Propofol Hemisuccinate Suppresses Cortical Spreading Depression

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Abstract
Propofol is a rapidly acting water-insoluble non-barbiturate anesthetic agent that is widely used as an intravenous sedative–hypnotic agent. Anecdotal evidence indicates that propofol may be effective at terminating intractable migraine headache. Cortical spreading depression (CSD) is believed to be the neural correlate of migraine aura and may be a trigger for migraine pain. Agents that block the induction or slow the spread of CSD may be of utility in treating migraine. Here we examined the ability of propofol hemisuccinate (PHS), a water-soluble prodrug of propofol, to affect CSD in mice. For comparison, we examined dizocilpine, an NMDA receptor antagonist, that is well recognized to inhibit CSD. When administered 15 min prior to activation of CSD by KCl application to the cortex, intraperitoneal PHS at doses of 120 and 200 mg/kg decreased the number of CSD deflections without an effect on CSD amplitude, and at 200 mg/kg caused a 77% reduction in CSD velocity. The minimally-effective dose of PHS (120 mg/kg) did not cause sedation or motor impairment and while some animals receiving 200 mg/kg did demonstrate motor impairment none exhibited loss-of-righting reflex (anesthesia). Dizocilpine produced comparable inhibition of CSD at doses of 0.5 and 2.5 mg/kg. We conclude that acute PHS treatment inhibits CSD. Our results indicate that propofol, or its prodrug PHS, are worthy of further investigation as a treatment for migraine.

1. Introduction
Several small open label trials and case reports indicate that acute administration of propofol at subanesthetic doses can rapidly terminate treatment refractory migraine [7,13,15,20,24,28]. These reports raise the possibility that propofol could have activity as a migraine abortive agent. However, propofol must be administered intravenously and therefore is of limited utility in the routine clinical treatment of migraine. Propofol hemisuccinate (PHS), a water soluble prodrug of propofol that can be administered by other routes including by inhalation, may be more broadly applicable [12]. In order to provide support for propofol-related agents in the acute treatment of migraine, we sought to determine if PHS can influence cortical spreading depression (CSD) in mice. CSD, a self-propagating wave of neuronal depolarization, is considered relevant to the neural mechanisms in migraine. Moreover, activity in inhibiting CSD may signify the potential of an investigational agent in migraine therapy [3,16].

Propofol itself is short acting and its central nervous system effects wane within approximately 20 min after intraperitoneal bolus administration [12]. This pharmacokinetic behavior is not ideal for studies of CSD where the frequency of repetitive CSD waves is assessed in recordings over a more prolonged period. A single intraperitoneal dose of PHS is longer acting because of the requirement for conversion into the active form. The duration of action (approximately 1 h) matches the typical CSD recording period.

In the present study, we sought to determine if PHS can affect CSD elicited in vivo in the mouse by cortical application of KCl. As an active comparator, we used the potent uncompetitive NMDA receptor antagonist dizocilpine (MK–801), which is well recognized to suppress CSD [18,21,23,30]. Our results demonstrate that a single acute dose of PHS is effective in suppressing CSD.

2. Materials and methods
2.1. Animals
Male NIH Swiss mice (22–30 g) were kept in a vivarium under controlled environmental conditions (temperature, 22–26 °C; humidity, 40–50%) with an artificial 12-h light/dark cycle. Wood chips were used in all cages. Experiments were performed during the light phase of the light/dark cycle after a minimum 30-min period of acclimation to the experimental room. The animal facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All studies were

Abbreviations: CSD, cortical spreading depression; PHS, propofol hemisuccinate.
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performed under protocols approved by the Animal Care and Use Committee of the University of California, Davis in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council [National Academy Press, Washington, DC; http://www.nap.edu/readingroom/books/labrats/].

2.2. Test substances

Propofol hemisuccinate ([PHS; 4-(2,6-diisopropylphenoxy)-4-oxobutanoic acid]) was synthesized as described in our earlier publication [12]. PHS was dissolved in 0.1 M sodium bicarbonate solution in double distilled water. The final pH of the solution was 7.5. Dizocilpine ([+]-MK-801 hydrogen maleate; Sigma–Aldrich, St. Louis, MO) was dissolved in double distilled water. The drug solutions were administered intraperitoneally 15 min before cortical application of KCl at doses of 0.1, 0.5 and 2.5 mg/kg. Inhibitory effects of dizocilpine on CSD in rats in vivo have been obtained at doses of 1–10 mg/kg [21,30]. The drugs were administered in a volume of 10 ml/kg. In an earlier study, we found that the peak anticonvulsant effect of intraarterial PHS in mice occurs at 15 min [12]. Therefore, the interval between drug administration and cortical CSD application was 15 min.

2.3. CSD recording

Mice were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Body temperature was maintained throughout the study at 37.0 ± 0.1°C using a circulating water warming pad (Gaymar Industries, Orchard Park, NY). The level of anesthesia was maintained by administering additional doses of pentobarbital sodium. Pentobarbital was chosen as an anesthetic because of its limited effect on CSD [19]. After exposing the skull, two Burr holes were made over the left hemisphere leaving the dura intact for KCl application (mm from Bregma: posterior 5 mm, lateral 1.5 mm) and recording electrode (posterior 1 mm, lateral 1.5 mm) [5]. Borosilicate glass recording electrode blanks (Kimble Chase, Vineland, NJ; OD 1.7 mm, ID 1.2 mm) were pulled to a resistance of 1–1.5 MΩ with a vertical electrode puller (Narishige Japan Model PP-830) and filled with artificial cerebrospinal fluid (aCSF). The electrode was fixed to a 1/100 CV-3 headstage (Axon Instruments, Union City, CA) with an E-series electrode holder (Warner Instruments, Hamden, CT) containing a Ag/AgCl wire. The headstage signal was fed to an amplifier (Axopatch 1C) controlled through a Digidata 1320A interface and operated in current clamp mode. The recording electrode was positioned 300 μm below the dural surface. A Ag/AgCl reference electrode was placed subcutaneously in the neck region. The cortex was allowed to recover for at least 30 min under saline irrigation. The dura in the floor of the first Burr hole was punctured and retracted. CSD was induced by placing cotton swabs (1.5 mm diameter) soaked with 3 M KCl upon the cortical surface. The cotton was refreshed every 15 min with 5 μl of KCl solution. The steady (DC) potential was digitally recorded for at least 1 h using pCLAMP 9.2 (Axon Instruments).

Recordings were analyzed off-line. The parameters measured were (a) amplitude of DC deflections (deflections <5 mV were ignored) and (b) number of DC deflections in 1 h. Average peak amplitude was determined for individual treatment groups and compared among different treatment groups. CSD velocity (mm/min) was calculated as the ratio: [distance (in mm) between the stimulation site and the recording electrode] ÷ [latency between simulation and the first negative CSD wave]. Ayata et al. have found that the propagation velocity determined by this method does not differ from that in which the velocity is determined by recording from two sites [4].

2.4. Motor toxicity test

Motor toxicity was evaluated using a modification of the horizontal screen test that determines an animal’s ability to support its own body weight by grasping an inverted grid for 1 min. Animals are scored for motor toxicity if they fall from the grid.

2.5. Loss-of-righting reflex test

Loss-of-righting reflex was assessed as described by Dhir et al. [12]. In brief, animals were placed on a filter paper stage and gently turned onto their backs every 10 s. Animals that were unable to regain normal posture 3 times within 30 s were scored as positive.

Fig. 1. Effects of propofol hemisuccinate (left traces) and dizocilpine (right traces) on cortical spreading depression deflections. Mice were pretreated with the test substance at the dose indicated and 15 min later KCl solution was applied to the cortical surface. The interval between drug administration and cortical KCl application corresponds to the peak anticonvulsant effect of PHS [12]. The traces show the entire 1 h recording period after KCl treatment. Each trace represents a separate animal.
Fig. 2. Compilation of cortical spreading depression deflection measurements in experiments similar to those of Fig. 1 with propofol hemisuccinate (A) and dizocilpine (B). Bars indicate mean ± S.E.M. of average peak CSD amplitude values in groups of 6–8 animals treated with each of the doses shown. Squares indicate the mean ± S.E.M. of average number of CSD deflections in 1 h recordings from the time of KCl application. *p < 0.05 compared to the vehicle treated group (ANOVA followed by Dunnett’s test).

2.6. Data analysis

For each group, CSD amplitude, number of CSDs, and CSD velocity are expressed as mean ± S.E.M. Comparisons between groups are made using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons versus control.

3. Results

Cortical application of KCl elicited repetitive negative waves (mean duration, 2.43 ± 0.09 min) at the recording site at a constant rate of about 6–8 per hour as shown in Fig. 1 (vehicle traces). Pretreatment with PHS (60–200 mg/kg, i.p.) 15 min before KCl application did not significantly affect the amplitude of the CSD deflections (Fig. 2A). However, there was a dose-dependent reduction in number of CSD deflections during the 1 h recording period that reached statistical significance for the 120 and 200 mg/kg doses. PHS also decreased the speed of propagation of CSD at all doses tested; the decrease was statistically significant only at the highest dose (200 mg/kg) (Fig. 3).

As was the case with PHS, dizocilpine (0.1–2.5 mg/kg, i.p.) did not affect the amplitude of the CSD deflections (Figs. 1 and 2B).

However, there was a significant reduction in the number of CSD deflections at the 0.5 and 2.5 mg/kg doses (Fig. 2B).

Groups of 6 unrestrained mice were injected with PHS at doses of 120 and 200 mg/kg. Animals in the 120 mg/kg group did not appear sedated and did not score positive for motor toxicity. Animals in the 200 mg/kg group began to appear ataxic about 6 min after injection and all animals scored positive for motor toxicity at 10 min after injection. None of the animals exhibited loss-of-righting reflex.

4. Discussion

Chronic administration of various established migraine preventive agents such as topiramate, valproate, propranolol, amitriptyline, and methysergide suppress CSD frequency in rats [4,8]. To date, the only migraine preventive agents that have been found to suppress CSD with acute administration are topiramate [1] and gabapentin [17]. The present study for the first time demonstrates an acute effect of propofol, as generated from the prodrug PHS, on CSD. Both the initiation and rate of propagation of CSD were suppressed by doses of PHS that are slightly higher than the minimal doses required to confer seizure protection [12] but still below those causing anesthesia as assessed by loss-of-righting reflex. Moreover, the minimal dose of PHS that suppressed CSD (120 mg/kg) did not cause sedation or motor impairment. Overall, the results support the utility of propofol-related agents in the treatment of CSD-associated disorders including migraine and perhaps also stroke and traumatic brain injury.

A variety of evidence supports the view that CSD represents the key neural event underlying migraine aura [9]. However, there is still uncertainty as to whether it is relevant to migraine without aura. Recent studies showing that CSD excites meningeal nociceptors that activate the trigeminovascular pain pathway provide support for a central role of CSD in migraine pain, whether or not there is aura [31]. Therefore, agents such as PHS that suppress CSD could have broad utility in preventing and aborting migraine attacks. Indeed, the anecdotal reports cited in the Introduction suggest that propofol is effective in terminating even treatment refractory migraine attacks. Whether propofol or PHS is similarly effective when administered early in the migraine attack remains to be determined. Assessing the utility of propofol-related agents in this scenario will require the development of non-intravenous forms that can be self-administered as are other migraine abortive therapies. Although an action on CSD is a plausible basis for the putative effect of propofol in migraine, other actions could
contribute as well, including its anxiolytic action [10]. Propofol is not considered to be an analgesic [14]. Nevertheless, subhypnotic doses do have transitory effects on pain perception [2,11] that could also be a factor in migraine and, as some patients included in the clinical reports may have had other types of headache, also in non-migraine headache.

The exact mechanism of the effect of PHS on CSD remains to be determined. PHS serves as a produg for propofol, which is believed to produce its anesthetic, hypnotic and anticonvulsant actions via GABA\(_A\) receptors [29]. At low concentrations, propofol acts as a positive modulator of GABA\(_A\) receptor responses and at higher concentrations directly activates GABA\(_A\) receptors. In addition, relatively low concentrations of propofol inhibit voltage-activated sodium channels [22,26]. While both of these actions could contribute to the anesthetic, hypnotic and anticonvulsant actions of propofol, neither is likely to account for the inhibitory effect on CSD reported here inasmuch as other drugs that potentiate GABA\(_A\) receptors or inhibit sodium channels have not been found to inhibit CSD. This implies that other pharmacological actions of propofol are responsible. Propofol does affect other receptors and ion channels, but the relevance of any of these actions to the clinical effects of the drug is uncertain [29]. In particular, in in vitro studies in cultured hippocampal neurons, propofol was found to weakly and incompletely inhibit NMDA receptor responses [25] and there was also a weak effect on NMDA responses in vivo [6]. Although NMDA receptor antagonists are highly effective in inhibiting CSD, as confirmed in the present study with dizocilpine, the limited effect of propofol on NMDA receptors raises doubt as to whether this action could account for the inhibition of CSD. Similarly, although inhibition of certain voltage-activated calcium channels coupled to glutamate release may inhibit CSD [27] and propofol does inhibit evoked glutamate release, the inhibition occurs through effects on voltage-activated sodium channels and not calcium channels [26]. Therefore, it is doubtful if effects of propofol on voltage-activated calcium channels account for the inhibition of CSD. Since the identity of the channels responsible for the majority of the ion flux in CSD has not yet been defined, an understanding of the mechanism of propofol inhibition of CSD may require a fuller understanding of the underlying physiological mechanisms of CSD.

Although the underlying mechanism is obscure, propofol is one of only a few treatments that are able to inhibit CSD acutely. The effect on CSD could account for the clinical effect of propofol in migraine described in anecdotal reports. Further study of propofol or propofol-related agents in migraine is warranted. Moreover, such agents could also be of value in the treatment of other neurological conditions in which CSD is believed to contribute to the pathology such as stroke and traumatic brain injury.

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