Published in final edited form as:

Anesth Analg. 2012 October ; 115(4): 837–840. doi:10.1213/ANE.0b013e318258b9db.

Receptor Specificity Defines Algogenic Properties of Propofol and Fospropofol

Amol Patwardhan, MD, PhD,

Department of Anesthesiology, University of Arizona Health Sciences Center, Tucson, Arizona

Rebecca Edelmayer, PhD,

Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona

Emil Annabi, MD,

Department of Anesthesiology, University of Arizona Health Sciences Center, Tucson, Arizona

Theodore Price, PhD,

Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona

Phil Malan, MD, PhD, and

Departments of Anesthesiology and Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona

Gregory Dussor, PhD

Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona

Abstract

Corresponding Author Gregory Dussor Department of Pharmacology University of Arizona P.O. Box 245050 1501 N. Campbell Ave Tucson, AZ 85724-5050 dussorg@email.arizona.edu..

Drs. Amol Patwardhan and Rebecca Edelmayer contributed equally to the manuscript.

DISCLOSURES:

Name: Amol Patwardhan, MD, PhD

Contribution: This author helped generate the hypothesis, design the study, analyze the data, and write the manuscript.

Conflicts of Interest: The author has no conflicts of interest to declare.

Name: Rebecca Edelmayer, PhD

Contribution: This author helped design the study, perform the experiments, and analyze the data.

Conflicts of Interest: The author has no conflicts of interest to declare.

Name: Emil Annabi, MD

Contribution: This author helped design the study

Conflicts of Interest: Emil Annabi serves as a consultant for Medtronics

Name: Theodore Price, PhD

Contribution: This author helped design the study

Conflicts of Interest: The author has no conflicts of interest to declare.

Name: Phil Malan, MD, PhD

Contribution: This author helped design the study

Conflicts of Interest: The author has no conflicts of interest to declare.

Name: Gregory Dussor, PhD

Contribution: This author helped design the study and write the manuscript.

Conflicts of Interest: The author has no conflicts of interest to declare.

This manuscript was handled by: Marcel E. Durieux, MD, PhD

See Disclosures at end of article for Author Conflicts of Interest.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Background—Propofol-evoked injection site pain is not observed with fospropofol. We hypothesized that unlike propofol, fospropofol does not activate the irritant receptor, transient receptor potential 1 (TRPA1).

Methods—We tested the hypothesis using electrophysiology and behavioral studies.

Results—Our data demonstrate that propofol (100 μ M) evokes an inward current only in TRPA1-expressing neurons. However, fospropofol (100 μ M and 1mM) is unable to evoke depolarizing currents in either TRPA1-positive or TRPA1-negative neurons. Both propofol and fospropofol produced general anesthesia.

Conclusions—The lack of algogenic activity in fospropofol is most likely the result of its inability to activate TRPA1 on nociceptors.

Introduction

The IV general anesthetic propofol has been widely used for induction and maintenance of general anesthesia for decades. One of the common side effects observed with the use of propofol as an induction drug is injection site pain.¹ Irritation evoked by propofol leads to undesirable patient movement at induction. Moreover, propofol injection/infiltration can lead to development of thrombophlebitis/tissue inflammation.² Interestingly, fospropofol, a recently approved prodrug of propofol, does not cause injection site pain.³ Multiple mechanisms have been proposed to explain the algogenic properties of propofol and they include the solvent used, emulsion formulation, etc.⁴ A more recent study demonstrated propofol-induced activation of an ion channel known as transient receptor potential A1 (TRPA1), that is expressed by nociceptors, as a more likely explanation for propofol-evoked pain.⁵

TRPA1 is a cation selective ion channel that belongs to a larger family of TRP channels, many of whom are expressed by nociceptors and serve as sensors for various harmful stimuli such as heat, chemicals, acid, etc.⁶ TRPA1 is activated by environmental irritants such as cigarette smoke, certain pungent chemicals (e.g., mustard oil [MO], wasabi, desflurane, etc.), and possibly by noxious cold.^{7,8}

In the current study, we hypothesized that the lack of injection site pain upon fospropofol administration is due to its inability to activate TRPA1. We conducted single cell electrophysiological studies on dissociated rat trigeminal ganglia (TG) neurons. Behavioral studies were also performed to assess the anesthetic efficacy of propofol and fospropofol.

Materials and methods

Animals

Adult male Sprague Dawley rats (200-225 g, Harlan) were maintained in a climate-controlled room on a 12-hr light/dark cycle (light on 7am, light off 7pm) with food and water *ad libitum*. All procedures were performed in accordance with the policies and recommendations of the International Association for the Study of Pain, the National Institutes of Health guidelines for the handling and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee of the University of Arizona.

Drugs

Allyl isothiocyanate (AITC; MO) was purchased from Sigma. Propofol and fospropofol disodium were obtained in clinical formulation from the University of Arizona pharmacy. For patch clamp experiments, MO and propofol were dissolved in dimethylsulfoxide to 10 mM as a stock solution and diluted to the 100 μ M concentration with external bath solution.

Final dimethylsulfoxide concentration never exceeded 0.1% for patch-clamp experiments. Fospropofol was diluted directly in external bath to final concentrations of 100 μ M and 1 mM. For the *in vivo* experiments, effective doses of propofol (10 mg/kg) and fospropofol (45 mg/kg) were administered IV.

Cell culture

TG were removed, enzymatically treated, and mechanically dissociated as previously described. Briefly, rats were anesthetized with isoflurane (Phoenix Pharmaceuticals) and killed by decapitation. The TG were removed and placed in ice-cold Hanks balanced-salt solution (divalent free). Ganglia were cut into small pieces and incubated for 25 min in 20 U/ml Papain (Worthington) followed by 25 min in 3 mg/ml Collagenase Type II (Worthington). Ganglia were then triturated through fire-polished Pasteur pipettes and plated on poly-d-lysine (Becton Dickinson) and laminin (Sigma) coated 35 mm plates. After several hours at room temperature to allow adhesion, cells were cultured in a room-temperature, humidified chamber in Liebovitz L-15 medium supplemented with 10% fetal bovine serum, 10 mM glucose, 10 mM HEPES, 50 U/ml penicillin/streptomycin, and 50 ng/ml nerve growth factor. Cells were used within 24 h post plating.

Electrophysiology

Whole cell patch-clamp experiments were performed on isolated rat TG using a MultiClamp 700B (Axon Instruments) patch-clamp amplifier and pClamp 10 acquisition software (Axon Instruments). Recordings were sampled at 5 kHz and filtered at 1 kHz (Digidata 1322A, Axon Instruments). Pipettes (OD: 1.5 mm, ID: 0.86 mm, Sutter Instrument) were pulled using a P-97 puller (Sutter Instrument) and heat polished to 2.5–4 M Ω resistance using a microforge (MF-83, Narishige). Series resistance was typically <7 M Ω and was compensated 60%. All recordings were performed at room temperature. A Nikon TE2000-S Microscope was used to identify TG cells. Data were analyzed using Clampfit 10 (Molecular Devices) and Origin 8 (OriginLab). Pipette solution contained (in mM) 140 KCl, 11 EGTA, 2 MgCl₂, 10 NaCl, 10 HEPES, 2 MgATP, and 0.3 Na₂GTP, 1CaCl₂ pH 7.3 (adjusted with *N*-methyl glucamine), and was ~320 mosM. External solution contained (in mM) 135 NaCl, 2 CaCl₂, 1 MgCl₂, 5 KCl, 10 glucose, 10 HEPES, pH 7.4 (adjusted with *N*-methyl glucamine), and was ~320 mosM. Solutions were rapidly changed during recordings (exchange time was ~20 ms) using gravity-fed flow pipes positioned near the cell and controlled by computer-driven solenoid valves. Four different test solutions were applied for 2 min to each cell. The order of the solutions was varied from cell to cell with no effect on the outcome of the recordings. All electrophysiological data are presented as cell counts or as a percentage of TRPA1-expressing neurons.

In vivo anesthesia

Intravenous tail vein injections were performed by placing the animal into a rat restrainer (VWR International, LLC, Radnor, PA, USA) and holding the tail in warm water for 5 sec to dilate the tail vein. A 30-gauge needle was inserted into the tail vein and propofol or fospropofol was injected. Bolus injections of the anesthetics were performed over a 10-sec period and were noted as positive by the presence of blood in the tip of the syringe before injection and the absence of an out-pocketing of the tail-skin at the site of injection. Immediately after injection, animals were removed from the restrainer and the time to anesthesia was recorded (as defined by the loss of righting reflex)⁹ and the time to first movement was observed to calculate duration of anesthesia. Anesthesia data are presented as means $\pm$ SEM.

Results

Patch clamp electrophysiology was performed on cultured TG neurons taken from rats. TRPA1-expressing sensory neurons were identified using application of the known specific TRPA1-agonist MO (100 μ M). Among 25 cells recorded, 52% expressed the TRPA1 receptor as evidenced by MO-evoked currents (Fig 1A). All 13 neurons that exhibited MO-evoked current also exhibited TRPA1-like current in response to propofol (100 μ M) (Fig 1A). Propofol concentrations were chosen based upon the dose responses performed in previous studies.^{5,10,11} We used the clinically available formulations of both propofol and fospropofol to keep our observations consistent with human studies. Although it has been reported that the aqueous portion of propofol may vary depending upon the formulation, the clinical efficacy and pharmacokinetics are unchanged.¹² The propofol current was similar to that previously reported by Matta et al. and therefore would unlikely be the result of TRPV1 or TRPM8 channel activation.⁵ Additionally, the carrier solvent for propofol is not the cause of TRPA1 activation because it has been shown that application of the carrier produces no increase in intracellular calcium when applied to cultured dorsal root ganglion neurons nor does it produce pain upon injection in human subjects.¹⁰ Cells expressing TRPA1-like currents were not activated by a comparable concentration of fospropofol (100 μ M) (Fig 1A). Clinically, fospropofol requires a higher concentration than propofol for effective anesthesia.¹³ To exclude the possibility that TRPA1 activation by fospropofol requires a higher effective concentration, a 1 mM fospropofol solution was applied to TRPA1-positive neurons and no apparent current was observed (Fig 1A). The 12 neurons identified as TRPA1-negative cells (defined by the absence of MO-evoked current) did not express current after either propofol or fospropofol solution application (Fig 1B). These data indicate that fospropofol is not an agonist for the TRPA1 receptor on sensory neurons.

To demonstrate that the clinical formulations used in our *in vitro* patch-clamp studies were biologically active, we performed an *in vivo* study to characterize general anesthesia induced by both drugs. Propofol (10 mg/kg) induced an immediate state of anesthesia in less than 10 sec after a single bolus IV injection with duration of anesthesia lasting 7.44 ± 0.32 min (N = 6). Fospropofol (45 mg/kg) induced full anesthesia more gradually in 2.57 ± 0.16 min with duration of anesthesia lasting 16.38 ± 1.19 min (N = 6). These data are consistent with previous clinical reports concerning both dosing and the pharmacokinetics of the two drugs.¹³ Moreover, some rats injected with propofol demonstrated pain-like behavior (vocalization, flicking of the tail, withdrawal responses) whereas those with fospropofol did not. However, these were qualitative observations and were not quantifiable given the very short duration of the behavior. We performed further studies to evaluate whether propofol evokes nocifensive behavior upon application to the nasal mucosa or the cornea and were unable to observe any nocifensive responses to either drug in any assay. The data indicate that the clinical formulation of fospropofol is biologically active in rats and the lack of efficacy at the TRPA1 receptor is unrelated to species difference.

Discussion

In the present study we demonstrated that unlike propofol, fospropofol does not activate TRPA1 providing a mechanistic explanation for its lack of algogenic properties. However, both drugs were effective in inducing general anesthesia demonstrating biological activity. Moreover, *in vivo* studies confirmed pharmacokinetic properties of fospropofol observed in humans, i.e., higher dose requirement, slower onset and offset.

Injection site pain is a very undesirable side effect of propofol. Although not very common, thrombophlebitis or tissue inflammation upon infiltration is a more serious consequence of irritation evoked by propofol.^{14,15} Various measures are taken to reduce this side effect and

they include lidocaine pre or co-injection, slower rate of injection, use of large bore IV etc.⁴ However, most of these measures are at best partly effective and may not be possible in all patients (e.g., lidocaine allergy, difficult IV access). Multiple mechanisms have been proposed to explain the irritation caused by propofol and they include the emulsion formulation, vehicle used etc. However, an elegant study by Matta et al. demonstrated a receptor-specific mechanism via activation of TRPA1 as a more likely explanation, given that the vehicle itself did not evoke any pain-like behavior in animals and propofol-evoked pain was virtually absent in TRPA1 knockout mice.⁵ TRPA1-expressing nerve terminals surrounding the vessels are the likely target of propofol. Our study demonstrates that fospropofol lacks the TRPA1 agonist activity characteristic of propofol and very likely explains the lack of algogenic properties of fospropofol in humans.

More recent studies have demonstrated that propofol also activates other ion channels expressed on nociceptors such as TRPV1.¹⁰ However, propofol-evoked nociceptor excitation and pain-like behavior in animals was absent in TRPA1 knockout mice and blocked by a TRPA1 antagonist suggesting that the contribution of other ion channels in propofol-evoked pain is minimal. Moreover, it should be noted that propofol did not evoke current in TRPA1-negative neurons in the current study and fospropofol was unable to evoke any current in any of the TG neurons (both TRPA1 expressing and nonexpressing) that were tested. Therefore, it is unlikely that fospropofol activates any other ion channels in sensory neurons.

Although fospropofol does not evoke pain at the injection site, the prodrug nature mandates hepatic metabolism before bioactivity is observed and results in slower onset and offset, not a desirable property in an ideal induction/maintenance drug. There are various possibilities that may account for the difference in the algogenic properties of propofol and fospropofol. 1. Stereospecificity: Although the binding site of propofol on TRPA1 is not known, our study demonstrates that addition of the phos group in fospropofol may hinder that interaction. 2. Solubility: If the propofol binding site on TRPA1 is intracellular, it is possible that lipophilic propofol reaches the intracellular compartment more readily than the hydrophilic fospropofol. 3. A distant possibility is that fospropofol has antagonist or cross-desensitizing activity on TRPA1 via action of some other receptor expressed by sensory neurons. A moiety in propofol structure that is responsible for TRPA1 activity but not essential for gamma-aminobutyric acid A binding could be identified. This information is of potential use in molecular modeling studies where the structural difference between propofol and fospropofol could serve as a template to design propofol-like molecules that are not prodrugs but nonalgogenic as well.

Finally, although not a major focus of the study, we observed that the pharmacokinetic profile of fospropofol in rats is similar to that seen in humans. To the best of our knowledge, this is the first published report of characterization of fospropofol in a rodent model. Future studies could use this model to study the interaction of various drugs that can potentially affect *in vivo* efficacy of fospropofol (e.g., hepatic inducers/inhibitors).

In conclusion, we demonstrated that the lack of algogenic properties of fospropofol is likely due to its inability to activate TRPA1 or any other ion channels on sensory neurons. Moreover, the pharmacokinetic profile of fospropofol was established in a rodent model that can be used for future studies.

Acknowledgments

Funding: This study was funded by NIH R01 NS072204 to GD.

References

1. Picard P, Tramer MR. Prevention of pain on injection with propofol: a quantitative systematic review. *Anesth Analg*. 2000; 90:963–9. [PubMed: 10735808]
2. Paul M, Dueck M, Kampe S, Fruendt H, Kasper SM. Pharmacological characteristics and side effects of a new galenic formulation of propofol without soyabean oil. *Anaesthesia*. 2003; 58:1056–62. [PubMed: 14616590]
3. Moore GD, Walker AM, MacLaren R. Fospropofol: a new sedative-hypnotic agent for monitored anesthesia care. *Ann Pharmacother*. 2009; 43:1802–8. [PubMed: 19826098]
4. Scott RP, Saunders DA, Norman J. Propofol: clinical strategies for preventing the pain of injection. *Anaesthesia*. 1988; 43:492–4. [PubMed: 3261547]
5. Matta JA, Cornett PM, Miyares RL, Abe K, Sahibzada N, Ahern GP. General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. *Proc Natl Acad Sci U S A*. 2008; 105:8784–9. [PubMed: 18574153]
6. Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. *Nat Rev Drug Discov*. 2009; 8:55–68. [PubMed: 19116627]
7. Story GM, Gereau RWt. Numbing the senses: role of TRPA1 in mechanical and cold sensation. *Neuron*. 2006; 50:177–80. [PubMed: 16630827]
8. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature*. 2007; 445:541–5. [PubMed: 17237762]
9. Cavazzuti M, Porro CA, Barbieri A, Galetti A. Brain and spinal cord metabolic activity during propofol anaesthesia. *Br J Anaesth*. 1991; 66:490–5. [PubMed: 2025477]
10. Fischer MJ, Leffler A, Niedermirtl F, Kistner K, Eberhardt M, Reeh PW, Nau C. The general anesthetic propofol excites nociceptors by activating TRPV1 and TRPA1 rather than GABAA receptors. *J Biol Chem*. 2010; 285:34781–92. [PubMed: 20826794]
11. Zhang H, Wickley PJ, Sinha S, Bratz IN, Damron DS. Propofol restores transient receptor potential vanilloid receptor subtype-1 sensitivity via activation of transient receptor potential ankyrin receptor subtype-1 in sensory neurons. *Anesthesiology*. 2011; 114:1169–79. [PubMed: 21364461]
12. Li X, Zhang Y, Fan Y, Zhou Y, Wang X, Fan C, Liu Y, Zhang Q. Preparation and evaluation of novel mixed micelles as nanocarriers for intravenous delivery of propofol. *Nanoscale Res Lett*. 2011; 6:275. [PubMed: 21711808]
13. Cohen LB. Clinical trial: a dose-response study of fospropofol disodium for moderate sedation during colonoscopy. *Aliment Pharmacol Ther*. 2008; 27:597–608. [PubMed: 18194506]
14. Dubey PK, Kumar A. Vascular complication following lipid free propofol injection. *J Postgrad Med*. 2005; 51:73–4. [PubMed: 15793348]
15. Tokumine J, Sugahara K, Tomori T, Nagasawa Y, Takaesu Y, Hokama A. Tissue necrosis caused by extravasated propofol. *J Anesth*. 2002; 16:358–9. [PubMed: 14517633]

Fig. 1.

In total, 25 trigeminal ganglion (TG) neurons were recorded. Thirteen cells tested positive for transient receptor potential 1 (TRPA1) expression and 12 were observed to be TRPA1-negative. (A) A representative TRPA1-expressing TG neuron. Mustard oil (MO, 100 μ M), propofol (Prop, 100 μ M), fospropofol (Fosprop, 100 μ M and 1 mM) were randomly applied to the neuron for 2 min.

MO and propofol produced robust TRPA1-like inward currents in this cell. Fospropofol did not produce TRPA1-like current in the same cell. (B) A representative TRPA1-negative cell. Mustard oil (MO, 100 μ M), propofol (Prop, 100 μ M), fospropofol (Fosprop, 100 μ M and 1

mM) were applied to the neuron for 2 min. TRPA1-like current was not produced after random application of any of the four solutions.