

## Local application of halothane, isoflurane or sevoflurane increases the response to an electrical stimulus in humans

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**Abstract :** Volatile anesthetics may interfere with pain perception. This study investigates the effect of halothane, isoflurane and sevoflurane when applied locally, to the response of an electrical stimulus.

**Methods :** In this randomized control double-blind crossover study 70 volunteers were studied. In experiment 1 (30 subjects), equipotent liquid volumes of halothane 1 ml, isoflurane 1.5 ml and sevoflurane 2.7 ml were randomly applied on one forearm for 30 minutes. The other forearm received water. Both forearms were exposed to an electrical stimulus. The experiment was repeated the following day in a reverse fashion. In experiments 2 (20 subjects) and 3 (20 subjects) the response to the same stimulus was tested after local application of 2, 4, and 6 ml of halothane or 5 ml of sevoflurane respectively.

**Results :** Low doses of the three anesthetics were associated with an increased response to the electrical stimulus ( $F = 8.940$ ,  $df = 1,174$ ,  $P = 0.003$ ). Higher doses of halothane and sevoflurane had no effect on the response ( $F = 2.358$ ,  $df = 1,114$ ,  $P = 0.127$  and  $t = 0.840$ ,  $df = 19$ ,  $P = 0.411$  respectively).

**Conclusions :** Low liquid volumes of volatile anesthetics, when applied locally to the skin enhanced the response to an electrical stimulus but higher volumes had no effect.

**Key words :** Anesthetics : volatile, halothane, isoflurane, sevoflurane ; volatile anesthetics : nociception, antinociception ; stimulus : electrical.

### INTRODUCTION

The impact of volatile anesthetics on pain perception and their possible analgesic effect has been investigated and assessed clinically, particularly in obstetrics (1, 13). Addition of isoflurane to 50% nitrous oxide in oxygen decreased the analogue pain scores for pain during the first stage of labor (21). The possible analgesic effect of methoxyflurane and halothane on pain threshold has been examined in healthy volunteers (18). Subanesthetic concentrations of these anesthetics increased the pain threshold but there was no linear relationship between pain threshold and blood

levels of the anesthetics (18). The authors attribute the increase in pain threshold to the onset of drowsiness.

Halothane and ether at clinical concentrations have been found to block about 50% of current through voltage-gated sodium channels in central and peripheral mammalian neurons (4). Also 1 MAC of halothane, enflurane, isoflurane or desflurane administered in rats inhibited the nociception induced spinal sensitization produced by the formalin test (14).

These findings are not consistent with other studies. In rats low concentrations of halothane increase the sensitivity to a noxious thermal stimulus and reduce the analgesic effect of intraventricular morphine (3). Isoflurane, halothane, nitrous oxide and diethyl-ether produce hyperalgesia at subanesthetic concentrations with maximal hyperalgesic effect at 0.1 MAC (22). Similarly, desflurane at 0.1 MAC shortened the tail-flick latency indicating a hyperalgesic effect. In contrast, at higher partial pressure concentrations desflurane exerted an antinociceptive effect (19).

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The results of the studies investigating the analgesic effects of volatile anesthetics are contradictory. Also there is a difficulty to differentiate between a sedative action of the volatile agents blunting the response to a noxious stimulus and a real analgesic effect. Volatile anesthetics, when inhaled, pass through the skin during the uptake and the elimination phase. Their percutaneous loss was found to be greater during the elimination than during the administration phase and greater for the more soluble agents (7). The skin partition coefficient of the volatile agents has not been measured. The skin uptake for the more soluble inhalational agents is higher than the less soluble ones. Nevertheless, the total percent losses through the skin represent a minimal fraction of the total quantity of the anesthetic uptaken by the lungs (7).

In a previous study we investigated in volunteers the analgesic effects of isoflurane when applied locally on the forearm (5), so the anesthetic passes through the skin from outwards inwards. In this way we could eliminate or at least minimize the effect of sedation produced concomitantly by these agents. The present study is the second part of a recent study (6). The goal of the present study was to investigate the effect of different doses and of different volatile agents, applied to the skin, on the response to a painful electrical stimulus.

## METHODS

The study was approved by the Local Ethics Committee. All experiments on volunteers were conducted in accordance with the Declaration of Helsinki. Subjects were ASA physical status I and were recruited from residents and nurses who were not familiar with the anesthetic drugs and were not working in the Department of Anesthesiology. All volunteers signed an informed consent, after adequate understanding of the study, according to which they were allowed to discontinue their participation in the study at any time and for any reason.

Exclusion criteria were body weight exceeding the 20% of their ideal body weight, allergies to cotton, metal or to previous anesthetics and left handed persons. Subjects treated with calcium channel blockers, beta-blockers, analgesics, sedatives or chronic pain conditions were also excluded. Women participants were asked about menstrual cycle history and contraceptive drugs. If the possibility of pregnancy could not be excluded they were not recruited for the study.

## Experiments

In all experiments the tests for each anesthetic were two days apart from the tests for the other anesthetics.

### Experiment 1

Volumes of 1 ml, 1.5 ml and 2.7 ml of halothane, isoflurane and sevoflurane were applied on the skin of the forearm of 30 volunteers in a randomized cross-over double-blinded manner. The subjects received on the contra lateral forearm equal volumes of water. Randomization was done using sealed opaque envelopes containing the order of the anesthetics to be tested as first, second and third. An independent anesthesiologist who did not participate in the study, opened the envelopes, recorded the order of exposure and prepared the syringes containing the water and the liquid anesthetic.

### Experiment 2

The experiment was performed in 20 subjects who received on their forearms 2, 4 and 6 ml of liquid halothane and equal volumes of water as control for the other forearm. All applications of halothane were done in a randomized, cross-over, double-blinded manner. As in Experiment 1, both arms were tested for the three doses of the anesthetic and equal volumes of water in a cross-over manner. Randomization was done using sealed envelopes. Opening of the envelopes and filling the syringes was done by an independent anesthesiologist.

### Experiment 3

Five ml of liquid sevoflurane or 5 ml of water were applied simultaneously on each forearm of 20 healthy subjects who were tested for pain perception. Tests were performed in a randomized double-blind manner as described in the previous experiments.

### Electrical Stimulus

The effect of application of volatile anesthetics versus water on the volar surface of the forearms was tested against an electrical stimulus. We used a peripheral nerve stimulator (DigiStim III, Organon Teknika® made by Neuro technology INC, Houston, TX) with the current (mA) to be delivered set at 60 mA. The two metal bar electrodes were placed to the lower third of the volar

surface of each forearm along the ulnar nerve, 5 to 6 cm apart. A square wave monophasic pulse (200- $\mu$ sec duration, 1 pulse/10 sec) electrical stimulus was applied once to the lower volar surface of each forearm. This stimulus provokes pain, which is acceptable by the volunteers and we have applied it in previous studies (6, 20). The VAS score for each forearm and the mA delivered were recorded.

#### Procedure

The data of the current study were acquired in the same volunteer population used in a previous study (6). The VAS score corresponding to the electrical stimulus was obtained after the response to the mechanical stimulus. Each subject recruited for one experiment did not participate in any other experiment. To limit the effect of learning the test subjects were exposed to the test without receiving anesthetic and water one or two days before starting the experiment. Bispectral Index was monitored during the exposure of the skin to the anesthetic in all experiments using a BIS Monitor Model A-2000™ (Aspect Medical Systems, Inc, Newton, MA).

The volunteer was in the sitting position with the forearms on a table exposing the volar surface to the anesthesiologist performing the test. The forearms were wrapped with cotton. Steps taken to assure blinding were use of mask by both the volunteer and the anesthesiologist as well as the simultaneous fast application of the liquids between the cotton surrounding the forearms and the skin, so the volunteer and the anesthesiologist could not differentiate from which forearm the smell was arising, if any. Since the anesthetic was applied under the cotton, between the cotton and the skin no particular smell could be detected. Consequently the forearms were covered with aluminum foil, which in turn was covered with transparent film. All layers were fixed with tape, allowing the elbow free.

The independent anesthesiologist prepared the syringes and determined the anesthetic, the dose and the treatment for each forearm. Thirty minutes later the covers were removed, the skin temperature in each forearm was measured using skin electrodes, and subsequently the response to the electrical stimulus was recorded as VAS score. The same anesthetic and the same dose was repeated the following day but in a cross-over manner regarding the treatment of each arm. The VAS scores obtained for the particular anesthetic and dose from both forearms was averaged and compared with the averaged VAS score obtained by its control (water).

#### Statistical analysis (6)

Sample size estimation for two-way analysis of variance was based on the instructions provided by the "Data analysis and biostatistics software and resources", GraphPad.com, GraphPad Software (San Diego, CA, USA). We found that to ensure power 0.80 of detecting 40-50% changes of the mean VAS scores after stimulation 30 subjects should be studied in experiment 1 and 20 subjects in experiment 2. Alpha error was assumed 0.05 and standard deviations of approximately 18-22 were estimated from initial pilot observations and previous publication (5). Sample size estimation for the experiment 3, performed using the GB-Stat™ PPC 6.5.4 software for Macintosh, showed that approximately 20 subjects should be needed in order to detect a 40% change from the mean VAS scores after stimulation. Alpha error was assumed 0.05 and standard deviation of approximately 20 was estimated from initial pilot observations.

The Kolmogorov-Smirnov test showed that all variables followed normal distribution. VAS scores were compared using two-way analysis of variance in experiments 1 and 2, and paired Student's t-tests in experiment 3. Paired Student's t-tests were also employed for pair-wise comparisons of VAS scores between each anesthetic treatment and its water-control measurements wherever appropriate (2).  $P \leq 0.05$  was considered significant. The Microsoft® Excel X, SPSS® 11, and GB-Stat™ PPC 6.5.4 software for Macintosh were used for statistical analysis.

#### RESULTS

The age of the volunteers was  $33 \pm 5$ ,  $33 \pm 4$  and  $34 \pm 4$  years in the experiments 1, 2 and 3 respectively. The body weight was  $66 \pm 12$ ,  $65 \pm 12$  and  $67 \pm 13$  kg and the height  $170 \pm 8$ ,  $171 \pm 9$  and  $170 \pm 8$  cm respectively. The ratio of men to women was 15/15 in experiment 1, 11/9 in experiment 2, and 10/10 in experiment 3. The temperatures in each experiment did not differ between the anesthetic and water applications except for the experiment 3 where for 5 ml sevoflurane versus water were  $29.0 \pm 1.0^\circ\text{C}$  and  $28.2 \pm 1.5^\circ\text{C}$  respectively, ( $p = 0.008$ ). After the 6 ml of halothane treatment two subjects presented irritation of the skin. As this dose was the last test for the two subjects the experiment was completed. None of the subjects presented BIS values below 93 during the experiments.

Table I

VAS scores (mm/mA) after application of 1 ml of halothane (HAL), 1.5 ml of isoflurane (ISO) or 2.7 ml of sevoflurane (SEVO) versus 1, 1.5 or 2.7 ml of water as responses to the electrical stimulus

VAS (mm/mA)	HAL 1 ml	Water 1 ml	ISO 1.5 ml	Water 1.5 ml	SEVO 2.7 ml	Water 2.7 ml	ANOVA
Mean	1.35*	1.18*	1.56†	1.11†	1.28‡	0.95‡	F = 8.940 df = 1,174 P = 0.003
SD	0.81	0.61	0.95	0.47	0.75	0.50	
Median	1.30	1.25	1.35	1.10	1.25	0.80	
CI	1.0-1.6	0.9-1.4	1.2-1.9	0.9-1.3	1-1.6	0.8-1.1	
Range	3.2	2.4	3.9	2.0	3.4	1.9	
D % vs water	≈ 13%		≈ 29%		≈ 26%		
Paired t-test	*t = 2.10, df = 29, P = 0.044		† t = 3.47 df = 29, P = 0.001		‡t = 3.63, df = 29, P = 0.001		

Table II

VAS scores (mm/mA) after application of 2, 4, and 6 ml of halothane (HAL), versus 2, 4, and 6 ml of water as responses to the electrical stimulus

VAS (mm/mA)	HAL 2 ml	Water 2 ml	HAL 4 ml	Water 4 ml	HAL 6 ml	Water 6 ml	ANOVA
Mean	1.53	1.33	1.31	1.23	1.53	1.29	F = 2.358 df = 1,114 P = 0.127
SD	0.86	0.45	0.64	0.49	0.69	0.44	
Median	1.5	1.3	1.3	1.3	1.6	1.4	
CI	1.1-1.9	1.1-1.5	1.0-1.6	1.0-1.5	1.2-1.8	1.1-1.5	
Range	3.4	1.7	2.4	1.8	2.2	1.5	
D % vs water	≈ 13%		≈ 6%		≈ 16		

### Experiment 1

As after the application of the electrical stimulus the current passing through the skin differed between the tests, we normalized the VAS values by dividing them by the mA recorded. After normalization in experiment 1, all the three anesthetics were associated with higher VAS values after application of the electrical stimulus versus the water applications. Multifactorial ANOVA (corrected model  $F = 2.642$ ,  $df = 5,174$ ,  $P = 0.025$ ) showed a significant difference for the effect of the three anesthetics versus their water-control applications ( $F = 8.940$ ,  $df = 1,174$ ,  $P = 0.003$ ) though there was no difference between the three anesthetics themselves ( $F = 1.556$ ,  $df = 2,174$ ,  $P = 0.214$ ) (Table I, Fig. 1).

### Experiment 2

In this experiment also, the current that passed through the skin was significantly higher after water than after halothane applications. For this reason we normalized the VAS responses to the electrical stimulus obtained after the three applications of halothane or water by dividing them by the current that passed through the skin after release of the electrical stimulus. Multifactorial ANOVA (correct-

ed model,  $F = 0.864$ ,  $df = 5,114$ ,  $P = 0.508$ ) showed no differences in the VAS values normalized for the current, regarding halothane applications versus water ( $F = 2.358$ ,  $df = 1,114$ ,  $P = 0.127$ ) or the doses of halothane ( $F = 0.798$ ,  $df = 2,114$ ,  $P = 0.453$ ) (Table II, Fig. 1).

### Experiment 3

After normalization of the VAS values for the current allowed to pass through the skin, the responses to the electrical stimulus did not differ between 5 ml of sevoflurane and water application ( $t = 0.840$ ,  $df = 19$ ,  $P = 0.411$ ) (Table III, Fig. 1).

The responses to the electrical stimulus normalized for the current amplitude (VAS mm/mA) to different volumes of volatile anesthetics versus equal volumes of water are shown in Figure 1.

### DISCUSSION

According to our results, the low but not the higher doses of the anesthetics tested enhanced the response to an electrical stimulus, when this response was normalized for the current passing through the skin.

Table III

VAS scores (mm/mA) obtained after application of 5 ml of sevoflurane (SEVO), versus 5 ml of water as responses to the electrical stimulus

VAS (mm/mA)	SEVO (5 ml)	Water (5 ml)
Mean	1.34	1.24
SD	0.756	0.486
Median	1.34	1.12
95% CI	0.99-1.69	1.01-1.46
Range	2.5	1.8
D % vs water	≈ 7%	

The effect of volatile anesthetics on pain perception is controversial. Subanesthetic concentrations of isoflurane as low as 0.1 MAC significantly increased the nociceptive reflex thresholds to single electrical stimulation but not to repeated electrical stimulations (16). This analgesic effect of subanesthetic doses of volatile anesthetics is not consistent with animal studies. In rats low partial pressures of isoflurane, halothane, nitrous oxide and diethyl-ether produced hyperalgesia with the maximal antanalgesic effect observed at 0.1 MAC (22). A decrease in the time to withdrawal of the rat hind paw exposed to heat was considered as hyperalgesia produced by exposure to the low concentration of the anesthetic. However, at 0.4 to 0.8 MAC the same anesthetics produced analgesia (22).

Also in rats halothane concentrations as low as 0.06 and 0.12% decreased the threshold to a thermal stimulus and attenuated the antinociceptive effect of intraventricular morphine (3). Similarly, desflurane at 0.1 MAC shortened the tail-flick latency indicating a hyperalgesic effect. In contrast, at higher partial pressure concentrations desflurane exerted an antinociceptive effect (19).

In the present study we found an enhanced response to the electrical stimulus we applied, which is significant only for the lower doses of the volatile anesthetics we tested. We did not observe different responses when we applied locally higher doses of halothane and sevoflurane. This is in contrast to the results of a previous study where 10 ml of isoflurane similarly applied attenuated the response to the electrical stimulus. The difference may be due to methodology and specifically to different electrical stimuli (11). The difference in skin temperature between the high dose of sevoflurane and water is not clinically significant. Otherwise, we found no significant differences in skin temperatures between the anesthetics and the water applications.

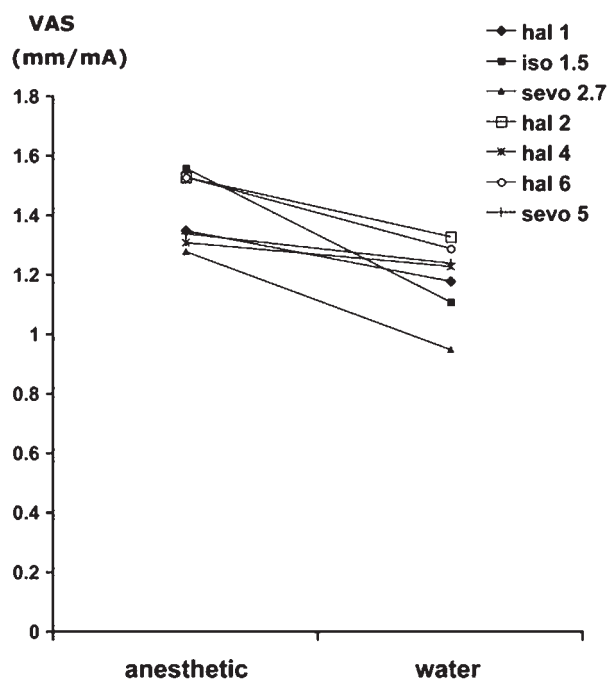


Fig. 1. — VAS scores (mm/mA) as responses to the electrical stimulus, after application of 1, 2, 4 and 6 ml of Halothane (hal), 1.5 ml of Isoflurane (iso), 2.7 and 5 ml of Sevoflurane (sevo) versus equal volumes of water for each volatile anesthetic respectively.

Specific differentiation between peripheral nerve fibers that convey pain signals (such as A $\delta$  and C) by transcutaneous electrical stimuli is difficult (15). It has been estimated that threshold is higher for the mechano-insensitive C-nociceptive fibers (60 mA) than for the polymodal C-nociceptors (2.0-5.5 mA, lower and upper quartile respectively) (10). Large-diameter fast-conducting afferents have electrical threshold lower than that of small-size C-fibers (9). Thus we selected current intensity 60 mA to ensure maximal stimulation of all nociceptive nerve fibers in all subjects. The use of increased intensity of transcutaneous electrical stimulus constitutes an acceptable, mainstream methodology in humans in order to induce evoked pain responses (9).

The antanalgesic effect of the lower doses of volatile anesthetics we found is in accordance with the results of the experimental studies by Drasner, Zhang and Sonner (3, 22, 19). However, our results regarding the response to the electrical stimulus are different from the results of a previous study where subanesthetic concentrations of isoflurane administered by inhalation increased the nociceptive reflex thresholds to single electrical stimulation (16).

The results of the present study are hard to compare with all the previous studies since we do not know the skin concentration of the anesthetics after local versus systemic administration. We also cannot compare these results with those of a previous study where we administered locally very high doses of isoflurane because of the different dose and the lack of recording of the current passing through the skin (5).

The nociceptive or antinociceptive effect of volatile anesthetics in the periphery is probably affected by differences in solubility as percutaneous losses of halothane have been found 6 and 2 times greater than those of desflurane and halothane respectively (7). It is also affected by the dose of the anesthetic applied and by the type of the stimulus. Indeed higher but not lower doses of the same anesthetics applied locally attenuated the response to a mechanical stimulus (6).

The responses we obtained after application of a mechanical stimulus (6) differ from those we obtained after the electrical stimulus in the present study. This may be explained by the fact that different noxious stimuli affect differently the A $\beta$ , A $\delta$  and C afferents. For example, cutaneous C fiber nociceptors are not sensitized by mechanical stimulation by von Frey hairs (17). On the contrary the A $\beta$  afferents were sensitized after both electrical and mechanical stimulation (8).

The antanalgesic effect of the inhaled anesthetics may be due to enhancement of C fiber activity. At 1 MAC, halothane increased spontaneous discharge frequency of C fibers and reduced the action potential amplitude and increased spike latency in A $\delta$  fibers (12). The A $\delta$  fibers are stimulated by mechanical pressure and their suppression may be associated with attenuated responses to the mechanical stimulation we observed after local application of the liquid anesthetics (6). The differential effects of volatile anesthetics we found on the mechanical and electrical stimulus may suggest either different mechanisms of response or/and different sensitivity to each type of stimulus. On the other hand, while local application of the volatile anesthetics minimizes the sedation effect, it also minimizes their effect on the central pain processing, which may be also clinically significant.

In conclusion, local application of the lower doses of halothane, isoflurane and sevoflurane tested in healthy volunteers enhanced the response to the electrical stimulus. The higher concentrations of these anesthetics when applied locally had no effect on the response to the same electrical stimulus versus the control. These results are consistent with

animal studies demonstrating the hyperalgesic effect of inhaled anesthetics at subanesthetic partial pressures.

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