Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation

M. Orth1, L. Barter1, C. Dominguez3, R. Atherley1, E. Carstens2 and J. F. Antognini12*

1Department of Anesthesiology and Pain Medicine and 2Section of Neurobiology, Physiology and Behavior, University of California, Davis, CA, USA. 3Department of Anesthesiology, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA

*Corresponding author: Department of Anesthesiology TB-170, University of California, Davis, CA 95616, USA. E-mail: jfantognini@ucdavis.edu

Background. Anaesthetics blunt neuronal responses to noxious stimulation, including effects on electroencephalographic (EEG) responses. It is unclear how anaesthetics differ in their ability to modulate noxious stimulation-evoked EEG activation. We investigated the actions of propofol and halothane on EEG responses to noxious stimuli, including repetitive electrical C-fibre stimulation, which normally evokes neuronal wind-up.

Methods. Rats were anaesthetized with halothane (n=8) or propofol (n=8), at 0.8× or 1.2× the amount required to produce immobility in response to tail clamping [minimum alveolar concentration (MAC) for halothane and median effective dose (ED50) for propofol]. We recorded EEG responses to repetitive electrical stimulus trains (delivered to the tail at 0.1, 1 and 3 Hz) as well as supramaximal noxious tail stimulation (clamp; 50 Hz electrical stimulus, each for 30 s).

Results. Under halothane anaesthesia, noxious stimuli evoked an EEG activation response manifested by increased spectral edge frequency (SEF) and median edge frequency (MEF). At 0.8 MAC halothane, the tail clamp increased the MEF from ≈25 to ≈28.5 Hz, and the SEF from ≈25.5 to ≈27 Hz. At both 0.8 and 1.2 MAC halothane, similar patterns of EEG activation were observed with the 1 Hz, 3 Hz and tetanic stimulus trains, but not with 0.1 Hz stimulation, which does not evoke wind-up. Under propofol anaesthesia, noxious stimuli were generally ineffective in causing EEG activation. At 0.8 ED50 propofol, only the tail clamp and 1 Hz stimuli increased MEF (≈8 to ≈10–10.5 Hz). At the higher propofol infusion rate (1.2 ED50) the repetitive electrical stimuli did not evoke an EEG response, but the tetanic stimulus and the tail clamp paradoxically decreased SEF (from ≈23 to ≈21.5 Hz).

Conclusions. Propofol has a more significant blunting effect on EEG responses to noxious stimulation compared with halothane.

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Furthermore, halothane appears to have a major action in the spinal cord, at least for the production of immobility. Propofol, however, has a major effect in the brain to produce anaesthesia,\(^3\) and blunts EEG activation responses to noxious stimulation.\(^3\) These data\(^3\)–\(^8\) suggest that propofol and halothane might differ in terms of their ability to modulate EEG responses to noxious stimulation.

In the present study we examined the effects of halothane and propofol anaesthesia on the ability of supramaximal noxious stimulation to cause EEG activation. We included repetitive electrical C-fibre stimulation because it induces wind-up of nociceptive spinal neurons, a mechanism contributing to temporal summation of pain that is depressed by some anaesthetic agents.\(^9\)–\(^11\) We hypothesized that propofol would blunt EEG responses to supramaximal and repetitive noxious stimulation more than halothane.

**Methods**

The local animal care and use committee approved this study. Adult male Sprague–Dawley rats (\(n=500\) g) were anaesthetized in a chamber with either halothane (\(n=8\)) or isoflurane (\(n=8\)). The rats were removed and placed on mask anaesthesia and tracheostomy was performed to permit placement of a 12- or 14-gauge tracheostomy tube, followed by mechanical ventilation. A catheter was placed into the internal jugular vein for fluid and drug administration, and a catheter was placed into a carotid artery to measure blood pressure. Ligation of a carotid artery does not affect cerebral metabolism or blood flow.\(^12\) Mean arterial pressure was maintained at 75 mmHg or greater using an infusion of lactated Ringer’s solution. Rectal temperature was measured with a thermometer and maintained at \(\approx37–38^\circ\)C using a heating pad and heating lamp as needed. End-tidal anaesthetic concentration was determined using a calibrated anaesthetic agent analyser (Rascal, Ohmeda, Salt Lake City, UT, USA; or Datex 254, Helsinki, Finland).

In the halothane group, the minimum alveolar concentration (MAC) needed to prevent movement was determined in each individual rat. The halothane concentration was equilibrated at 0.9–1% and maintained for at least 15–20 min. A clamp was applied at the base of the tail and oscillated at 1–2 Hz for up to 1 min or until the rat displayed gross and purposeful movement. Depending on the initial response, the halothane concentration was increased or decreased by 0.2%, stabilized for 15 min, and the tail clamp was applied again. This process was continued until two halothane concentrations were found that just prevented and just permitted gross and purposeful movement. MAC was the average of these.

In one of the isoflurane-anaesthetized rats and in an additional five rats, we determined the propofol requirements to prevent movement in response to supramaximal noxious stimulation. In brief, the six rats were anaesthetized with isoflurane and ventilated via tracheostomy. A jugular catheter was inserted and a propofol infusion initiated at \(\approx400\) \(\mu\)g kg\(^{-1}\) min\(^{-1}\). The isoflurane was discontinued and after 30–45 min, when the expired isoflurane was \(<0.2\%\), a clamp was applied to the base of the tail and oscillated at 1–2 Hz for up to 1 min or until the rat displayed gross and purposeful movement. Depending on the response, the infusion rate was increased or decreased by 20%, and after 15–20 min the tail clamp was reapplied. This process was repeated until two infusion rates were found that just permitted and just prevented movement; the median effective dose (ED\(_{50}\)) for the infusion rate was the average of these rates. The average of these values for all six rats was used as the population ED\(_{50}\) for the propofol EEG studies.

The electroencephalogram was recorded via four stainless steel screws placed into the skull. Two screws were placed 0.5 cm from the midline on each side near lambda. One screw was placed near the midline in the frontal region while the fourth screw was placed near the base of the skull. Leads from an Aspect 1050 EEG machine (Aspect Medical Systems, Newton, MA, USA) were attached to the rats to record EEG responses. The EEG signals were digitized at 256 Hz and filtered at 2–70 Hz. The A-1050 monitor performed a power analysis to generate the median edge frequency (MEF) and spectral edge frequency (SEF), which are the frequencies below 50 and 95% of the EEG power, respectively. MEF and SEF were downloaded every 5 s to a computer. The EEG monitor used a rolling average of the previous 30 s when generating these numbers. In addition, the raw EEG was recorded onto a computer hard drive using Chart5 (AD Instruments, Colorado Springs, CO, USA).

In the halothane rats, the halothane concentration was stabilized at 0.8 or 1.2 MAC for 15–20 min before application of noxious stimuli. The animals anaesthetized with isoflurane were administered propofol via infusion, starting at 0.8 or 1.2 \(ED_{50}\) (\(\approx480\) \(\mu\)g kg\(^{-1}\) min\(^{-1}\) or \(\approx720\) \(\mu\)g kg\(^{-1}\) min\(^{-1}\)), and the isoflurane was discontinued. We waited at least 30–45 min to permit the expired isoflurane concentration to decrease to less than 0.2% before beginning the noxious stimulation.

Noxious stimuli were applied in the following manner. After two needle electrodes (E-2; Grass Instruments, West Warwick, RI, USA) had been inserted into the skin at the base of the tail, trains of 20 C-fibre strength electrical stimuli (40 V, 0.5 ms pulse duration) were delivered at 0.1, 1 and 3 Hz, with 3–4 min between each train. In addition, two supramaximal stimuli were used: a tetanic stimulus (50 Hz, 60 mA current passed via the electrodes) and a tail clamp, each applied for 30 s. Pancuronium (0.2–0.3 mg kg\(^{-1}\) every 1–2 h) was administered intravenously to eliminate electromyographic artefacts. Once EEG responses had been recorded at one anaesthetic concentration (or propofol infusion rate), the anaesthesia was switched to the other concentration (or infusion rate) and stabilized for 15–20 min, and the noxious stimuli were applied as described above. The order in which the anaesthetic concentrations were
administered was alternated between experiments. When data collection was complete, the animals were euthanized with additional anaesthesia and i.v. potassium chloride.

The MEF and SEF data were evaluated using repeated measures analysis of variance for the 30 s period before stimulation and the 200 s period after initiation of stimulation. Post hoc testing was performed using the Student–Newman–Keuls test. Baseline MEF and SEF at 0.8 MAC (average of 30 s before stimulation) were compared with the respective values at 1.2 MAC using a paired t-test when comparing within an anaesthetic or an unpaired t-test when comparing between anaesthetics. *P*<0.05 was considered significant.

**Results**

Halothane MAC was 1.0 (0.1)% and propofol ED₅₀ was 600 (130) μg kg⁻¹ min⁻¹. During halothane anaesthesia, the prestimulus SEF decreased [from 24.3 (2.2) to 21.8 (2.7) Hz, *P*<0.01], as did MEF [from 5.8 (0.9) to 4.5 (1.0) Hz, *P*<0.002], in the transition from 0.8 to 1.2 MAC. During propofol anaesthesia, prestimulus MEF decreased [from 9.0 (1.3) to 7.8 (1.0) Hz, *P*<0.05] in the transition from 0.8 to 1.2 ED₅₀, while the SEF was unchanged [23.2 (1.5) to 23.3 (1.3) Hz, *P*>0.05]. Prestimulus MEF values at 0.8 and 1.2 MAC for halothane were significantly different from the MEF values for propofol at 0.8 and 1.2 ED₅₀, respectively (*P*<0.001). Prestimulus SEF values for halothane were not significantly different from the SEF values for propofol.

Figure 1 shows individual examples of the electroencephalogram under propofol anaesthesia. At 0.8 ED₅₀ propofol (Fig. 1A), both repetitive electrical stimulation (upper trace) and the noxious tail clamp (lower trace) produced limited activation responses in the EEG. At 1.2 ED₅₀ propofol, the same noxious stimuli did not evoke EEG changes (Fig. 1B). The EEG pattern during propofol anaesthesia included large spikes (Fig. 1C). Data are summarized in Fig. 2, where filled symbols represent EEG responses at 0.8 ED₅₀ propofol and the open symbols 1.2 ED₅₀ propofol. At 0.8 ED₅₀, significant EEG activation in the MEF occurred for the 1 Hz stimulus train (Fig. 2A) and the tail clamp (Fig. 2E). At 1.2 ED₅₀ propofol, none of the noxious stimuli resulted in EEG activation; in fact, the tetanic electrical stimulus and the tail clamp evoked a paradoxical decrease in SEF (Figs 2D and E).

In contrast to the depressant effect of propofol, halothane had very little effect on EEG activation evoked by noxious stimulation. Figure 3 shows examples of EEG traces at 1.2 MAC halothane. Electrical stimulation at 3 Hz produced EEG activation (upper trace in Fig. 3), and the supramaximal tail clamp (middle trace) and tetanic stimulus (lower trace in Fig. 3) evoked EEG spikes (Fig. 3C).
Fig 2 Summary data (mean, SD) for propofol at 0.8 and 1.2 effective dose (ED$_{50}$) showing the median edge frequency (MEF) and spectral edge frequency (SEF) for 20 electrical stimuli applied at (a) 0.1 Hz, (b) 1 Hz and (c) 3 Hz, as well as responses to tetanic electrical stimulus (d) and (e) tail clamp (each 30 s). Data points are plotted every 5 s. The stimuli were applied at the 50 s time point (arrow). The duration of each stimulus is noted by the black bar to the right of each arrow. Significance markers (*) show the SEF and MEF values after initiation of each stimulus that are significantly different from pre-stimulus (control) values. In contrast to the results with halothane, few significant changes occurred: the MEF increased with the 1 Hz stimulus and tail clamp stimulus at 0.8 ED$_{50}$; there was a paradoxical decrease in the SEF at 1.2 ED$_{50}$ when the tetanic electrical stimulus and the tail clamp were applied (d and e). For clarity, not all significant changes are shown, e.g. differences between the peak electroencephalographic change and the recovery values after stimulus. n=8.
1.2 MAC or ED50) and cannot comment on any possible unchanged, while we observed a slight decrease in MEF (Fig. 4B).

The main finding of the present study is that propofol, given at infusion rates bracketing the ED50 for movement in the greater propofol dose. Some studies have described EEG activation after propofol administration in low doses associated with the transition from consciousness to unconsciousness. Halothane also appears to cause EEG activation, followed by depression. Unlike other more commonly used volatile anaesthetics, such as isoflurane, halothane will usually induce burst suppression only at concentrations that exceed the clinically relevant range.

Interestingly, at 1.2 ED50 for propofol, we observed a paradoxical decrease in the SEF during application of the tetanic stimulus and the tail clamp. The mechanism by which this occurs is unknown, but has been reported before with noxious stimuli applied during isoflurane anaesthesia. In the present study with halothane, the 1 and 3 Hz stimuli increased SEF and MEF in a manner similar to those occurring with the tail clamp and electrical tetanic stimulus. The 0.1 Hz stimulus does not normally cause neuronal wind-up and did not evoke significant EEG changes, probably reflecting much lower temporal summation compared with the 1 Hz, 3 Hz tail clamp and tetanic stimuli.

While many previous studies have reported anaesthetic effects on spontaneous EEG activity, few studies have examined the effect of noxious stimulation on cerebral activity and the electroencephalogram during anaesthesia. In general, when clinically relevant concentrations of anaesthetic are administered, noxious stimulation causes EEG activation, although less EEG activation occurs during propofol anaesthesia. We found that propofol blunted EEG activation resulting from noxious stimulation.

Hofbauer and colleagues investigated in humans the relationship between propofol administration, subjective pain ratings and cerebral activation, as determined by positron emission tomography. When propofol was infused at doses that caused mild sedation, pain ratings of noxious heat increased, as did neural activity in the anterior cingulate cortex and thalamus. As the propofol dose was increased, the pain rating decreased, as did the neural responses; however, even when unconsciousness occurred, noxious heat evoked increased activity in the cingulate cortex and thalamus. Greater propofol concentrations, however, are associated with blunted EEG responses to noxious stimulation, suggesting that this depressant effect occurs between propofol concentrations that produce unconsciousness and those needed to produce immobility.

Although the exact mechanisms by which propofol and halothane produce anaesthesia are unclear, emerging evidence suggests that action at specific ligand-gated receptors might be critically involved. Propofol acts at the GABA_A receptor to enhance the effect of GABA. A mutation in the β3 subunit of the GABA_A receptor renders mice resistant to propofol, but only slightly increases halothane requirements. Halothane probably acts at several receptors, including GABA_A, glycine and NMDA receptors. Furthermore, propofol and halothane might have different modes of action with respect to sites within the central nervous system. The sedative effect of propofol probably occurs...
Fig 4 Summary data (mean, SD) for halothane at 0.8 and 1.2 minimum alveolar concentration (MAC). Median edge frequency (MEF) and spectral edge frequency (SEF) for twenty electrical stimuli applied at (A) 0.1 Hz, (B) 1 Hz and (C) 3 Hz, and responses to the (D) tetanic electrical stimulus and (E) tail clamp (each 30 s) are shown. Data points are plotted every 5 s. The stimuli were applied at the 50 s time point (arrow). The duration of each stimulus is noted by the black bar to the right of each arrow. Significance markers (*) show SEF and MEF values after initiation of each stimulus that are significantly different from prestimulus (control) values. The SEF and MEF increased for almost all of the stimuli, except for the 0.1 Hz stimulus and the MEF for the 1 Hz stimulus at 0.8 MAC, although in the latter example the MEF trended to increase. For clarity, not all significant changes are shown; e.g. differences between the peak electroencephalographic change and the recovery values after stimulus. n=8.
by actions at discrete supraspinal sites, including a sleep-promoting pathway in the tuberomamillary nucleus.\(^8\) In addition, propofol and halothane may act in the septohippocampal system to induce anaesthesia.\(^{25}\) Halothane appears to have an action in the spinal cord to ablate movement that occurs in response to noxious stimulation.\(^7\) Furthermore, propofol and volatile anaesthetics such as isoflurane can suppress nociception in the spinal cord and thereby affect EEG responses to noxious stimulation.\(^2\) Thus, in the present study, propofol and halothane could have acted in the brain directly to suppress the EEG response, and in the spinal cord to indirectly suppress the response.

The lower propofol infusion rate may have already surpassed the infusion rate needed to blunt the EEG responses. If so, this was not because of excessive EEG depression. We used the same fractions (0.8 and 1.2) of the amount needed to produce immobility, and our values for halothane MAC and propofol ED\(_{50}\) are similar to those previously published.\(^{27,28}\) We found that SEF and MEF during propofol anaesthesia were greater than those during halothane anaesthesia. At the greater propofol infusion rate (720 \(\mu g\) kg\(^{-1}\) min\(^{-1}\)), the MEF was \(\approx\) 8 Hz. Tzabazis and colleagues\(^{29}\) used a modified MEF that incorporated the occurrence of spikes and burst suppression; these authors maintained a modified MEF of 3 Hz by infusing propofol at a dose similar to the higher dose used in the present study (730 (200) \(\mu g\) kg\(^{-1}\) min\(^{-1}\)). Although we did observe spikes in the EEG during propofol infusion, we did not routinely observe burst suppression. Antunes and colleagues\(^{14}\) observed burst suppression at infusion rates greater than those used in our study (1000 \(\mu g\) kg\(^{-1}\) min\(^{-1}\)). Because of the modified MEF used by Antunes and colleagues, it is difficult to make a direct comparison with our data. Nonetheless, we believe that excessive EEG depression does not explain the stronger blunting effect of propofol on EEG activation by noxious stimuli.

In summary, we found that propofol, in a dose range that prevents movement, caused significant depression of EEG responses to noxious electrical and mechanical stimulation, while halothane did not.

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References

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