Seizure Protection by Intrapulmonary Delivery of Propofol Hemisuccinate

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ABBREVIATIONS: PHS, propofol hemisuccinate

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ABSTRACT

The lung provides a portal of entry for drug delivery that could be used to administer anticonvulsant substances to prevent or abort seizures. Here we demonstrate that intrapulmonary propofol hemisucinate (PHS) rapidly confers seizure protection in various rodent chemoconvulsant models. Propofol is a powerful anticonvulsant substance at subanesthetic doses but it is a viscous, water-immiscible oil that is not suitable for intrapulmonary administration. We found that PHS can be formulated as an aqueous solution that is well tolerated when instilled into the lung. High dose intraperitoneal PHS induced loss-of-righting reflex in rats and mice. The onset of action of PHS was delayed in comparison with propofol, suggesting that conversion to propofol is required for activity. A lower dose of PHS (40 mg/kg, i.p.) did not cause general anesthesia, but protected against pentylenetetrazol (PTZ)-induced seizures in rats. Intrapulmonary administration of an aqueous PHS solution via a tracheal cannula at lower doses (5 and 10 mg/kg) conferred equivalent seizure protection without acute motor toxicity. In mice, intraperitoneal PHS (60–80 mg/kg) was associated with an elevation in PTZ, bicuculline, picrotoxin, and kainic acid seizure thresholds. Intratracheal PHS was markedly more potent, producing seizure threshold elevations at doses of 10–15 mg/kg. In the PTZ threshold model in mice, PHS was active at 5 min, maximally active at 10 min and no longer active at 20 min. Intratracheal PHS also prolonged the onset of 4-aminopyridine-induced convulsions but did not affect the threshold for NMDA-induced convulsions. We conclude that intratracheal administration of an aqueous solution of PHS, a putative propofol prodrug, provides potent seizure protection of rapid onset and brief duration. Intrapulmonary PHS may be useful to prevent the spread of seizures or to abort seizure clusters without causing prolonged sedation.
Inhaled therapeutic agents have been in clinical use for more than a century, but the intrapulmonary route has been applied nearly exclusively for the delivery of agents to treat pulmonary diseases (Crompton, 2006). Only recently has it been recognized that the lung offers certain advantages over conventional routes of administration as a portal of entry for systemic therapies (Patton and Byron, 2007). These advantages include more rapid systemic delivery than with oral, intranasal or rectal dosing; avoidance of first pass metabolism in the liver; and improved bioavailability for certain agents that are poorly absorbed by the intestine (Schanker, 1978). The lung is highly vascularized and the thin alveolar epithelium represents a large absorptive area estimated at 100 m² that presents a minimal barrier to drug absorption (Patton, 1996). It is therefore feasible to rapidly and with high efficiency deliver larger drug quantities through the lung than by other nonconventional portals of entry such as the nose. The blood exiting the lungs is directly carried to the brain via the carotid arteries. Consequently, pulmonary delivery may be of particular utility for agents that are targeted to the central nervous system. Other than volatile anesthetics, however, no drugs are marketed in a form suitable for pulmonary delivery although an abortive treatment for migraine is in late stage development (Aurora et al., 2009).

Recognizing the potential of the lung as a route for the rapid delivery of therapeutic drugs to the brain, we sought to investigate pulmonary delivery of treatments for epilepsy. Inhaled epilepsy treatment approaches could be used in situations where speed of action is critical, such as to abort an impending seizure or for the out-of-hospital treatment of seizure clusters or status epilepticus when intravenous access is not readily available. In the present study, we have characterized the antiseizure activity of a putative propofol (2,6-diisopropylphenol) prodrug delivered by the pulmonary route. Propofol is commonly used as an intravenous sedative and for the induction and maintenance of general anesthesia (Reves et al., 2009). In addition to its sedative and anesthetic properties, at low (non-anesthetic) doses propofol confers seizure protection. The anticonvulsant activity of propofol has been demonstrated in diverse animal seizure models (Lowson et al., 1990; Hasan et al., 1991;...
Micklethwaite and Pleuvry, 1993) and in vitro preparations (Rasmussen et al., 1996; Ohmori et al., 2004; Borowicz and Czuczwar, 2003). Propofol is especially active in animal models of status epilepticus (De Riu et al., 1992; Ahuja and Germano, 1998; Lee and Cheun, 1999), and it is commonly used in the treatment of refractory status epilepticus in humans (Prasad et al., 2001; Arif and Hirsch, 2008). Propofol protects against seizures, at least in part, via its action as a powerful GABA<sub>A</sub> receptor positive modulator with greater potency than barbiturates (Peduto et al., 1991; Hales and Lambert, 1991; Hara et al., 1994; Lingamaneni and Hemmings, 2003).

Propofol is a challenging compound to formulate because it is an oil at room temperature that is poorly miscible with water and because it does not form a pharmaceutically suitable salt (Baker and Naguib, 2005). Neither pure propofol nor the soybean oil emulsion form that is used for intravenous administration can be safely administered into the lung (unpublished observations). We therefore have chosen to investigate a water-soluble prodrug form that combines the active propofol moiety with a cleavable hemisuccinate group (Fig. 1). Only limited information is available regarding the biological activity of propofol hemisuccinate (PHS) (Sagara et al., 1999; Vansant et al., 2007) and to our knowledge the compound has not previously been used as a propofol prodrug or administered by the pulmonary route. In the present study, we delivered an aqueous solution of PHS directly into the lung by intratracheal instillation. Recognizing that the lung is rich in esterase enzymes (Vatter et al., 1971), we hypothesized that PHS would be enzymatically cleaved to propofol so that active propofol could be absorbed into the pulmonary circulation for delivery to the brain.

**Materials and Methods**

**Preparation of Propofol hemisuccinate (PHS).** PHS [4-(2,6-diisopropylphenoxy)-4-oxobutanoic acid] was synthesized according to the scheme shown in Fig. 1. In brief, 178 g (1.0 mole) of 2,6-diisopropylphenol (propofol; Sigma-Aldrich, St. Louis, MO, USA) was combined with
120 g (1.2 mole) of succinic anhydride in 300 ml of triethylamine under argon in a flask equipped with a mechanical stirrer. A catalytic amount (100 mg) of 4-dimethylaminopyridine (Sigma-Aldrich) was added and the material was stirred for a 48-h period. At the end of this time, triethylamine was removed on the rotary evaporator to yield oil, which was taken up with 300 ml ethyl acetate. This solution was washed with $3 \times 300$ ml of 1 M hydrochloric acid, $3 \times 300$ ml portions of saturated sodium bicarbonate, and $3 \times 300$ ml portions of deionized water. The resultant solution was dried with anhydrous magnesium sulfate, treated with activated charcoal, and purified by flash chromatography. The product was crystallized from a mixture of heptane and 2-propanol to yield 228 g (82%) of analytically pure material. The structure of propofol hemisuccinate was confirmed by NMR.

**Animals.** Male Sprague Dawley rats (Taconic; 150–200g) and 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 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, D.C.; http://www.nap.edu/readingroom/books/labrats/).

**Test Substances and Chemoconvulsants.** PHS was dissolved in slightly basic water (pH 7.5). Propofol was administered as a commercially available injectable emulsion (Diprivan 1%, AstraZeneca) that contains soybean oil (100 mg/ml), glycerol (22.5 mg/ml), egg lecithin (12 mg/ml), and disodium edetate (0.005%) with sodium hydroxide to adjust the pH 7–8.5. Pentylenetetrazol (PTZ), picrotoxin, 4-aminopyridine, N-methyl-D-aspartate (NMDA), and kainic acid were dissolved
in 0.9% saline while bicuculline was dissolved in slightly acidic water (pH 5.0). All test substances other than Diprivan were from Sigma-Aldrich. The volumes used for intraperitoneal injection were 10 ml/kg in the case of mice and 1 ml/kg in the case of rats. PHS was administered via the intratracheal route in a volume of 0.25 ml/kg in mice and 0.75 ml/kg in rats. Intraperitoneal PHS was administered 15 min before challenging the animals with convulsant agents; this interval corresponds to the onset of loss-of-righting reflex in rats and 1.7 times this value in mice (Fig. 2). Intratracheal PHS was administered 10 min (or in a few experiments with rats 7 min and in the time course experiment with mice 5, 10 and 20 min) before beginning the challenge with convulsant agents; in the threshold experiments the infusion typically required 20–35 s, so the exact time threshold values were recorded is variably delayed from the nominal pretreatment time. A 10 min pretreatment interval was routinely used to insure that the animals had fully recovered from the isoflurane anesthesia required to permit intratracheal intubation as described below. Animals appeared to be conscious at 3–5 min following isoflurane and there was no effect of isoflurane at 5 min on motor function as assessed in the horizontal screen test (see Results) or on seizure threshold (Fig. 4). Each experimental group consisted of a minimum of 6 animals.

**Intratracheal Drug Delivery.** Intratracheal administration of propofol hemiscuccinate was carried out as described by Oka et al. (2006). In brief, rats and mice were briefly anesthetized using isoflurane anesthesia (4% isoflurane). The animals were immediately placed on a surgical board held at a 60° angle. The animal’s mouth was kept open by hanging the upper incisors on a hook to facilitate detection of the epiglottis. An operating light was used to illuminate the view of the pharynx after displacement of the tongue with a spatula. A syringe fitted with a blunted 18-gauge/50-mm needle (in case of rats) and 24-gauge/25-mm (in case of mice) was pushed against the soft palate to enter the trachea past the vocal cords. When the tracheal cartilage ring is felt, the needle is considered properly placed within the tracheal lumen. The needle was inserted almost to the bottom of the trachea. When the animals began to show signs that the anesthesia was waning, the liquid
sample was gently injected at a dose volume of 0.25 ml/kg (in case of mice) or 0.75 ml/kg (in case of rats). After the injection of solution, air was blown inside the lungs to uniformly distribute the drug in the lungs (2 ml air in case of rats and 1 ml in case of mice). Following the injection, the needle was gently removed and animal was held vertically for 2 min to facilitate the downward movement of liquid in the lungs. The animal was allowed 10 min to recover fully from the anesthesia as confirmed by normal spontaneous exploratory behavior. Seizure testing then commenced. For each of the seizure tests, we verified that there was no residual effect of the anesthetic on responsiveness.

**Loss-of-righting Reflex Test.** Loss-of-righting reflex, an indication of profound sedation/anesthesia, was assessed in rats and mice following intraperitoneal injection of different doses of either propofol or PHS (Ozbakis-Dengiz and Bakirci, 2009). Following injection, animals were returned to their cage and when they started showing signs of ataxia, they were taken out of the cage and placed on a filter paper stage. Animals were gently turned onto their backs every 10 s. Untreated animals immediately regained normal posture. For drug treated animals, onset of the loss-of-righting-reflex was the time after injection when the animal was unable to right itself. Recovery from loss-of-righting reflex was the time after injection when the animal regained the righting reflex (able to right itself three times within 30 sec). The total time for loss-of-righting reflex (“total sleep time”) was calculated as the difference between the onset and recovery times.

**PTZ-induced Convulsions in Rats.** PTZ was administered intraperitoneally at a dose of 80 mg/kg, which causes clonic convulsions in >97% of rats (Dhir et al., 2006). Animals were observed for a period of 30 min following injection. The time of onset of myoclonic jerks, clonus and tonic extension; the severity of clonus; and the incidence of lethality was recorded. The severity of the clonus was scored as either mild (1), moderate (2) or severe (3), where mild indicates brief and transitory episodes of limb clonus, moderate indicates longer but not continuous episodes of limb clonus where at least one episode lasts at least 10 s, and severe indicates continuous limb clonus.
Mean convulsion severity was calculated as the sum of the individual scores divided by the number of animals.

**PTZ, Bicuculline and Picrotoxin Seizure Threshold Tests in Mice.** The thresholds for various behavioral seizure stages induced by the GABA receptor antagonists PTZ, bicuculline and picrotoxin were determined by infusing the convulsant drugs via a 27 gauge-¼ inch “butterfly” needle inserted into the lateral tail vein. The needle was secured to the tail vein by a narrow piece of adhesive tape and the animal was permitted to move freely inside an inverted 2 litre glass beaker with free aeration from the top. PTZ (10 mg/ml) (Akula et al., 2008), bicuculline (0.1 mg/ml), and picrotoxin (1 mg/ml) (Chan et al., 2006) was infused at a constant rate of 0.5 ml/min using a Beckton Dickinson (1 ml) syringe mounted on an infusion pump (Model ‘11’ plus syringe pump; Harvard Apparatus, Holliston, MA, USA). The syringe was connected to the needle by polyethylene tubing. The infusion was stopped at 3 min or at the onset of tonic extension, whichever occurred first. The thresholds to the following endpoints were determined: (i) the first myoclonic jerk; (ii) the onset of generalized clonus with loss-of-righting reflex; and (iii) the onset of tonic extension. Latencies were measured from the start of convulsant infusion to the onset of all these three events. The threshold value (mg/kg) for each endpoint was determined according to the following formula: (infusion duration [sec] \times infusion rate [ml/min] \times convulsant drug concentration [mg/ml] \times 1000)/(60 [sec] \times weight of mouse [g]). Fractional change in threshold values were calculated by dividing each of the threshold values for the three endpoints by the corresponding vehicle control threshold values.

**N-Methyl-D-aspartate (NMDA) and Kainic Acid Seizure Threshold Test in Mice.**

Threshold determinations for the excitatory amino acid agonists NMDA (8 mg/ml; infusion rate 0.5 ml/min) (Finn and Crabbe, 1999) and kainic acid (7.5 mg/ml; infusion rate 0.15 ml/min) (Kaminski et al., 2005) when administered intravenously were obtained according to the same protocol as described for the GABA receptor antagonists.
4-Aminopyridine (4-AP)-induced Convulsions in Mice. The 4-AP seizure test was carried out as previously described (Yamaguchi and Rogawski, 1992). In brief, 4-AP was administered intraperitoneally at a dose of 13 mg/kg, which causes tonic seizures in 100% of control mice. Onset time of myoclonic jerks, clonus and tonic extension was noted.

Motor toxicity test. Motor toxicity was evaluated using a modification of the horizontal screen test (Coughenour et al., 1977) that determines an animal’s ability to support its own body weight by grasping an inverted grid for 1 min. Unless intoxicated, mice do not fall from the grid.

Data Analysis. Results are expressed as mean ± S.E.M.; the significance of the difference in the responses of treatment groups with respect to control is based on one-way analysis of variance (ANOVA) followed by specific post-hoc comparisons using Tukey’s test. Differences were considered statistically significant when the probability of error was less than 0.05 ($p < 0.05$). Control values in the threshold tests were the mean threshold values for vehicle-treated groups.

Results

Comparison of Loss-of-Righting Reflex Following Intraperitoneal Propofol and PHS in Rats and Mice. To assess the central nervous system activity of systemically administered PHS, we determined if intraperitoneal PHS influences the righting reflex; for comparison, we administered aqueous propofol emulsion. In rats, 70 mg/kg PHS caused total sleep time (2163 ± 324 s) that was roughly equivalent to the sleep time obtained with 60 mg/kg propofol (2116 ± 101 s) (Fig. 2A). However, onset and recovery occurred earlier with propofol than PHS. In an additional experiment with mice, we administered equimolar doses of the two agents. As shown in Fig. 2B, the onset of loss-of-righting reflex occurred earlier with propofol (200 mg/kg) than PHS (312 mg/kg) but the total sleep time was more prolonged with PHS (3842 ± 202 s) than with propofol (1367 ± 283 s).
Comparison of Intraperitoneal and Intratracheal PHS on PTZ-induced Seizures in

**Rats.** Intraperitoneal PTZ (80 mg/kg) induced clonic seizures, tonic seizures and mortality in all vehicle pretreated animals (Table 1). When administered 15 min prior to PTZ, intraperitoneal PHS (40 mg/kg) provided marked seizure protection against clonic seizures and completely prevented tonic seizures and the mortality that invariably accompanies tonic seizures. At lower doses, intratracheal PHS administered 10 and 7 min before PTZ provided similar seizure protection. The effect of intratracheal PHS was dose-dependent: the 5 mg/kg dose failed to provide complete protection against tonic seizures and mortality whereas the 10 mg/kg dose protected all animals in both the 10 and 7 min pretreatment time experiments.

**Comparison of Intraperitoneal and Intratracheal PHS in the PTZ Threshold Test in Mice.** Intravenous infusion of PTZ (10 mg/ml) produced a sequence of myoclonic jerks, clonus and tonic extension followed by death. Pretreatment (15 min) with PHS by the intraperitoneal route at doses of 60 and 80 mg/kg but not 40 mg/kg caused a significant elevation in the thresholds for all seizure signs with respect to the values in vehicle-treated animals (Fig. 3A). In contrast, pretreatment (10 min) with PHS by the intratracheal route was effective at lower doses of 10 and 15 mg/kg but not 5 mg/kg (Fig. 3B).

Having established that a 10 mg/kg intratracheal dose of PHS was effective with a 10 min pretreatment time, we next sought to assess the time course of action of PHS administered by the intratracheal route. As shown in Fig. 4, an elevation in threshold with respect to the corresponding threshold values in vehicle pretreated animals was obtained with 10 mg/kg intratracheal PHS at 5 and 10 min but not 20 min. The fractional increase with respect to the corresponding threshold values in vehicle-treated animals for all three seizure measures was numerically greater at 10 min than at 5 min although there was no statistical significance between the 10 min and 5 min values (not shown). This suggests that the maximal effect of intratracheal PHS occurs near 10 min although a significant threshold change is observed as early as 5 min. It was not practical to assess shorter pretreatment
times due to the confounding effect of the isoflurane anesthesia that was required for intratracheal cannulation. Indeed, the small but non-significant increase in mean threshold values obtained at the 5 min time point in the vehicle pretreatment group is likely due to residual anesthesia effects.

**Motor toxicity testing.** The horizontal screen test was used to assess the acute motor toxicity of intratracheal PHS (10 mg/kg) in the time course experiment of Fig. 4. No motor toxicity was observed with PHS at any time point.

**Comparison of Intraperitoneal and Intratracheal PHS in the Bicuculline and Picrotoxin Seizure Threshold Tests in Mice.** Intravenous infusion of bicuculline (0.1 mg/ml) produced a similar sequence of seizure signs as PTZ. Pretreatment (15 min) with PHS by the intraperitoneal route at doses of 60 and 80 mg/kg caused a significant elevation in the thresholds for all seizure signs; the 40 mg/kg dose only affected tonic extension significantly (Fig. 5A). In contrast, pretreatment (10 min) with PHS by the intratracheal route was effective at lower doses of 15 and 20 mg/kg but not 10 mg/kg (Fig. 5B).

Intravenous infusion of picrotoxin (1 mg/ml) also produced the same sequence of seizure signs. Pretreatment (15 min) with PHS by the intraperitoneal route at dose of 60 and 80 mg/kg but not 40 mg/kg caused a significant elevation in the thresholds for all seizure signs (Fig. 6A). In contrast, pretreatment (10 min) with PHS by the intratracheal route was effective at lower doses of 10, 15 and 20 mg/kg (Fig. 6B).

**Effects of Intratracheal Administration of PHS in the NMDA and Kainic Acid Seizure Threshold Tests in Mice.** Intravenous infusion of the excitatory amino acid agonists NMDA (8 mg/ml) (Fig. 7) and kainic acid (7.5 mg/ml) (Fig. 8) induced a similar sequence of seizure signs as did the GABA<sub>A</sub> receptor antagonists PTZ, bicuculline and picrotoxin. However, with NMDA infusion, intratracheal pretreatment (10 min) with PHS at doses of 10 and 15 mg/kg did not modify the thresholds for any of the seizure signs. In contrast, with kainic acid infusion, PHS at doses of 10 and 15 mg/kg but not 5 mg/kg did elevate the thresholds for all seizure signs.
Effects of Intratracheal Administration of PHS in the 4-AP Seizure Test in Mice.

Intraperitoneal 4-AP (13 mg/kg) induced a sequence of seizure signs consisting of myoclonic jerks, clonus and tonic extension followed by death (Fig. 9). PHS pretreatment (10 min) at 10 mg/kg but not 5 mg/kg delayed the onset of all seizure signs.

Discussion

This study for the first time demonstrates the anticonvulsant activity of intrapulmonary (intratracheal) administration of PHS, a putative propofol prodrug. High systemic doses of PHS were found to cause anesthesia as assessed by loss-of-righting reflex in rats and mice. In rats, PHS was slightly less potent than propofol itself in inducing loss-of-righting reflex but the onset and recovery of the righting reflex was delayed in comparison with propofol, consistent with the requirement for PHS to be activated by deacetylation. Equimolar doses of PHS resulted in longer total sleep time than with propofol presumably because it acts as a depot-like form that is released over a longer period of time than propofol itself. The ability of PHS to cause loss-of-righting reflex demonstrated that it is active as a propofol prodrug when administered systemically by the intraperitoneal route.

We next compared the activity of PHS when administered by the intraperitoneal and intratracheal routes in various rodent seizure models. PHS was effective by both routes of administration. However, the potency with intratracheal dosing was greater. Thus, in the maximal PTZ test in rats (Table 1), intratracheal PHS at a dose of 10 mg/kg demonstrated efficacy similar to that of a 40 mg/kg intraperitoneal dose. Similarly, in the PTZ seizure threshold model in mice, an intraperitoneal dose of 60 mg/kg but not 40 mg/kg was associated with a significant fractional change in threshold in all measures whereas when administered by the intratracheal route the 10 mg/kg but not 5 mg/kg dose was associated with a change in threshold in all measures. The onset of the action of intrapulmonary PHS was remarkably rapid. Maximal efficacy occurred at 10 min and a nearly
maximal effect was obtained by 5 min. It was not possible to determine the minimum onset time due to the requirement for general anesthesia with intratracheal delivery but it may be substantially less than 5 min. For comparison, it is noteworthy that the time to peak functional (central nervous system) effect of propofol following bolus intravenous administration in humans is about 100 sec (Flaishon et al., 1997; Schnider et al., 1999) and this corresponds with peak blood levels of 30–120 sec as observed in other species (rat, dog and rabbit) (Simons et al., 1991; Shyr et al., 1993). Recovery was similarly rapid, with a return to baseline seizure threshold values at 20 min. The rapid time course of the anticonvulsant action of intrapulmonary PHS is consistent with the known pharmacokinetic properties of propofol (Cockshott, 1985). The pharmacokinetics of propofol is generally described by two-compartment or three-compartment models in which blood levels after a single bolus fall according to 2 or 3 exponentials. The half-time of the first exponential component, representing extensive redistribution from a large central compartment into a volume much greater than total body volume, is 3.5 min in rats (Cockshott et al., 1992) and in the range of 1.6–4.2 min in humans (Saint-Maurice et al., 1989). This initial redistribution likely accounts for the rapid recovery seen in the present study. A second slow distribution phase may follow the first (with half-time of 33 min in rats and 34–69 min in humans) followed by metabolism and clearance proceeding with a half-time of 6.4 h in rats and 8–14 h in humans (Cockshott, 1985; Saint-Maurice et al., 1989). At the minimum intratracheal dose effective in raising seizure threshold (10 mg/kg), there was no apparent behavioral effect and an objective measure of motor impairment (horizontal-screen test) failed to demonstrate acute neurological toxicity. Consequently, there is a separation between the subanesthetic doses of propofol that confer seizure protection and the higher doses that would induce neurobehavioral side effects. Interestingly, a similar threshold dose of intravenous propofol (10 mg/kg) has been reported to confer a transient analgesic effect and suppression of electroencephalographic activity with maintained cardiovascular performance (Shyr et al., 1993).
As was the case for PTZ, PHS raised the threshold for seizures induced by bicuculline and picrotoxin, which like PTZ act by blocking GABA<sub>A</sub> receptors. With all three convulsant agents, PHS was 6-fold more potent when administered by the intratracheal route than by intraperitoneal injection. The potency difference may relate to a high capacity of the lungs to activate PHS by deacetylation, given the high abundance of non-specific esterase enzymes in the bronchiolar mucosa and alveolar walls (Chessick, 1953), but could also relate to greater bioavailability of PHS itself when administered by the pulmonary route. It is noteworthy that although most of the biotransformation of propofol to its inactive metabolites occurs in the liver, the lung extracts (Matot et al., 1993) and metabolizes (Dawidowicz et al., 2000) a substantial fraction of propofol. While such metabolism could occur with intrapulmonary PHS administration thus reducing the available propofol, the extent must be relatively minor given the high potency of intrapulmonary PHS.

Intratracheal PHS at doses effective in the PTZ, bicuculline and picrotoxin threshold models also raised the threshold for seizure signs in the kainic acid but not the NMDA infusion models. The results with kainic acid are consistent with a prior study showing that propofol is protective against kainic acid-induced seizures in rats (Ahuja and Germano, 1998). The failure of PHS to affect NMDA-induced seizures is in accordance with the limited information available that propofol does not block NMDA receptors (Zhan et al., 2001).

The efficacy of PHS in the diverse seizure models we examined likely results in part from the well-recognized action of propofol as a GABA<sub>A</sub> receptor positive modulator (Peduto et al., 1991; Hales and Lambert, 1991; Hara et al., 1994) and a direct activator of GABA<sub>A</sub> receptors (Hara et al., 1993). Seizures induced by GABA<sub>A</sub> receptor antagonists are sensitive to GABA<sub>A</sub> receptor positive modulators (Rastogi and Ticku, 1986) as are seizures induced by kainic acid whereas NMDA-induced seizures are relatively resistant to such agents (Kokate et al., 1996). In each of the models we studied, PHS was more effective in blocking tonic extension than myoclonic jerks and clonus. Tonic extensor seizures are highly susceptible to sodium channel blocking antiseizure agents and it is...
conceivable that the effect on tonic extension could be due to previously described actions of propofol on voltage-gated sodium channels, in which binding occurs preferentially to the fast inactivated state as occurs with sodium channel blocking anticonvulsants (Rehberg and Duch, 1999; Ouyang et al., 2003). The effects on sodium channels occur in the same clinically relevant range of concentrations as the effect on GABA<sub>A</sub> receptors so that actions on sodium channel could contribute in a major way to the anticonvulsant activity of propofol.

Schanker et al. (1986) have observed that lipid-soluble drugs are absorbed from the lung following intratracheal administration at the same rate in diverse species, suggesting that the data obtained here is transferable to humans. If so, intrapulmonary PHS could have a variety of applications in human epilepsy therapy. For example, nebulized PHS could be inhaled to abort the occurrence of a full-blown seizure by a patient experiencing an aura or warned of an impending seizure by a seizure prediction device. In another application, a facemask inhaler could be used in an out-of-hospital situation to abort seizure clusters or status epilepticus. The pharmacokinetic properties of intrapulmonary PHS may be well suited for these applications inasmuch as rapid onset is clearly necessary and rapid recovery would ensure that any sedative effects would be transitory.

In order to be useful in these applications, an inhaler device is required that reliably delivers sufficient quantities of nebulized PHS. The device would need to tightly regulate exposure levels so as to obtain consistent therapeutic effects without delivering excessive doses that could lead to respiratory depression or adverse hemodynamic effects. High technology inhalers have become available in recent years that meet the requirements of these applications (Vanbever, 2005). Such devices efficiently deliver a uniform distribution of appropriately sized droplets to the deep lung, avoiding filtration in the throat and upper airways. This allows the full area of the alveolar epithelium to be used as an absorptive surface. In contrast, other nonconventional drug portals, such as the nose, do not have the same efficiency and do not provide a means of achieving sufficient drug exposure. The nasal mucosa provides much less absorptive surface area and the geometry does not allow
trapping of fluid droplets as occurs in alveolar sacs. Moreover, while highly vascularized, the nose is exposed to only a fraction of the blood supply in contrast to the lung that receives the entire cardiac output. Therefore, the lung has unique characteristics that make it particularly promising as a portal for the delivery of agents such as PHS for epilepsy treatment applications and inhaler technologies exist that make it feasible to investigate the use of intrapulmonary PHS in such applications.
REFERENCES


Footnotes

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FIGURE LEGENDS

**Fig. 1.** Synthesis of propofol hemisuccinate.

**Fig. 2.** Loss-of-righting reflex induced by intraperitoneal administration of propofol or propofol hemisuccinate (PHS) in rats (A) and mice (B). Thick vertical lines indicate the mean ± S.E.M. for 6–8 animals of the latency to loss-of-righting reflex and recovery from loss-of-righting reflex; total sleep time (open squares) is the difference between these two values. Doses in rats for propofol and PHS were 60 mg/kg (0.34 mmol/kg) and 70 mg/kg (0.25 mmol/kg), respectively; doses in mice were 200 mg/kg (1.12 mmol/kg) and 312 mg/kg (1.12 mmol/kg). In mice, the mean latency to and recovery from loss-of-righting reflex and total sleep time values were significantly different in the PHS and propofol groups ($p < 0.05$; ANOVA followed by Tukey’s test).

**Fig. 3.** Effect of intraperitoneal (A; 40–80 mg/kg) and intratracheal (B; 5–15 mg/kg) propofol hemisuccinate (PHS) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous PTZ infusion in mice. PHS was administered 15 min and 10 min prior to the beginning of the PTZ infusion for the intraperitoneal and intratracheal experiments, respectively. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the thresholds in the vehicle-treated groups (V), which (in mg/kg) for myoclonic jerk, clonus and tonic extension, respectively, were 37.1 ± 3.8, 41.9 ± 3.9, 47.9 ± 3.5 for intraperitoneal and 37.8 ± 1.2, 45.1 ± 3.9, 47.9 ± 3.5 for intratracheal. *$p < 0.05$ as compared to vehicle control group (ANOVA followed by Tukey’s test).

**Fig. 4.** Time course for the action of 10 mg/kg intratracheal propofol hemisuccinate (PHS) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous PTZ infusion in mice. PHS was administered 5, 10 and 20 min prior to the beginning of the PTZ infusion. Closed (■) and open (□) symbols indicate mean ± S.E.M. of values from 6–8 mice pretreated with PHS or
vehicle, respectively. *$p < 0.05$ as compared to vehicle control group (ANOVA followed by Tukey’s test).

**Fig. 5.** Effect of intraperitoneal (A; 40–80 mg/kg) and intratracheal (B; 10–20 mg/kg) propofol hemisuccinate (PHS) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous bicuculline infusion in mice. PHS was administered 15 min and 10 min prior to the beginning of the bicuculline infusion for the intraperitoneal and intratracheal experiments, respectively. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the thresholds in the vehicle-treated groups (V), which (in mg/kg) for myoclonic jerk, clonus and tonic extension, respectively, were 0.55 ± 0.02, 0.60 ± 0.02, 0.84 ± 0.02 for intraperitoneal and 0.56 ± 0.04, 0.62 ± 0.04, 0.92 ± 0.06 for intratracheal. *$p < 0.05$ as compared to vehicle control group (ANOVA followed by Tukey’s test).

**Fig. 6.** Effect of intraperitoneal (A; 40–80 mg/kg) or intratracheal (B; 10–20 mg/kg) propofol hemisuccinate (PHS) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous picrotoxin infusion in mice. PHS was administered 15 min and 10 min prior to the beginning of the picrotoxin infusion for the intraperitoneal and intratracheal experiments, respectively. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the threshold in the vehicle-treated groups (V), which (in mg/kg) for myoclonic jerk, clonus and tonic extension, respectively, were 14.6 ± 0.3, 18.1 ± 0.3, 23.1 ± 0.5 for intraperitoneal and 17.3 ± 0.7, 22.3 ± 0.7, 31.3 ± 1.3 for intratracheal. *$p < 0.05$ as compared to vehicle control group (ANOVA followed by Tukey’s test).

**Fig. 7.** Effect of intratracheal propofol hemisuccinate (PHS; 10 and 15 mg/kg) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous NMDA infusion in mice. PHS was
administered 10 min prior to the beginning of the NMDA infusion. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the threshold values in the vehicle-treated control group (V), which (in mg/kg) for myoclonic jerk, clonus and tonic extension, respectively, were 39.8 ± 4.1, 56.1 ± 6.1, 91.9 ± 17.7. The values in the treated groups were not significantly different from those in the control group (ANOVA).

**Fig. 8.** Effect of intratracheal administration of propofol hemisuccinate (PHS; 5–15 mg/kg) on myoclonic jerk, generalized clonus and tonic extension in response to intravenous kainic acid infusion in mice. PHS was administered 10 min prior to the beginning of the kainic acid infusion. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the threshold in the vehicle-treated group, which (in mg/kg) for myoclonic jerk, clonus and tonic extension, respectively, were 29.8 ± 2.8, 79.1 ± 3.7, 111.2 ± 7.1. *p < 0.05 as compared to vehicle control group (ANOVA followed by Tukey’s test).

**Fig. 9.** Effect of intratracheal administration of propofol hemisuccinate (PHS; 5 and 10 mg/kg) on the mean onset time to myoclonic jerk, generalized clonus and tonic extension in response to 4-aminopyridine (13 mg/kg, i.p.) in mice. PHS was administered 10 min prior to 4-aminopyridine. Bars indicate mean ± S.E.M. of values from 6–8 mice normalized with respect to the times in the vehicle-treated group, which (in min) for myoclonic jerk, clonus and tonic extension, respectively, were 7.12 ± 0.28, 10.30 ± 0.98, 10.35 ± 0.94. *p < 0.05 as compared to vehicle control group (ANOVA followed by Tukey’s test).
Table 1

Comparison of intraperitoneal and intratracheal propofol hemisuccinate (PHS) on PTZ-induced convulsions in rats.

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Pretreatment Time (min)</th>
<th>Number of animals</th>
<th>Percent showing myoclonic jerks</th>
<th>Percent showing clonus</th>
<th>Mean clonus severity</th>
<th>Percent showing tonic extension</th>
<th>Percent showing mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraperitoneal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>15</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PHS (40 mg/kg)</td>
<td>15</td>
<td>6</td>
<td>33*</td>
<td>33*</td>
<td>0.5</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td><strong>Intratracheal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PHS (5 mg/kg)</td>
<td>10</td>
<td>6</td>
<td>83</td>
<td>83</td>
<td>1.3</td>
<td>17*</td>
<td>16*</td>
</tr>
<tr>
<td>PHS (10 mg/kg)</td>
<td>10</td>
<td>7</td>
<td>57*</td>
<td>29*</td>
<td>0.5</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td><strong>Intratracheal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PHS (10 mg/kg)</td>
<td>7</td>
<td>6</td>
<td>50*</td>
<td>33*</td>
<td>0.3</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

PTZ was administered at a dose of 80 mg/kg, i.p. The second column indicates the pretreatment interval between PHS administration and the subsequent PTZ injection. The solution volume for intraperitoneal PHS was 1 ml/kg and for intratracheal PHS was 0.75 ml/kg. Values indicate percent of animals exhibiting indicated seizure sign or mortality, except mean clonus severity which is the average of the clonus scores rated either 1, 2 or 3. *p < 0.05 as compared to vehicle treated control group (ANOVA followed by Tukey’s test).
Fig. 1

Propofol

Succinic anhydride

Propofol hemisuccinate
Fig. 2
Fig. 3

A

Fractional Change in Threshold

Myoclonic Jerk  Clonus  Tonic Extension

Intracritonal Dose (mg/kg)

V  40  60  80  V  40  60  80  V  40  60  80

B

Fractional Change in Threshold

Intratracheal Dose (mg/kg)

V  5  10  15  V  5  10  15  V  5  10  15

Fig. 3
Fig. 4

**Myclonic Jerk**

**Clonus**

**Tonic Extension**

![Graphs showing seizure threshold (mg/kg) over time (min)](image)

*Fig. 4*
Fig. 5

A

Myoclonic Jerk  Clonus  Tonic Extension

Fractional Change in Threshold

V  40  60  80  V  40  60  80  V  40  60  80

Intraperitoneal Dose (mg/kg)

B

Fractional Change in Threshold

V  10  15  20  V  10  15  20  V  10  15  20

Intratracheal Dose (mg/kg)
Fig. 6

A

Myoclonic Jerk

Clonus

Tonic Extension

Fractional Change in Threshold

Intraperitoneal Dose (mg/kg)

0 1 2

V 40 60 80 V 40 60 80 V 40 60 80

B

Fractional Change in Threshold

Intratracheal Dose (mg/kg)

0 1 2

V 10 15 20 V 10 15 20 V 10 15 20

Fig. 6
Fig. 7

[Diagram showing the fractional change in threshold with different intratracheal doses and conditions: Myoclonic Jerk, Clonus, Tonic Extension.]

Intratracheal Dose (mg/kg)

V 10 15 V 10 15 V 10 15
Fig. 8
Fig. 9

Bar graph showing fractional change in onset time for different intratracheal doses (mg/kg) for myoclonic jerk, clonus, and tonic extension.