Effects of propofol and sevoflurane on the excitability of rat spinal motoneurones and nociceptive reflexes in vitro

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Background. Spinal actions of halogenated ethers are widely recognized, whereas spinal actions of intravenous anaesthetics like propofol are less clear. The aim of this study was to compare the spinal effects of propofol and sevoflurane.

Methods. We used an isolated spinal cord in vitro preparation from rat pups and superfused the anaesthetics at known concentrations. Responses of motoneurones to single and repetitive C-fibre intensity stimulation (trains of 20 stimuli at 1 Hz) of a lumbar dorsal root were recorded from the corresponding ventral root via a suction electrode.

Results. Stimulation trains produced a wind-up of action potentials in motoneurones. Both propofol and sevoflurane produced a significant concentration-dependent depression of the evoked wind-up, although at clinically relevant concentrations sevoflurane exhibited a larger intrinsic efficacy. Applied at anaesthetic concentrations, sevoflurane 250 μM abolished action potentials whereas propofol 1 μM only produced a reduction close to 50%. At these concentrations, sevoflurane produced a large depressant effect on the monosynaptic reflex whereas propofol was ineffective.

Conclusions. Sevoflurane produces large inhibitory effects on nociceptive and non-nociceptive reflexes which are likely to contribute to immobility during surgery. Compared with sevoflurane, propofol appears to have much weaker effects on spinal reflexes such as those recorded in an isolated preparation.

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provide additional information on the spinal action of these compounds. Preliminary results have been presented in abstract form.12

Materials and methods
All experimental procedures were performed according to European Union and Spanish Government regulations and were supervised and approved by the University Animal Care Facility.

Preparation of the in vitro hemisected spinal cord
Experiments were performed in hemisected spinal cords from newborn (7–10-day-old) Wistar rats. Rats were anaesthetized with intraperitoneal urethane (2 g kg\(^{-1}\)). The spinal cord was rapidly extracted following a dorsal laminectomy and handled according to a standard procedure.13 The hemisected spinal cord was placed in a recording chamber where it was continuously superfused at 4–5 ml min\(^{-1}\) with aerated (\(O_2/CO_2\), 95:5%; carbogen) artificial cerebrospinal fluid (ACSF) at pH 7.4 and room temperature (23 (SD 1) °C). The composition of the ACSF was NaCl (128 mM), KCl (1.9 mM), KH\(_2\)PO\(_4\) (1.2 mM), MgSO\(_4\) (1.3 mM), CaCl\(_2\) (2.4 mM), NaHCO\(_3\) (26 mM) and glucose (10 mM). A period of 60 min was allowed for the preparation to stabilize before testing spinal reflexes.

Stimulation and recording conditions
The L4 or L5 lumbar dorsal root and the corresponding ventral root were placed on tight-fitting glass suction electrodes. Responses to electrical stimulation of the lumbar dorsal root (L4–5) were recorded extracellularly from the corresponding ventral root via a suction electrode. The signal was then split and amplified with two separated AC–DC amplifiers (Digitimer Ltd, UK) set to AC and DC modes respectively, digitized at 3.3 kHz and stored for off-line computer-aided analysis using CED hardware and software (Cambridge Electronic Design Ltd; Cambridge, UK).

Spinal reflexes were elicited with electrical high intensity dorsal root stimuli (200 \(\mu\)s and 300 \(\mu\)A), previously shown to be about three times the threshold for C-fibre stimulation.14 The stimulation pattern consisted of three simple stimuli applied at 45 s intervals followed by a train of 20 stimuli at a frequency of 1 Hz. Test stimuli were repeated at 30 min intervals and at least three tests were performed prior to drug perfusion.

Quantification of electrophysiological recordings
The AC channel recorded fast events (\(\approx 2\) ms) of variable amplitude originated by the firing of action potentials at motoneurones.11 A single stimulus produced motoneurone firing in only a few cases. In contrast, repetitive dorsal root stimulation produced a typical wind-up effect in the majority of experiments. Activity in this channel was quantified as (i) the integrated area of the response and (ii) the number of spikes crossing a threshold.11 Both measurements were performed on a time window consistent with C-fibre-mediated responses (between 100 and 990 ms from stimulus artefact) and responses to each one of the 20 stimuli were summed.

The DC channel recorded slow motoneurone depolarizations reflecting the compound excitatory postsynaptic potentials (EPSPs) generated by synaptic activation. Single dorsal root stimulation produced a short latency monosynaptic reflex (MSR) which is related to the myotatic reflex and due to activation of rapidly conducting A\(\alpha\) fibres. This is followed by a slow ventral root potential (VRP) which is caused by the activation of A\(\beta\) and C fibres and polysynaptic circuits. Repetitive stimulation of the dorsal root produces a cumulative depolarization (CD) or “subthreshold wind-up”14 due to summation of successive VRPs which appears only if C fibres are activated. Activity in this channel was quantified as follows. The mean amplitude of the MSR was taken from three consecutive responses to single stimuli. The integrated area of the CD was measured with a cut-off at 24 s from first stimulus artefact.14

Drug administration
Propofol (Sigma Chemical Co., St Louis, MO, USA) was administered dissolved in ACSF for a period of 30 min at 0.4, 1, 5 and 10 \(\mu\)M. The effective concentration 50% (\(EC_{50}\)) of propofol in clinical anaesthesia ranges from 0.4 to 1 \(\mu\)M.15 In most cases, up to three concentrations were applied to a preparation in a cumulative fashion since pilot experiments had shown that recovery after propofol is extremely slow.

Sevoflurane (Abbott Laboratories, Kent, UK) was vaporized into carbogen at known volume percentages (0.25%, 0.75%, 2% and 4%) via a specific vaporizer (Quick Fil\textsuperscript{TM} Draeger Vapor\textsuperscript{TM} 19.1 n). The gas mixture was bubbled into an ACSF-containing reservoir. According to the pilot experiments, sevoflurane-containing ACSF was superfused to the preparation for 30-min periods in order to allow for equilibration. The concentration of sevoflurane in the ACSF at the recording chamber was assessed by gas chromatography using a Hewlett-Packard (HP-5890 series II) gas chromatograph with a procedure previously described.5 A linear correlation was found between vaporizer readings and molar concentrations of sevoflurane in ACSF (\(r^2=0.995\)) (Fig. 1). The sevoflurane \(EC_{50}\) equivalent to 1 MAC in clinical anaesthesia ranges from 280 \(\mu\)M to 350 \(\mu\)M.17

Data management
For each variable considered, the difference between the value found during propofol or sevoflurane administration and control was analysed. Differences obtained for a range of propofol and sevoflurane concentrations were analysed by
means of one-way ANOVA with Dunnett’s post-hoc tests (Graph-Pad Prism 3.0, Graph-Pad Software, USA). Two-way ANOVA was used to compare pairs of curves (GB-Stat, Dynamic Microsystems Inc., USA). All statistical analyses were run on raw data. Data are represented in the figures as mean (SEM) percentage of control values.

**Results**

A total of 22 hemisected spinal cord preparations were used for the study. All cords showed low-frequency DC spontaneous activity (typical values were frequency 0.3–0.5 Hz, duration 100–120 ms and amplitude 0.05–0.07 mV), but spontaneous spikes reflecting the firing of action potentials (recorded in the AC channel) were rare. Most preparations (n=15) showed stable responses to repetitive dorsal root stimulation, but seven failed to fire action potentials or showed a progressive loss of response during control testing, and were discarded from statistical analysis.

**Effect of the anaesthetics on C-fibre-mediated ‘wind-up’**

The total number of spike counts to the train of stimuli was 808 (SEM 164) (range 236–2435, n=15), and the integrated area under the AC signal was 43.9 (11.1) μV·s (range 15.5–173 μV·s, n=15). The frequency of spikes detected increased towards the final stimulus of the train producing the typical ‘wind-up’ effect (initial number of spikes 12 (8) versus final number 62 (32), n=15). The mean integrated area of CD was 16.8 (3.9) mV·s (range 7.1–37.1 mV·s, n=15). Original simultaneous recordings of these responses are shown in Figure 2.

Propofol showed a small but long-lasting depressant effect on motoneurone wind-up (Fig. 2A). In contrast, sevoflurane produced a strong and reversible depression of wind-up (Fig. 2B). Quantitative effects of both anaesthetics on the integrated area of the AC (action potential firing) and DC (cumulative depolarization) responses are shown in Figure 3. Three to six observations per data point were pooled for the analysis. Overall, ANOVA analysis showed that both propofol and sevoflurane produced a concentration-dependent effect on these variables (P<0.0001 for both drugs and variables). The effect of propofol on action potential wind-up was significant for concentrations >1 μM, whereas significant reductions of the underlying CD were only found for concentrations of propofol >5 μM. At 1 μM propofol reduced the number of spikes from 650.2 (169.7) to 374 (165.3) (n=6; P<0.01), and the integrated area of the CD from 9.6 (1.5) to 9.3 (1.4) mV·s (n=6; P>0.05). At the largest concentration used (10 μM), propofol reduced the number of spikes from 650.2 (169.7) to 95.5 (96) (n=6; P<0.01), and the integrated area of the CD from 9.6 (1.5) to 6.4 (1.2) mV·s (n=6; P<0.01).

Sevoflurane caused a significant depression of both the action potential firing and the underlying CD from low (subanaesthetic) concentrations (Fig. 3B). When applied at ~250 μM (2 vol%), sevoflurane essentially abolished spike firing (from 914.3 (259.2) to 17.2 (10.8); P<0.01) and the underlying CD (from 12 (1.8) to 2.8 (0.7) mV·s; P<0.01). These effects were very close to the maximal effects attained with sevoflurane applied at ~450 μM (4 vol%).

When compared using two-way ANOVA, the effects of sevoflurane on action potential wind-up and the CD were significantly larger from those of propofol at subanaesthetic concentrations (~30 μM or 0.25 vol%) than that produced by 0.4 μM propofol (P<0.01) and similar
to that of 1 μM propofol (not significant). Similarly, sevoflurane applied at ~100 μM (0.75 vol%) produced a greater effect on the CD than propofol applied at maximal concentration (10 μM) (P<0.01).

**Discussion**

The experimental arrangement used in the present experiments allowed quantification of drug effects on the monosynaptic reflex and on polysynaptic nociceptive reflexes, both of which are involved in the generation of movement during surgery. The results presented here constitute a direct comparison between the effects of two classes of anaesthetics using the same model and experimental conditions. Our results show that both propofol and sevoflurane alter spinal processing of afferent inputs but detect qualitative and quantitative differences between their effects. These differences are probably related to the mechanisms of action and the clinical effects of each compound.

Propofol and sevoflurane reduced motoneurone action potential wind-up in response to repetitive activation of nociceptive afferents, and these effects were concentration dependent. However, when compared at concentrations close to their reported EC₅₀, sevoflurane shows a much stronger inhibitory effect than propofol. The anaesthetic EC₅₀ values reported for propofol are within the range 0.4–1 μM¹⁵ and those of sevoflurane are within the range 280 μM¹⁶ to 350 μM.¹⁷ Within these concentration ranges, propofol reduced motoneurone action potential firing to about 50% of control, whereas sevoflurane produced an almost complete abolition of response. These differences were statistically significant when compared by two way ANOVA. In addition to this, sevoflurane reduced the monosynaptic reflex whereas propofol, if anything, showed a non-significant tendency to potentiate this reflex. Collectively, these results suggest that sevoflurane should show a greater immobilizing effect during anaesthesia than propofol. This is consistent with clinical observations showing that sevoflurane administered at 1 MAC requires very little neuromuscular blockade to maintain immobility during surgery.¹⁸ ¹⁹ In apparent contradiction to this view, a recent experimental report has shown that the F wave, an indicator of motoneurone excitability, is reduced in a similar way by subanaesthetic concentrations of propofol and sevoflurane in humans.⁹ It is possible that this latter effect was due to suppression of the descending facilitating signals rather than to a direct action of propofol on motoneurones.

It is interesting to note that the cumulative depolarization which underlies action potential firing is also reduced by both anaesthetics to different extents (the reduction caused by sevoflurane is stronger than that produced by propofol). This subthreshold signal is generated by the summation of compound excitatory and inhibitory postsynaptic potentials.
(EPSPs and IPSPs) at motoneurones upon arrival of sensory inputs through polysynaptic circuits. Therefore the power of this signal depends strongly on the correct transmission of nociceptive inputs through each of the neurons and synapses comprising the polysynaptic circuit. Since a number of these interneurones are likely to redirect information towards supraspinal centres, the cumulative area of depolarization has been used to estimate the potential analgesic effect of compounds.20 21 According to this view, it would be expected that sevoflurane had a greater analgesic action than propofol at the spinal level. Again in agreement with this, clinical evidence has been obtained showing that sevoflurane reduces the haemodynamic response to nociceptive stimulation during surgery22 and potentiates opioid analgesia. 23 In vivo experiments indicate that propofol has analgesic actions and can depress spinal sensitization,24 although a supraspinal descending inhibitory action cannot be ruled out in these experiments.

The details of the inhibitory actions of these compounds can be explained on the basis of their cellular mechanisms. The action of propofol has been shown to rely on the potentiation of GABA<sub>A</sub>-receptor-mediated responses.15 25 Sevoflurane, in addition to potentiation of the GABA<sub>A</sub> receptor function,26 may modulate some K<sup>+</sup> conductances27 and depresses responses mediated by NMDA and AMPA receptors<sup>5</sup> which are key elements in the spinal transmission of nociceptive and non-nociceptive signals.13

In conclusion, we have shown that sevoflurane has a powerful and concentration-dependent inhibitory effect on spinal nociceptive and non-nociceptive transmission. This effect is detectable at subanaesthetic concentrations and becomes maximal at anaesthetic concentrations. In contrast, propofol shows an appreciably lower inhibitory effect on spinal nociceptive transmission which only emerges at anaesthetic concentrations. Our in vitro model supports a greater effect of sevoflurane than propofol at the level of the spinal cord.

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