Changes in axonal excitability of primary sensory afferents with general anaesthesia in humans

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Key points

- Intraoperative neuromonitoring can be affected by specific general anaesthetics.
- Changes in axonal excitability of primary sensory afferents with propofol and sevoflurane were investigated using threshold tracking technique.
- Both anaesthetics similarly affected nerve excitability to a small but significant extent.
- Possible mechanisms include direct anaesthetic effects on neuronal excitability and temperature change due to vasodilation.

Background. Intraoperative monitoring of neuronal function is important in a variety of surgeries. The type of general anaesthetic used can affect the interpretation and quality of such recordings. Although the principal effects of general anaesthetics are synaptically mediated, the extent to which they affect excitability of the peripheral afferent nervous system is unclear.

Methods. Forty subjects were randomized in a stratified manner into two groups, anaesthetized with either propofol or sevoflurane. The threshold tracking technique (QTRAC®) was used to measure nerve excitability parameters of the sensory action potential of the median nerve before and after induction of general anaesthesia.

Results. Several parameters of peripheral sensory afferent nerve excitability changed after induction of general anaesthesia, which were similar for both propofol and sevoflurane. The maximum amplitude of the sensory nerve action potential decreased in both groups (propofol: 25.3%; sevoflurane: 29.5%; both P<0.01). The relative refractory period [mean (SD)] also decreased similarly in both groups [propofol: −0.6 (0.7) ms; sevoflurane: −0.3 (0.5) ms; both P<0.01]. Skin temperature at the stimulation site increased significantly in both groups [propofol: +1.2 (1.0) °C; sevoflurane: +1.7 (1.4) °C; both P<0.01].

Conclusions. Small changes in excitability of primary sensory afferents after the induction of anaesthesia with propofol or sevoflurane were detected. These effects, which were non-specific and are possibly explained by changes observed in temperature, demonstrate possible anaesthetic effects on intraoperative neuromonitoring.

Keywords: anaesthetics i.v., propofol; anaesthetics volatile, sevoflurane; ions, ion channels, nerve, membrane; nerve, transmission

Accepted for publication: 28 June 2010

The aims of this investigation were to test whether propofol and sevoflurane have different effects on axonal excitability of peripheral sensory afferents after induction of general anaesthesia and how sensory nerve monitoring might be influenced during anaesthesia.

We used the technique of threshold tracking, a diagnostic tool which assesses axonal nerve excitability of myelinated peripheral nerves in a non-invasive manner. In contrast to conventional nerve conduction studies, threshold tracking uses subthreshold currents to provide information about nerve excitability and ion channel function.

Methods

Study subjects and randomization

Study subjects. The study was performed at the University Hospital Zurich after approval by the local ethics committee.
Assessed for eligibility (n=502)

Excluded (n=462)
- Not meeting inclusion criteria (n=434)
- Refused to participate (n=21)
- Other reasons (n=7)

Enrolment

Stratified randomization

Allocated to PROPOFOL group (n=20)
- Received allocated intervention (n=20)
- Did not receive allocated intervention (n=0)

Allocated to SEVOFLURANE group (n=20)
- Received allocated intervention (n=20)
- Did not receive allocated intervention (n=0)

Analysis

Analysed (n=20)

Excluded from analysis (n=0)

Discontinued intervention (n=0)

Fig 1 Enrolment and randomization of subjects recruited to the study.

General anaesthesia changes nerve excitability

(University Hospital Zurich, StV. 5-2008) in accordance with the Declaration of Helsinki 2008 (registration number NCT00696254, www.clinicaltrials.gov). All study subjects gave written informed consent after careful instructions concerning the study details. Specifically, they agreed on the omission of premedication with a tranquilizer to exclude as many unknown influencing factors as possible. Inclusion criteria were: German-speaking patients undergoing surgery under general anaesthesia; age 18–70 yr; weight 50–100 kg; and signed informed consent. Exclusion criteria were: known peripheral neuropathy; diabetes mellitus; neuro-psychiatric diagnosis; pregnant/breast-feeding women; congestive heart disease; more than two out of four risk factors for postoperative nausea and vomiting (PONV): female, non-smoker, known PONV, and planned opioids after operation; participation in other studies; and inability of verbal expression. Forty subjects were randomized in a stratified manner into two equally sized groups of 20 subjects (Fig. 1). Induction was performed either with propofol or sevoflurane.

Threshold tracking

We used a computer-assisted threshold-tracking program (QTRAC, © Institute of Neurology, London, UK) to investigate the excitability parameters of myelinated axons and ion conductances in the median nerve. This technique provides different information than conventional nerve conduction studies which use supramaximal stimuli and provide information about conduction velocity and amplitude. The threshold-tracking technique uses subthreshold
Excitability measurements during anaesthesia induction in patients

The study was performed in the anaesthesia induction room. After completion of the first nerve excitability measurement immediately before induction, anaesthesia was administered exclusively with propofol or sevoflurane. No local anaesthetics, neuromuscular blocking agents, or opioids were used until the second measurement was completed. Pulse oximetry (Draeger Infinity Delta Systems, Draeger Medical Systems, Danvers, MA, USA) was measured using a probe attached on a finger on the same arm used to measure nerve excitability. Subjects were monitored with ECG, non-invasive arterial pressure, and end-tidal gas analysis using a KION anaesthesia workstation (KION workstation and Maquet SC 7000 screen, Maquet-Siemens, Rastatt, Germany). During the first measurement, baseline end-tidal CO₂ values were determined during quiet spontaneous respiration using a tight face mask. In order to reach a comparable depth of anaesthesia in both groups we aimed at a stable bispectral index (BIS) value below 40 (BIS QuatroTM sensors with Infinity® BISx Pod for Draeger; Software version 1.03, Aspect Medical Systems, Inc., Norwood, MA, USA). According to this parameter, we varied the target concentration of the propofol infusion (using a software-controlled infusion pump with the pharmacological model of Schnider and colleagues17 programmed to plasma concentration of propofol, constant calculated plasma concentration of propofol, or difference in inspired and expired concentration of sevoflurane <0.3%.

The second nerve excitability measurement was then performed at steady state. After the second measurement was recorded, the study ended. Standard anaesthesia techniques were carried out by the addition of a neuromuscular blocking agent, opioid, and benzodiazepine where indicated. The anaesthesia was continued according to institutional standards for the respective procedures.

Statistical analysis

All values are given as mean and standard deviation (SD) and in Supplementary Figures S2 and S3 where standard errors of the mean (SEM) were used to visualize the significance between groups. To analyse data we used the software QtracP (Version 3/4/2009, © Institute of Neurology, London, UK). Data were tested for a normal distribution with the Lilliefors test for normality. Gender was compared between groups using Fisher’s exact test. Data before and after the induction of anaesthesia within the same group were analysed with a paired t-test. To compare excitability changes between the two groups after the induction of anaesthesia, we used an unpaired t-test. A Bonferroni correction was performed to address multiple comparisons for the same variable. Thus, comparisons before and after the induction of anaesthesia within the same group and between groups after intervention were considered significant if \( P<0.013 \).

Results

Changes of general parameters during anaesthesia

We enrolled 17 female and 23 male subjects (Fig. 1), all of whom completed the study. The characteristic data of the studied population before starting anaesthesia are shown in Table 1. After inducing anaesthesia, a comparable anaesthesia depth was achieved during the second measurement in both groups (propofol: BIS 25 (8); sevoflurane: 27 (10); \( P=0.42 \). The calculated plasma concentration of propofol during the second measurement was 6.6 (1.3) \( \mu g \) ml\(^{-1}\). The end-expiratory sevoflurane concentration was 5.5 (1.3%). The concentration of end-expiratory CO₂ during and after the induction of anaesthesia was not significantly higher than the value during spontaneous breathing before

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristic data. Values are presented as number (n) or as mean (range) or mean (sd). There were no significant patient characteristic differences between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Propofol</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43 (18–66)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 (10)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177 (7)</td>
</tr>
<tr>
<td>Female/male</td>
<td>6/14</td>
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anaesthesia induction [propofol spontaneous: 4.5 (0.5) kPa; anaesthetized: 4.6 (0.5) kPa, P=0.09; sevoflurane spontaneous: 4.6 (0.4) kPa; anaesthetized: 4.6 (0.4) kPa, P=0.72]. There was no difference in the end-expiratory CO₂ levels between the two groups before (P=0.59) or after the induction of anaesthesia (P=0.84) (Fig. 2A). Skin temperature at the stimulation site increased significantly in both groups [propofol before: 32.5 (0.9) °C; after: 33.7 (1.1) °C, P<0.01; sevoflurane before: 32.4 (1.2) °C; after: 34.1 (1.0) °C, P<0.01]. No difference existed between the groups after the induction of anaesthesia (P=0.17) (Fig. 2C). Mean arterial pressure decreased significantly in both groups [propofol before: 101 (13) mm Hg; after: 81 (12) mm Hg, P<0.01; sevoflurane before: 101 (14) mm Hg; after: 78 (12) mm Hg, P<0.01] but was similar when compared between the groups (P=0.55) after the induction (Fig. 2D). Arterial pressure stabilized after reaching equilibrium of anaesthetic depth, end-expiratory CO₂ concentration, stable BIS value, and stable SO₂. We did not need to take any pharmacological or other measures in any of the subjects to stabilize arterial pressure.

Changes in excitability parameters during anaesthesia

A summary of the excitability changes during the anaesthesia induction is given in Tables 2 and 3. We observed significant changes in only three excitability parameters after the induction of anaesthesia.

First, the maximum amplitude of the SNAP decreased in both groups significantly [before propofol: 39.9 (5.8) μV; after: 29.8 (4.4) μV, P<0.01; before sevoflurane: 47.1 (7.8) μV; after: 33.2 (5.3) μV, P<0.01], which corresponds to a decrease of 25.3% and 29.5%, respectively (Fig. 3). No difference was observed between the two groups after the induction of anaesthesia (P=0.44). As a result, the stimulus–response curve

Fig 2 Ventilation parameters and temperature during anaesthesia induction. (a) End-expiratory CO₂ remained stable after the induction of anaesthesia. We therefore assume that tissue pH was unaffected as well. (b) Peripheral oxygen saturation—measured with pulse oximetry on the arm where the recording was made—did not change after induction, indicating that no tissue ischaemia occurred at the stimulation and recording sites. (c) The temperature at the stimulation site increased similarly and significantly during the induction with both general anaesthetics. (d) Mean arterial pressure decreased significantly in both groups. The changes occurred in all subjects within a physiological range.
shifted downwards on the stimulus–response plot. In the group with sevoflurane, a distinctive shift of the curve to the right was observed. For this group, this implies that more current was needed to elicit the target action potential.

Secondly, during the early phase of the recovery cycle, the curve shifted to the left in both groups (Fig. 4). Consequently, the first intersection with the control threshold (the current that is normally needed to elicit the targeted size of the action potential, plotted as a straight line at 0% on the y-axis) and representing the end of the relative refractory period (RRP; see Supplementary Fig. S1C) occurred earlier. The RRP decreased similarly in both groups [before propofol: 4.4 (1.2) ms; after: 3.9 (1.1) ms, \( P < 0.01 \)], with a distinctive shift of the curve to the right was observed. For this group, this implies that more current was needed to reach the target threshold after the end of the hyperpolarizing conditioning stimulus.15 It corresponds to the activities of certain voltage-gated membrane ion channels (mainly slow potassium currents) which counteract the changes of the membrane potential induced by the hyperpolarizing conditioning stimulus. Consequently, we would also expect a decrease in excitability at the end of the hyperpolarizing stimulus [e.g. at \( \text{TEh}(90–100 \text{ ms}) \)]. However, this parameter remained stable after induction, which cannot be conclusively explained at present.

**Discussion**

This study shows for the first time that general anaesthesia affects excitability of primary sensory afferents determined by threshold tracking. Using subthreshold currents rather than supramaximal currents we could detect smaller changes of nerve excitability properties than conventional nerve conduction studies or somatosensory evoked potential.
Possible intrinsic effect of anaesthetics on excitability parameters

Previous studies have shown that both propofol and sevoflurane alter nerve excitability at clinically relevant concentrations by modulating voltage-gated sodium channels, voltage-gated potassium channels, or both in the central and peripheral nervous system. In our investigation, parameters sensitive to changes in membrane potential remained almost unchanged after the induction of anaesthesia. However, we found three parameters that could be explained by an intrinsic effect of the general anaesthetics on peripheral nerve excitability.

(i) The RRP was shorter: investigations with the same threshold-tracking technique have shown that blocking sodium channels results in a shift of the recovery cycle curve to the left combined with a decrease in superexcitability in sensory afferents. The latter parameter, however, remained unchanged in our investigation.

(ii) The amplitude of the maximum peak response decreased; the size of a compound action potential decreases by temporal dispersion caused by different underlying mechanisms: differential slowing of individual fibres, availability of the largest-diameter fibres, and changes in the amplitude of individual spikes. An intrinsic blocking of voltage-gated sodium channels could have led to a reduced size of peak response. However, this conclusion could only be drawn in stable recording conditions. In our study, the temperature at the stimulation site changed significantly and, therefore, was likely to have an important influence on the response size (see below).

(iii) We observed no change in latency: according to the previously published data, we would expect a...
pronounced decrease in latency, hence an increase in conduction velocity, of about 3% caused by the temperature change detected at the stimulation site. But, the latencies remained unchanged before and after the induction of anaesthesia. This could imply that the faster kinetics of the voltage-gated ion channels induced by the higher temperature was counteracted by a partial block of sodium channels.

None of the above findings, however, is specific for an intrinsic effect of the investigated general anaesthetics on nerve excitability. Skin temperature at the stimulation site increased significantly and, therefore, temperature must be taken into account when interpreting these results.

Role of temperature in changes in excitability during anaesthesia

Temperature variation results in several changes in peripheral nerve excitability. Although in our study, temperature differences between the first and the second measurements were small, they were significant, probably due to a decrease in sympathetic activity, resulting in vasodilation with an increase in skin temperature. Temperature changes affect all nerve excitability indices measured with threshold tracking to some extent. The most prominent effect is a decrease in refractoriness with increasing temperature. Our results are in line with this finding, reflected by the leftward shift of the recovery cycle curve, indicating a shortening of the RRP. The underlying mechanism is a faster recovery from inactivation of voltage-gated sodium channels at higher temperatures.
Increased temperature also leads to a linear increase in nerve conduction velocity.\textsuperscript{30} \textsuperscript{31} According to the previously published data, we would expect a decrease in latency of about 3% caused by the temperature change measured in our study.\textsuperscript{26} However, latencies were not different, nor did we find a linear relationship of refractoriness and latency changes described by Burke and colleagues.\textsuperscript{28} Therefore, we might assume that the temperature change of $\sim 1.5$°C was too small to affect excitability. Also, temperature changes affect conditioned evoked potentials much more than unconditioned potentials.\textsuperscript{28} Refractoriness—in contrast to conduction velocity—was measured with a supramaximal conditioning stimulus; therefore, our findings would fit well with this theory.

In our investigation, the size of the compound action potential decreased after the induction of anaesthesia with both anaesthetics. The effect of temperature on the size of the compound action potential is more complex to explain. On the one hand, the compound action potential is sensitive to temporal dispersion of individual action potentials of nerve axons. Increasing the temperature, therefore, decreases the amount of dispersion of the compound action potential and results in a greater amplitude.\textsuperscript{31} On the other hand, an increase in temperature decreases the duration of the action potential and leads to a smaller action potential. Hence, the measured size of the action potential at higher temperatures is a combination of both effects. In a study by Kiernan and colleagues\textsuperscript{24} of motor nerve fibres, peak amplitude increased only when raising the temperature from 32 to 35°C, although the overall effect of temperature increase was a decrease of SNAP. Similar non-linear relationships between SNAP size and temperature changes have been described by Ludin and Beyeler.\textsuperscript{30}

### Anaesthesia depth, ventilation, and haemodynamic changes did not affect excitability

Our study endpoint was to compare the effect on peripheral nerve excitability of two different general anaesthetics at an equipotent dosage. Clinically, at first glance, these concentrations appear to be high because propofol and sevoflurane are usually used in combination with co-anaesthetics (e.g. opioids or benzodiazepines). In our study, however, patients were not premedicated, and until the end of the second measurement, we did not use any other co-medication. Consequently, the target plasma concentrations of propofol and end-expiratory sevoflurane concentrations required to reach BIS values below 40 were higher.

Several parameters which could have influenced nerve excitability were controlled very closely. Anaesthesia induction was performed exclusively by one senior anaesthesiologist and normoventilation was achieved throughout the recording period. We cannot fully exclude alveolar hypercapnia. However, according to the previously published data, strength–duration time constant would probably be the most sensitive parameter to detect hypercapnia.\textsuperscript{32} Since this parameter also remained unchanged we may assume that alveolar $\text{PCO}_2$ did not influence our measurements. The depth of anaesthesia was equivalent in both groups and therefore contributed comparably—if at all—to the measured excitability changes assuming conserved mechanisms.

Although the induction of anaesthesia caused a significant decrease in arterial pressure values, they never reached low physiological values. Furthermore, the oxygen saturation in the same arm remained stable. Therefore, we are sure that the perfusion pressure at the stimulation site was high enough to prevent hypoperfusion or hypoxaemia of the nerve and did not cause previously described changes in excitability.\textsuperscript{33}–\textsuperscript{35}

### Conclusion

The induction of general anaesthesia with propofol and sevoflurane resulted in a change of excitability of primary sensory afferents. These changes were subtle at relatively high concentrations of the anaesthetics. A direct effect of general anaesthetics on excitability could not be excluded but was minimal at most; it is more likely that the significant changes we found were caused by an increase in temperature at the site of stimulation. Further investigations are needed to elucidate the differentiation between these two mechanisms. However, our findings demonstrate possible interference by general anaesthetics during intraoperative neuromonitoring of the peripheral nervous system.

### Supplementary material

Supplementary material is available at British Journal of Anaesthesia online.

### Acknowledgements

We are grateful to the staff of the Institute of Anaesthesiology, University Hospital Zurich, Switzerland. Prof. Hugh Bostock, UCL Institute of Neurology, UCL, London, UK.

### Conflict of interest

K.M. has received travel support for consulting or lecturing from the following companies: Pfizer, Zurich, Switzerland; Bristol–Myers Squibb, Baar, Switzerland; Mundipharma, Basel, Switzerland; Janssen-Cilag, Baar, Switzerland; UCB, Bulle, Switzerland; and Medtronic, Bern, Switzerland. D.R.S. has received honoraria or travel support for consulting or lecturing from the following companies: Abbott AG, Baar, Switzerland; AstraZeneca AG, Zug, Switzerland; Bayer (Schweiz) AG, Zürich, Switzerland; B. Braun Melsungen AG, Melsungen, Germany; Boehringer Ingelheim (Schweiz) GmbH, Basel, Switzerland; Bristol–Myers Squibb, Rueil-Malmaison Cedex, France; CSL Behring GmbH, Hattersheim am Main, Germany and Bern, Switzerland; Curacyte AG, Munich, Germany; Ethicon Biosurgery, Sommerville, NJ, USA; Fresenius SE, Bad Homburg v.d.H., Germany; Galenica AG, Bern, Switzerland (including Vifor SA, Villars-sur-Glâne, Switzerland);
GlaxoSmithKline GmbH & Co. KG, Hamburg, Germany; Janssen-Cilag AG, Baar, Switzerland; Novo Nordisk A/S, Bagsværd, Denmark; Octapharma AG, Lachen, Switzerland; Organon AG, Pfaeffikon/ZG, Switzerland; Oxygen Biotherapeutics, Costa Mesa, CA, Pentapharm GmbH (now Tem International), Munich, Germany; Roche Pharma (Schweiz) AG, Reinach, Switzerland; and Schering-Plough International, Inc., Kenilworth, NJ, USA.

Funding
This study was funded by: Swiss Foundation for Anaesthesia Research (SFAR), Swiss National Science Foundation (grant no. SPUM 33CM30_124117), and Institute of Anaesthesiology, University Hospital Zurich, Switzerland.

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