (50–100 μM, $n=6$) for 35–40 min inhibited the stimulus-evoked seizure-like activity of veratridine (Fig. 1). The propofol inhibition was reversed after washing with veratridine–ACSF. The inhibitory effect of the drug (100 μM) was associated with membrane hyperpolarization [after veratridine, -66 ± 0.71 mV (mean ± S.E.M.), and after propofol, -77 ± 2.15 mV; paired t test, $P<.05$; Fig. 2, Panel A] and an increase in input resistance [after veratridine (37.8 ± 1.2 MΩ) and after propofol (43 ± 1.3 MΩ); paired t test, $P<.05$; Fig. 2, Panel B). This concentration of propofol also increased the firing threshold of the bursting (Fig. 3).

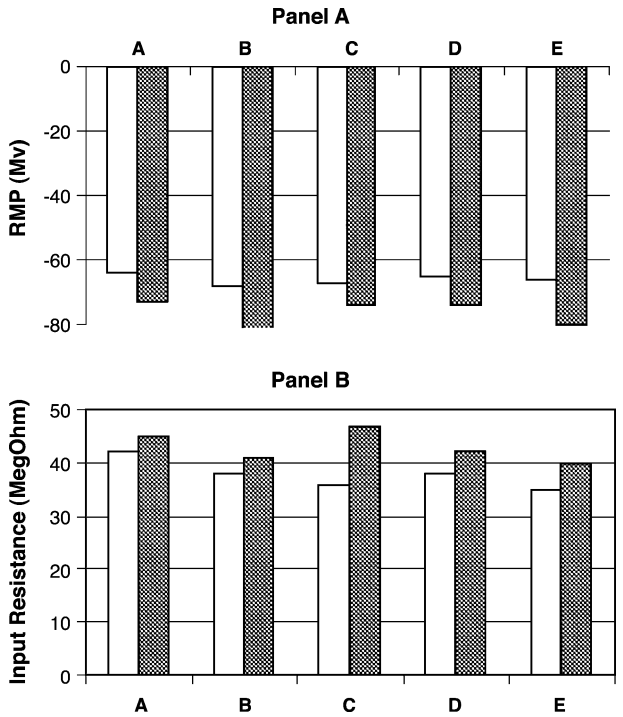

Fig. 2. Effect of propofol on passive neuronal parameters during the inhibitory effect of the drug. A, B, C, D and E are data from five different neurons. The neurons were treated with 0.3 μM veratridine to induce seizure-like activity. RMP (Panel A) and input resistance (Panel B) were measured after the addition of veratridine for 25–30 min (open bars) and after the addition of propofol for 35 min (closed bars). In all the experiments, propofol was associated with membrane hyperpolarization and with an increase in input resistance (paired t test, $P<.05$).

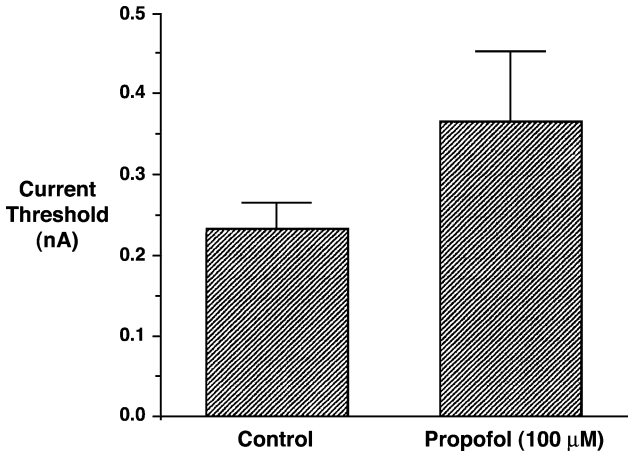

Fig. 3. Effect of propofol on the membrane parameters. The drug (100 μM) increased the intracellular current threshold (paired t test, $P<.05$). Bars represent the means of six neurons. Vertical lines are S.E.M.

The effect of propofol on spontaneous seizure-like activity was also studied. We tested three concentrations of veratridine: 0.1, 0.3 and 1 μM. While the experiments performed using 0.1 μM require long period (>1 h) to induce bursting, all the experiments performed using 1 μM veratridine produced spontaneous bursting within 1 h of superfusion. However, it was noticed that the cell, when using this concentration, became electrically unstable. Based on this, we decided to use 0.3 μM concentration of veratridine to induce spontaneous bursting in hippocampal slices. This concentration of veratridine induced spontaneous bursting within 45–50 min of superfusion. Propofol at 100 μM and after 35 min of superfusion completely blocked spontaneous bursting produced by veratridine (Fig. 4).

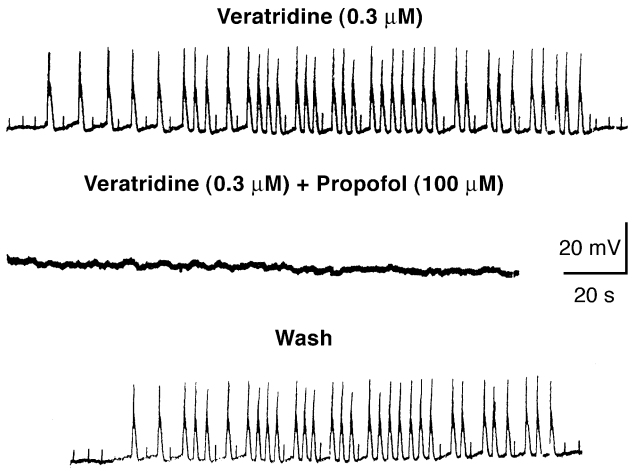

Fig. 4. The inhibition of veratridine-induced spontaneous bursting activity by propofol. After treatment with 0.3 μM veratridine, the neuron exhibited rhythmic spontaneous bursting activity (top trace), which was completely blocked by propofol (100 μM, middle trace) and reversed after wash-out with veratridine–ACSF (lower trace).

4. Discussion

Our study indicates that propofol concentrations (up to 100 μM) inhibit both evoked and spontaneous seizure-like activity induced by veratridine in the hippocampal pyramidal neurons. The inhibitory effect of the drug was associated with hyperpolarization and an increase in input resistance and current threshold.

The role of sodium channels in the pathogenesis of epilepsy was well studied (Mousnier and Couraud, 1993). In the hyperexcitable membrane, blocking sodium channels stabilize the membrane. Thus, drugs such as phenytoin and carbamazepine, which decrease repetitive firing by the inactivation of sodium channels, can be used in the treatment of epilepsy (Macdonald and Kelly, 1994). This suggests a possible involvement of sodium channels in the genesis of abnormal activities in epilepsy. In fact, a number of drugs and toxins, such as veratridine, batrachotoxin and grayanotoxin, which are known to produce persistent activation or opening of sodium channels (Catterall, 1980; Alkadhi and Tian, 1996), can produce convulsions similar with those of epileptic seizures. Furthermore, we have shown that endogenous seizure-like activity can be produced in the hippocampal CA1 pyramidal neurons when certain sodium channels are altered by veratridine at the concentration used in this study. It is shown that veratridine augments slow subthreshold sodium current $I_{Na(s)}$, which leads to the generation of a negative slope resistance observed in bursting neurons (Alkadhi and Tian, 1996). Veratridine bursting was sensitive to the action of propofol by showing inhibition at wide range of concentrations of this drug. The antiepileptic effect of propofol may be related to interference with sodium channels. However, further studies using a patch-clamp technique are required to investigate this prediction.

Our results are in agreement with previous studies that demonstrate the effect of propofol on sodium channels. In rat cerebrocortical synaptosomes and in using ion-specific spectrofluorimetry, propofol inhibited veratridine-induced sodium influx (Saint and Tang, 1998). In the human brain cortex tissue, propofol showed a voltage-independent decrease in the fractional channel open-time and an interaction with the steady-state activation of sodium channels at concentrations equivalent to the serum level of propofol when used in the induction of general anesthesia (Frenkel and Urban, 1991). It was suggested that propofol may interact with hydrophobic domains in the alpha helices of the sodium channel protein. In frog myelinated axons and in using a voltage-clamp technique, propofol showed a negative shift of the steady-state activation and inactivation of sodium and potassium current. Additionally, the drug blocked the sodium channel in a voltage-independent way (Veintemilla et al., 1992). The drug was also found to block sodium current in rat isolated myocardial cells (Saint and Tang, 1998).

Our data can also be explained by the action of propofol on potentiating the action of GABA. This potentiation will result in the GABA opening of chloride channels and hyperpolarizing the membrane (Fig. 2, Panel A), increasing the input resistance (Fig. 2, Panel B) and the firing threshold (Fig. 3). Different studies showed that the anesthetic, anticonvulsant and neuroprotective effects of propofol are related to the potentiation of GABA or the inhibition of glutamate. Propofol showed an increase in the intensity and duration of GABA-mediated inhibition in rat hippocampal slices. This effect was reversed by the $GABA_A$ antagonist, such as picrotoxin, and by the chloride channel antagonist DIDS, but not by the benzodiazepine antagonist flumazenil (Albertson et al., 1996). Investigations also identified a binding site for [3H]propofol in rat brain, which differs from either the steroid or barbiturate binding sites (Concas et al., 1991, 1992). Moreover, the drug was shown to enhance GABA-induced inhibition in bovine chromaffin cells, cell cultures of spinal neurons and in oocytes that express $GABA_A$ receptors (Albertson et al., 1996). The effect of propofol on glutamate release is not clear, although propofol was shown to inhibit glutamate release in synaptosomes (Ratnakumari and Hemmings, 1997). Another study showed that this drug enhanced NMDA-mediated glutamate excitotoxicity in rat hippocampal slices (Zhu et al., 1997).

In conclusion, seizure-like activity induced by veratridine in the hippocampal CA1 pyramidal neurons was sensitive to inhibition by propofol. The inhibitory effect of propofol was associated with hyperpolarization and with an increase in input resistance and current threshold. The results of this study indicate that propofol exhibits antiepileptic activity and point to the importance of the veratridine model in screening for potential antiepileptic drugs.

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