Volatile Anesthetics Excite Mammalian Nociceptor Afferents Recorded In Vitro

M. Bruce MacIver, Ph.D.,* Darrell L. Tanelian, M.D., Ph.D.†

The present study investigated the actions of halothane, isoflurane, and enflurane on spontaneous discharge and evoked action potential activity in mammalian A-δ and C fiber nociceptors from the in vitro rabbit cornea. At 1 MAC halothane, isoflurane, and enflurane significantly (P < 0.001) increased spontaneous discharge frequency of C fibers to 410%, 388%, and 569% of control, respectively. The anesthetics produced burst discharge activity over the concentration range of 0.25–1.5 MAC and depressed discharge activity at higher concentrations (>3.0 MAC). Similar excitatory effects were produced by the potassium channel blocker 4-aminopyridine (250–500 μM). Variable effects on evoked discharge activity of A-δ fibers were observed. Halothane reduced action potential amplitude (77.3 ± 4.5% of control ± SD; n = 6 at 1 MAC) and increased spike latency (0.42 ± 0.075 ms). In contrast, the others decreased both spike latency (isoflurane by 0.31 ± 0.064 ms and enflurane by 0.35 ± 0.058 ms) and action potential amplitude. Halothane and the ether anesthetics produced a common excitatory action on C fibers; however, the differential depressive effects on A-δ fibers suggest that different membrane mechanisms of action are involved. (Key words: Anesthetics, volatile; enflurane; halothane; isoflurane. Nociceptors, pain; activation; burst discharge; depression.)

VOLATILE GENERAL ANESTHETICS have been reported to produce excitatory actions on squid giant axon,1–3 crayfish giant axon,4 crayfish stretch receptor,5,6 and frog myelinated axons.7 In addition, halothane has been shown to increase the sensitivity of nociceptors in monkeys8 and to increase conduction velocity in human and cat peripheral nerves in vivo.9,10 Both excitatory and depressive anesthetic effects have been reported on mammalian baroreceptors11,12 and chemoreceptors.13,14

The excitatory effects reported in vivo could result from changes in vascular permeability,15 PO2, PCO2, and/or pH in adjacent tissue rather than reflecting a direct anesthetic effect on nerve fibers. The present study investigated the effects of volatile anesthetics on mammalian nociceptor fibers using an in vitro preparation of rabbit cornea. Because the cornea is avascular and nonkeratinized, the possibility that an observed anesthetic effect is secondary to vascular or nonneuronal changes is eliminated. The in vitro preparation also allowed control of PO2, PCO2, and pH in the perfusate. Thus, anesthetic effects on corneal nociceptors can be attributed to direct actions on neuronal elements.

Corneal nerve electrophysiology is well characterized16 in both the in vivo and in vitro rabbit cornea. In both models identical electrophysiology has been described: 1) a rapidly adapting A-δ mechanoreceptor; 2) an A-δ bimodal receptor that responds to high threshold mechanical stimuli and temperatures above 40°C; 3) a C fiber cold receptor; and 4) a C fiber chemosensitive receptor. Corneal tissue is innervated only by these four nociceptor afferent classes with cell bodies located in the trigeminal ganglion.16 As a model to study sensory nerves, the cornea is ideal because the anatomy and physiology of corneal A-δ and C fibers are similar to those described in other animal and human tissues.16

A growing number of reports demonstrate that general anesthetics produce a variety of agent specific effects both in the central nervous system17–19 and on isolated preparations of single axons.20–22 Selective, anesthetic specific actions have even been described for the relatively homologous series of volatile agents2,23–25 and alcohols.26,27 These findings do not support the widely held view that general anesthetics act via a common or "unitary" mechanism at the membrane level.28,29 The primary objective of the present study was to investigate anesthetic effects on A-δ and C fiber sensory afferents. In addition, similarities and differences between agents were compared and contrasted. Localization of effects to the transducer or axonal portion of afferents was also studied.

Materials and Methods

Tissue Isolation

The experimental protocol was approved by the Institutional Animal Care Committee at Stanford University, and care was taken at all stages of handling to minimize stress and discomfort for the animals. Thirty-two albino rabbits (New Zealand White) weighing 2.5–3 kg were obtained from Nittabel Inc. and housed on a 12-h light/dark cycle. Animals were anesthetized with urethane (1.5 g/kg ip) and killed with an overdose of pentobarbital.

The cornea and 3–5 mm of sclera were rapidly excised using a precleaned razor blade and placed in cooled (5°C) and oxygenated artificial aqueous humor solution (AQR). Excreaneous conjunctive and ocular musculature were carefully removed with dissecting scissors. The lens, iris, and retina were removed with fine forceps, and care

* Research Associate.
† Assistant Professor.
was taken not to damage the delicate endothelium on the inside of the cornea. Tissue was carefully washed with several changes of clean AQH solution. The isolation procedure was carried out in cold AQH and required less than 10 min.

**TISSUE CHAMBER**

The tissue chamber consisted of a modified brain slice bath fitted with an inner compartment, which allowed continuous perfusion with AQH behind the cornea at a constant temperature (35°C) and pressure (18 mmHg) to reproduce *in vivo* conditions. The cornea was placed over a small Plexiglas ring, and a pressure tight seal was formed by securing the sclera with suture (fig. 1). The ring with cornea attached was pressure fitted into a Plexi-

![Diagram of the isolated cornea preparation showing attachment of sclera to the inner chamber and placement of stimulating probe (STIM) on free nerve ending (FNE) terminals and extracellular recording electrodes (EC) on fine nerve bundles (NB). Anesthetics were applied as small drops in solution to the corneal surface or as vapors in the O₂/CO₂ gas stream (3.5 l/min), and concentrations were continuously monitored with a small sampling catheter (SAMP). Pressure (PRESS) and temperature (TEMP) were held constant and monitored with probes placed in the inner chamber. Artificial aqueous humor (AQH) solution was perfused through the chamber at a constant rate (1.0 ml/min).](diagram)

The AQH solution had the following composition: Na, 145 mM; K, 4.5 mM; Ca, 1.5 mM; Mg, 0.6 mM; Cl, 126.3

Electrophysiology

Extracellular recordings of nociceptor fiber action potentials were obtained using standard electrophysiological techniques. Suction electrodes (WPI Inc.; thin wall 1.0 mm OD fiberfill glass, filled with AQH) were placed in or near the small corneal nerve bundles near the cornea-

were viewed with a variable magnification dissecting mi-

sorticulum (Wild M 32; X15–X80).

Materials

The AQH solution had the following composition: Na, 145 mM; K, 4.5 mM; Ca, 1.5 mM; Mg, 0.6 mM; Cl, 126.3
mm; SO₄, 0.6 mm; HPO₄, 0.6 mm; HCO₃, 25 mm; and glucose, 10 mm. All chemicals were reagent grade or better and were obtained from J. T. Baker Chemical Co. as the hydrated salt. 4-Aminopyridine (4-AP) was obtained from Sigma Chemical Co. Water for solutions was HPLC and spectrophotometry grade and obtained from EM Science. AQH solutions were chilled to 5°C and saturated with O₂/CO₂ prior to use. After warming to 35°C solutions had a pH of 7.2–7.4. Anesthetics were obtained from the following: halothane (FLUOTHANE), Ayerst Laboratories; isoflurane (AERRANE) and enflurane (ETHRANE), Anaquest.

ADMINISTRATION AND ANALYSIS OF ANESTHETICS

The anesthetics were administered in the O₂/CO₂ gas stream surrounding the corneal surface, using calibrated vaporizers (Foregger). Agents were applied for at least 30 min at each concentration to ensure that steady state was achieved. Effects on discharge activity were apparent with 1 min, and steady state effects were observed within 5 min for low concentrations (<1 MAC) and 20 min for the highest concentrations studied. Anesthetic concentrations in the vapor phase were continuously measured using a Puritan-Bennett agent monitor (Mod. 222) with a sampling catheter placed within 3 mm of the corneal surface. Each preparation was exposed to only one anesthetic; however, several (1–4) concentrations (usually beginning with low levels) were studied, separated by at least 30 min of wash to allow full recovery.

4-AP was applied as a 20-μl drop of 500 μM in AQH solution. The solution was warmed to 35°C to prevent activation of thermosensitive (cold) fibers, and the force component was less than the threshold for activating mechanosensitive fibers. Effects on discharge frequency were observed within 2.0 s, and recovery occurred following approximately 15 min as the 4-AP was gradually washed out of the tissue by the continuous perfusion of AQH solution.

Results

ANESTHETIC-INDUCED EXCITATION OF C FIBERS

Corneal C fibers normally exhibited a low level of spontaneous (unstimulated) discharge at 35°C; typically 1–4 individual units could be discriminated and discharged at frequencies of 2 to 7 spikes/second per unit (mean of 5.3 ± 0.6 SD; n = 86 units). Control recordings from individual nerve fibers remained stable for several hours (5–7 h) and exhibited only a small variation in discharge frequency (<5%/h). The volatile anesthetics produced a marked increase in discharge frequency from all C fibers studied (86 nerve fibers from 32 preparations) at clinically relevant concentrations (1 MAC in rabbits: halothane, 1.2 vol%; isoflurane, 2.1 vol%; enflurane, 2.9 vol%). Increased discharge resulted from both an increase in the firing frequency of tonically active thermosensitive units and recruitment of previously inactive chemosensitive fibers. C fibers were readily distinguished from A-δ fibers by their small spike amplitude (<0.5 mV), slow conduction velocity (<1.0 m/s), and lack of response to mechanical stimulation. Spontaneously active A-δ fibers were not observed in control conditions, and A-δ fibers were not activated in the presence of anesthetics.

Figure 2 shows the effects of increasing concentrations of halothane on spontaneous discharge activity. The lowest effective concentration was 0.45 vol% (±0.08 SD; n = 6) and at 0.6 vol% (0.5 MAC) increased firing frequencies of 200–250% were observed. Concentrations between 0.5 and 1.0 MAC produced an altered pattern of discharge such that bursts or groups of action potentials were apparent (e.g., at 0.8 vol% in fig. 2A). Firing frequency increases of 380–500% (410 ± 56; n = 12; P < 0.001 vs. control) were produced by 1 MAC of halothane. Concentrations above 1.5 MAC produced progressive depression of discharge, and at 3 MAC activity was reduced to 50% of control (e.g., 3.7 vol% in fig. 2A). Total depression of spontaneous discharge was not observed up to the maximal concentration studied (4.8 vol%; 4 MAC; limited by the vaporizer). The effects produced by halothane were reversible and complete recovery was observed within 20 min.

The halogenated ethers, isoflurane and enflurane, produced effects comparable to halothane. Figure 2B provides examples of enflurane-induced activation and burst firing. The lowest effective concentrations were 0.3 MAC for both ethers, and burst discharges occurred between 0.5 and 1.5 MAC. Enflurane produced a significant increase to 569 ± 53% (SD; n = 14; P < 0.001) of control and isoflurane increased frequency by 388 ± 59% (SD; n = 10; P < 0.001) at concentrations of 1 MAC. The ethers were less effective at depressing discharge activity than was halothane; at 3 MAC frequencies of 187 ± 14% and 179 ± 10% of control were observed for isoflurane and enflurane, respectively. Reduction of discharge frequencies to below control levels could not be obtained at the maximal vapor concentrations.

To determine whether complete block of discharge activity could be produced by the ethers, isoflurane and enflurane were applied as solutions of high concentration (saturated, 10 mM; approximately 17 MAC) directly on the corneal surface. Figure 3 shows the biphasic (excitation/depression) effects produced by a 20-μl drop of solution containing isoflurane; the concentration reaching nerve elements is not known due to diffusion and evaporation but would be considerably less than 17 MAC. Increased discharge activity was evident within a few seconds following exposure to the anesthetic. Within 30 s marked
FIG. 2. A. One-second records of ongoing action potential discharge activity recorded from a single nerve showing the effects of various concentrations of halothane applied as a vapor from calibrated commercial vaporizers. Concentrations in the clinical range (1.2 vol% = 1 MAC) produced marked excitation (increased discharge frequency) and burst discharge activity. Higher concentrations produced depression of spike activity (e.g., 3.7 vol%). These effects were reversed within 20 min of discontinuing the halothane and full recovery was observed. B. Enflurane produced marked activation and burst discharge of action potentials at clinically relevant concentrations (2.9 vol% = 1 MAC). Full recovery was observed within 15 min of terminating the anesthetic. Each record is 1 s long; data in A and B are from different preparations.

Increases in frequency and burst discharge activity were apparent, similar to effects observed following exposure to the vapor. Block of discharge activity was observed within 40 s, and often an abrupt rather than a gradual decrease in frequency occurred. Complete recovery occurred within 1 or 2 min, preceded by a brief period of burst firing and high frequency discharge.

The concentration-dependent biphasic anesthetic actions on spontaneous discharge frequency are summarized in figure 4. Enflurane was the most effective agent for producing increases in discharge frequency. All three anesthetics produced significant ($P < 0.001$) increases in discharge activity at clinically relevant concentrations. Depression of spike activity only occurred at high concentrations.

**ANESTHETIC-INDUCED EXCITATION MAY INVOLVE POTASSIUM CURRENT BLOCK**

Excitatory and burst-inducing effects produced by the anesthetics could come about by either increases in sodium and/or calcium currents or by depression of chloride and/or potassium currents. The first possibility did not seem likely because preliminary experiments using low sodium or calcium containing solutions failed to alter the anesthetic responses. Similarly, an effect on a chloride current was unlikely because the chloride channel blocker, picrotoxin (100 μM), did not produce any noticeable effects on spontaneous or stimulated discharge activity. Thus, it appeared likely that depression of a potassium current accounts for the increased discharge activity observed.

The potassium blocker 4-AP produced a profile of effects that were indistinguishable from the actions of the anesthetics. Figure 5 shows the effects produced by 4-AP applied as a 20 μl drop (500 μM) to the corneal surface. 4-AP produced an almost immediate increase in discharge frequency (325 ± 13.7%; $n = 6$; $P < 0.001$ vs. control) followed by burst firing within a few minutes and depression of discharge by 10 min. Gradual recovery was observed within 30 min following this brief exposure to 4-AP.
Fig. 3. Isoflurane (ISO) produced activation and burst-like discharge of noceceptor afferents when applied as a small drop (20 μl) of saturated AQH on the corneal surface. This high concentration of isoflurane produced total depression of discharge within 45 s (each record is 1 s long). Complete recovery was observed within 1.5 min.

Fig. 4. Bar graphs summarizing the effects of anesthetics on spontaneous discharge activity of noceceptors. Each bar represents the mean ± SD from at least ten experiments. Control (preanesthetic) discharge activity was normalized to 100% and data expressed as a per cent of control. Note the marked increase in discharge frequency produced by all anesthetics at 1 MAC. Higher concentrations (e.g., 3 MAC) produced depression; however, only isoflurane depressed discharge to below control levels at this concentration. The data shown in each bar were significantly (P < 0.005) different from control and from each other (ANOVA).

Fig. 5. One-second records showing the activation and burst discharge produced by 4-AP (500 μM). This concentration produced a long lasting (several minutes) increase in discharge activity followed by depression (10 min). Complete recovery was observed within 40 min.

ANESTHETIC EFFECTS ON A-Ł MECHANORECEPTOR DISCHARGE

Corneal noceceptors respond to both mechanical and electrical stimuli presented to the terminal field of free nerve endings. Thus, it is possible to isolate individual fibers and study anesthetic effects on single action potentials in detail. Figure 6 shows the effects produced by halothane on an isolated spike discharge recorded in response to a mechanical stimulus. Halothane produced a concentration-dependent depression of action potential amplitude (77.3 ± 4.5% of control; SD; n = 6 at 1 MAC), accompanied by an increase in spike latency (0.42 ± 0.075 ms; 25% increase of control), increased spike half-width, and a depression of spike after potential amplitude (compare lower records in fig. 6). Relatively high concentrations (i.e., 3.0 vol%) of halothane were required to block mechanoreceptor responses, and recovery required up to 30 min in preparations exposed to these high concentrations. Isoflurane at 1 MAC produced a comparable depression of spike amplitude (76.4 ± 5.8% of control; n = 7); however, spike latency was decreased by −0.31 ± 0.064 ms (18% decrease of control). Enflurane at 1 MAC was significantly less effective (P < 0.01 vs. isoflurane; P < 0.05 vs. halothane) at depressing spike amplitude (81.2 ± 5.3% of control; n = 11) and also produced a decrease in spike latency (−0.35 ± 0.058 ms; 21% decrease of control). The others also produced increased spike half-width and depressed after potential amplitudes similar to the effects of halothane.
stimulation bypasses the normal generator potential in the terminals. In general, anesthetic effects on electrically evoked action potentials were similar to actions on mechanically activated fibers. Example records of anesthetic effects (at 1 MAC) on electrically excited units are shown in figure 8A. All anesthetics depressed spike amplitude and reduced the after potential. Halothane produced an increased latency, which was only about half as large as that seen with mechanical stimulation. In contrast, the ethers produced decreased latency to a level comparable to those seen with mechanical activation (fig. 8B). Thus, most anesthetic effects can be attributed to effects on action potential conduction because comparable effects were observed with both mechanical and electrical stimulation. The more pronounced effect of halothane on spike latency following mechanical stimulation when compared with electrical stimulation indicates an additional action on sensory transduction for this anesthetic.

Anesthetic-induced excitatory effects were not observed on mechanically or electrically evoked action potentials, except for a few experiments (three of 11) in which burst discharges of 3–5 spikes were observed in the presence of enflurane following mechanical stimulation. The decreased latency produced by isoflurane and enflurane on A-δ fibers indicates an enhancement of axonal conduction that could also be considered excitatory.9

Discussion

ANESTHETIC-INDUCED EXCITATION OF PERIPHERAL AXONS

Results of the present study confirm and extend previous reports that volatile general anesthetics increase the

4-AP EFFECTS ON EVOKED ACTION POTENTIALS

4-AP produced effects on A-δ fibers that were similar to the anesthetics (e.g., increased spike half-width and depressed after potential amplitudes) but also produced marked differences (increased spike amplitude and little or no change in spike latency, fig. 7). Spike amplitude was increased to 116 ± 5.7% of control (SD; n = 5; P < 0.01) following application of a 20-μl drop of 250 μM 4-AP. Spike after potential amplitudes were significantly depressed by 4-AP (51 ± 5% of control; n = 8; P < 0.001) compared with the modest reductions produced by the anesthetics (76 ± 6.3% of control; n = 21; P < 0.001 combined anesthetic data).

EFFECTS ON ELECTRICALLY EVOKED ACTION POTENTIALS

Electrical activation of nociceptors, when compared with mechanical activation, provides a convenient means of separating anesthetic effects at the sensory transduction stage from effects on fiber conduction because electrical

---

**Fig. 6.** Single isolated unit, which follows mechanical stimulation (STIM) at a short latency (3.0 ms), was progressively depressed by increasing concentrations of halothane (a to d). At 1.2 vol% (1 MAC), the spike amplitude was depressed, half-width increased, latency increased, and the spike after potential was reduced (lower superimposed traces). Responses were blocked at 3.0 vol% (d) and full recovery was observed within 30 min (e and f).

**Fig. 7.** Superimposed records of an A-δ mechanoreceptor unit before (control) and after exposure to 250 μM of 4-AP. Spike amplitude increased, accompanied by an increase in half-width and a reduction in the spike after potential. Latency following stimulation was not appreciably altered by this concentration.
FIG. 8. A. A series of superimposed records showing the effects of anesthetics (1 MAC) on electrically activated single A-δ units compared with control (preanesthetic) responses. Halothane (HAL) depressed spike amplitude, increased half-width, increased latency, and reduced the spike after potential. Isoflurane (ISO) and enflurane (ENF) depressed spike amplitude, shortened latency, and depressed spike after potential. Data shown for each anesthetic were from different preparations. B. Bar graphs summarizing the effects of anesthetics (1 MAC) on spike latency (upper) and amplitude (lower). Each bar represents the mean ± SD for effects recorded from at least six experiments. All effects were significantly (P < 0.001) different from control (ANOVA), but only halothane significantly (P < 0.01) increased latency for mechanical stimulation compared with electrical activation; isoflurane and enflurane decreased spike latency. All three anesthetics reduced spike amplitude to approximately 80% of control regardless of whether responses were electrically or mechanically elicited.

excitability of some nerve fibers at clinical concentrations. In particular, halothane, isoflurane, and enflurane activated C fiber nociceptors, evidenced by an increase in their spontaneous discharge frequency (figs. 2 and 3). In contrast, A-δ mechanoreceptor fibers were depressed by these same agents at 1 MAC, although burst discharges were induced by enflurane in a few experiments. The altered pattern of discharge activity seen in the present study has also been observed on other nerve fiber preparations, and burst discharge activity induced by enflurane has been described in the central nervous system. Thus, the notion that general anesthetics produce a universal depression of neuronal activity has not received support from electrophysiologic investigations. Instead, anesthetics appear to produce combinations of excitatory and depressant effects that are dependent on the preparation and/or type of neuron investigated. Differential actions on A-δ and C fibers may result from differences in protein (e.g., receptors, ATPase, and ion channels) or lipid distribution in the membrane of these modality-specific afferents.

Haydon et al. have undertaken detailed investigations...
of anesthetic actions on ionic currents in squid axon, and found that a number of agents, including halothane, isoflurane, and enflurane, depolarized the membrane. In addition, voltage clamp experiments on squid axon demonstrated that these agents block a resting potassium conductance. Both of these actions could contribute to the excitatory effects observed in the present study. Our methodology does not permit a detailed description of mechanism(s), but the similar effects produced by the anesthetics and 4-AP (fig. 5) suggest a depression of potassium conductance comparable to effects reported on squid axon.

**Clinical Significance**

Anesthetic-induced excitation of sensory afferents could contribute to the clinical profile of anesthesia in several ways. First, anesthetic with volatile agents is associated with “tingling,” “tickling,” and/or “buzzing” sensations on both induction and during recovery. These are the type of sensations that would be expected to result from excitation of somatosensory afferents. Second, most volatile agents induce a biphasic effect on the cortical EEG; activation occurs at low concentrations, followed by depression at higher levels. Increased discharge activity in sensory afferents could contribute to EEG activation and enhance cortical evoked potentials. Finally, random high frequency discharge of sensory afferents would disrupt normal neuronal processing and lead to misinterpretation of sensory information by the central nervous system.

The different volatile anesthetics produce agent-specific effects on cardiac, respiratory, vascular, and muscle physiology. These systems receive inputs from A-δ and C fiber sensory receptors, which provide feedback for normal homeostasis. Differences in A-δ and C fiber discharge, together with the various effects on action potential parameters produced by the volatile anesthetics, may partly account for anesthetic-specific effects on physiology.

**Differential Effects of Anesthetics**

The mechanism of action of volatile anesthetics is thought to involve nonspecific interactions between anesthetic molecules and hydrophobic sites, most likely comprising the lipids of neuronal membranes. Increasing evidence points to selective, anesthetic-specific actions at both the cellular and molecular level. Direct anesthetic–protein interactions have even been postulated to account for selective actions observed on isolated biologic systems. The results of the present study support a multisite agent-selective hypothesis of anesthetic action. The similarities in action (excitation of C fibers, depression of spike amplitude, depression of spike afterpotential, and spike broadening) produced by halothane, isoflurane, and enflurane suggest common actions at some membrane sites, whereas the differential effects (opposite shifts in spike latency produced by the ethers vs. halothane, and burst firing of A-δ units induced only by enflurane) implicate discrete actions on other functional membrane components.

The authors wish to thank Drs. J. R. Trudell, J. J. Kendig, and H. B. Fairley for their helpful comments on the manuscript.

**References**

19. MacIver MB, Tauck DL, Kendig JJ: General anaesthetic modifi-
23. Landau EM, Richter J, Cohen S: The mean conductance and open time of the acetylcholine receptor changes can be independently modified by some anesthetic and convulsant ethers. Mol Pharmacol 16:1075–1081, 1979
26. Gage PW, McBurney RM, Schneider GT: Effects of some aliphatic alcohols on the conductive change caused by a quantum of acetylcholine at the toad neuromuscular junction. J Physiol (Lond) 244:409–429, 1975
34. MacIver MB, Kendig JJ: Enflurane-induced burst discharge of hippocampal CA1 neurones is blocked by the NMDA receptor antagonist APV. Br J Anaesth 63:296–305, 1989