Halogenated Inhalation Anesthetic Agents Decrease Transepithelial Electrical Measurements Across the Isolated Iris–Ciliary Body

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Transmembrane electrical measurements were performed on the isolated rabbit iris–ciliary body (I–CB) to study the direct effects of halogenated inhalation anesthetic agents on the ciliary epithelium. Addition of either halothane, enflurane, or isoflurane to the control 95% O2:5% CO2 gas mixture resulted in a dose-dependent decrease in the short-circuit current (SCC) and potential difference (PD). This response was reversible after the anesthetic gas was discontinued. Pretreatment with either α-adrenergic or β-adrenergic antagonists (phentolamine or timolol) had no effect on the halothane-induced decrease in SCC. Delivery of the analgesic gas N2O did not alter baseline electrical measurements across the isolated I–CB. Invest Ophthalmol Vis Sci 32:1912–1915, 1991

Halogenated anesthetic agents have a direct inhibitory action on in vitro ciliary epithelial transport that can explain their in vivo effect to reduce intraocular pressure (IOP).

Halogenated inhalation anesthetic agents lower IOP in man.1–7 IOP falls further as deeper levels of anesthesia are attained. The decrease in IOP is attributed to an increase in outflow facility,8 a relaxation of the extraocular muscles,8 and a decrease in the rate of aqueous humor formation.7 Studies on the human ocular mechanisms of general anesthesia-induced decreases in IOP are restricted due to the concomitant use of muscle relaxants9,10 and premedications11 that can alter IOP.

Halothane anesthesia decreases IOP in normal rabbits12 and monkeys.12,13 The decrease in IOP is not associated with alterations in outflow facility as determined by tonography.12 In laboratory animals, halothane anesthesia reduces aqueous humor flow as estimated by tonography,12 slit-lamp fluorophotometry,13 or measurement of aqueous humor ascorbate concentration in the posterior and anterior chambers.12

Inhalation anesthesia has numerous systemic effects that may indirectly alter IOP. Hypercapnia, hyperventilation, and asphyxia increase IOP, whereas hyperventilation decreases IOP.14 Increased central venous pressure and coughing, or other Valsalva maneuvers, can cause marked increases in IOP.15 Alterations in acid–base balance16 and the central nervous system11 may affect aqueous humor dynamics. In these studies, we used an in vitro iris–ciliary body (I–CB) preparation to eliminate the systemic in vivo alterations associated with general anesthesia and thereby study the effects of inhalation anesthetic agents directly on ciliary body transport.

**Materials and Methods**

Adult male pigmented rabbits weighing 2–3 kg were killed with intravenous (marginal ear vein) sodium pentobarbital (150 mg/kg) and intravenous air (5 ml). The eyes were enucleated promptly and placed in modified Tyrode’s solution that was bubbled with 95% O2:5% CO2. This solution had a pH of 7.3–7.4 and an osmolality of 294 mOsm/l with this composition (in mM): NaCl 103, KCl 4, MgCl2 1.2, CaCl2 1.8, NaHCO3 30, Na2HPO4 0.8, and glucose 5.6. The enucleated globe was bisected 3–4 mm posterior to the limbus, and the anterior half was placed with the corneal surface down in a petri dish. With the use of a surgical microscope, the posterior lens capsule was opened, the lens removed, and the zonules incised with curved microscissors to remove the lens cap-

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sule.\textsuperscript{17} Using a lamellar blade, the choroid–retina was dissected to the ora serrata and the ciliary body separated from the sclera. The I–CB was transferred on a flat spatial (with the ciliary process side up) to a mounting block consisting of two Lucite circular plates. The inner plate had a central 12-mm-wide opening with a recessed rubber O-ring. Nylon mesh was tightly stretched over the inner plate and held in place with an outer rubber O-ring. The tissue was placed, with the ciliary process side up, onto the nylon mesh and centered over the central opening. The pupil and the area of the iris between the pupil, as well as the area of the iris between the pupil and ciliary processes, were occluded with the use of a thin Lucite disc that was attached with Eastman 910 adhesive (Eastman Kodak, Rochester, NY). The outer Lucite mounting plate, with a 12 mm diameter central opening, was placed over the tissue. The tissue and mounting block were then placed between two halves of an Ussing–Zerahn-type chamber, with the ciliary process side facing the right-side chamber half.

The assembled chamber was supported in a horizontal position between two aluminum posts with adjustable screws to prevent leaking. Each chamber half was connected to a glass bubbler that was open to atmospheric pressure. The potential difference (PD) was measured through 3 M KCl–calomel cells connected to the chamber via 4% agar bridges, with the tips of these bridges placed within 1 mm of the mounted tissue. Current was passed through the tissue via another set of 4% agar bridges to voltage clamp the tissue. An automatic voltage-clamp amplifier (Biomedizinische Instruments, Munich, Germany) measured the PD, voltage clamped the tissue, and compensated for the resistance of the bathing solution. Tissue resistance was measured by recording the change in transepithelial current (delta I\textsubscript{t}) in response to 30-msec pulses of 0.2–0.3 mV (delta V\textsubscript{t}).

Transepithelial electrical measurements were obtained with both chamber halves (10 ml each) filled with identical bathing solution. Bath temperature was 24°C. The tissue was continuously short circuited except when the circuits were opened, for not more than 10 seconds, to measure PD. Stable baseline tracings of SCC were made for at least 30 min with the control gas mixture of 95% O\textsubscript{2}:5% CO\textsubscript{2} to both chamber baths. The gas mixture was then changed for both baths to include the anesthetic agent, starting with the lowest concentration. SCC was allowed to stabilize for 20–30 min before it was increased to the next concentration of the anesthetic agent. Gases were delivered via an anesthetic machine using a Copper Kettle Vaporizer. A flow meter was also used to control drug and 95% O\textsubscript{2}:5% CO\textsubscript{2} flow rates and to achieve various concentrations of the anesthetic gas. The maximum drug concentration studied was similar to the clinically used inhaled concentrations of these agents. Gas mixtures were passed through the experimental setup for 30 min before initial attachment to the chamber bubblers to minimize losses into the plastic connecting tubing.\textsuperscript{18} At the conclusion of each experiment, the anesthetic gas was discontinued and recovery was determined by bubbling the preparation with control 95% O\textsubscript{2}:5% CO\textsubscript{2}. Anesthetic agents studied included halothane (Halocarbon Laboratories, Inc., North Augusta, SC), enflurane (Anaquest, Madison, WI), and isoflurane (Anaquest). In addition, the effects of nitrous oxide (N\textsubscript{2}O), an analgesic inhalation gas that has minimal effect on IOP,\textsuperscript{12} was studied.

In separate groups of experiments, adrenergic blocking agents (5 × 10\textsuperscript{−5} M) were delivered to both chamber baths before the administration of halothane. Concentrated stock solutions of the blocking drug were prepared in Tyrode’s solution and added to the chamber in 50-μl volume. Drugs studied were the nonselective α-adrenergic antagonist phentolamine HCl (Ciba Geigy, Summit, NJ) and the nonselective β-adrenergic antagonist timolol maleate (Merck, Sharp, and Dohme Research Laboratory, West Point, PA).

Analysis of variance was used to compare results within an individual drug experiment and the unpaired t-test was used for intergroup analysis. Results were reported as the mean ± SEM. Our experiments adhered to the ARVO Resolution on the Use of Animals in Research.

Results

Baseline PD of the preparation had a consistent polarity, and the aqueous side of the tissue was negative with respect to the blood side of the tissue. Addition of the halogenated anesthetic agent, halothane, (N = 8) to the 95% O\textsubscript{2}:5% CO\textsubscript{2} gas mixture resulted in a dose-dependent reduction in the SCC (F = 5.88, P < 0.00001) and PD values (F = 7.42, P < 0.00001) (Fig. 1). Baseline SCC (13.9 ± 0.9 μA) and baseline PD (−1.04 ± 0.12 mV) were significantly (P < 0.05, Newman–Keuls multiple-range test) reduced at halothane concentrations of 1.27% or higher. Stopping the halothane flow and returning to control 95% O\textsubscript{2}:5% CO\textsubscript{2} caused a recovery of SCC (12.9 ± 1.4 μA) and PD (−0.78 ± 0.15 mV) to baseline values (P > 0.2).

Addition of either the halogenated anesthetic agent enflurane or isoflurane (Table 1) to the bubbling gas decreased the SCC in a dose-dependent manner (for enflurane, F = 6.17, P < 0.0005; for isoflurane, F = 22.4, P < 0.00001). The response to either agent was similar (P < 0.005) to that seen with halothane. The percent decrease in SCC after enflurane 3.54%
Decrease (75% reduction) in SCC after halothane decrease) was greater than that after the highest concentration of halothane (P < 0.02), whereas the decrease seen with isoflurane 2.88% (87% decrease) was similar (P > 0.3) to the decrease after the highest halothane concentration. Baseline measured tissue resistance was not altered by the administration of either halothane, enflurane, or isoflurane.

The addition of N2O to the control bubbling gas had no effect on transepithelial measurements. Baseline SCC (10.9 ± 0.9 μA, N = 6) was not altered (P > 0.7) after increasing concentrations of N2O up to 30% (9.9 ± 1.2 μA).

Baseline SCC (9.7 ± 1.0 μA, N = 8) was not altered by the addition of the nonselective β-adrenergic antagonist timolol to both chamber sides (10.2 ± 1.3 μA). Delivery of halothane resulted in a dose-dependent decrease in the SCC (F = 7.70, P < 0.0001). The decrease (75% reduction) in SCC after halothane 3.19% (2.6 ± 0.4 μA) was similar (P > 0.5) to the decrease seen with halothane without timolol pretreatment (73% reduction). SCC returned to baseline value (P > 0.5) after halothane administration was stopped.

Baseline SCC (10.6 ± 1.4 μA, N = 9) was unchanged (11.2 ± 1.7 μA) after the bilateral chamber pretreatment with the nonselective α-adrenergic antagonist phentolamine. Halothane administration caused a dose-dependent decrease in the SCC (F = 5.72, P < 0.001). The decrease in SCC after halothane 3.19% with phentolamine pretreatment (3.3 ± 0.5 μA, 71% reduction) was similar (P > 0.5) to that after halothane alone. Discontinuing the halothane resulted in a return of the SCC to baseline value (P > 0.7).

**Discussion**

Transepithelial electrical parameters across the isolated I-CB are dependent on the ionic composition of the bathing media.17-19,20 The aqueous side of the preparation is consistently negative relative to the blood side when bicarbonate is present in the bathing solution. The orientation of the PD and the direction of the SCC suggest that there is a net translocation of negative charges toward the aqueous side from the blood side of the chamber.

This study has used the isolated I-CB preparation to study direct effects of inhalation anesthetic agents on the actively driven transport system that underlies secretion of aqueous humor by the ciliary process epithelium. The halogenated inhalation anesthetic agents—halothane, enflurane, and isoflurane—decrease the transpithelial PD and SCC across the in vitro I-CB preparation. The decrease in SCC for each drug is dose dependent, and the effect is reversible after the anesthetic agent is discontinued (Fig. 1 and Table 1). The induced decrease in SCC is greatest after isoflurane 3.6% delivery. Isoflurane produces a greater reduction in IOP than halothane in adult patients.21 These findings show that the halogenated anesthetic agents have a direct inhibitory action on the ciliary epithelium and the active production of aqueous humor. This action would account for the reduction in IOP seen clinically,1-7, and in laboratory animals,12,13,15 regardless of these agents exerting other in vivo systemic physiologic effects.14,16 The analgesic inhalation agent N2O that does not reduce IOP in laboratory animals12 has no effect on in vitro transepithelial electrical measurements.

Both α-adrenergic and β-adrenergic agonists decrease SCC across the isolated rabbit I-CB.22,23 In this study, pretreatment with either the nonselective α-adrenergic antagonist phentolamine or the nonselective β-adrenergic antagonist timolol, at concentra-

| Table 1. Effect of enflurane or isoflurane on the short-circuit current across the isolated rabbit iris–ciliary body |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Enflurane       | SCC (μA ± SEM)  | Isoflurane      | SCC (μA ± SEM)  |
| (N = 8)         | (N = 8)         | (N = 8)         | (N = 8)         |
| 0% (baseline)   | 8.1 ± 0.7       | 0% (baseline)   | 10.1 ± 0.6      |
| 1.11%           | 6.1 ± 1.2       | 1.08%           | 9.0 ± 1.0       |
| 2.83%           | 2.8 ± 1.0       | 2.15%           | 11.0 ± 1.0      |
| 3.74%           | 1.6 ± 0.8       | 2.88%           | 3.0 ± 0.5       |
| 0% (recovery)   | 7.1 ± 0.6       | 0% (recovery)   | 9.5 ± 0.6       |

SCC, short-circuit current. Baseline and recovery SCC measured with 95% O2:5% CO2. Anesthetic agents delivered in 95% O2:5% CO2.
tions that block the adrenergic agonist-induced decrease in SCC, do not change the halothane effect. Therefore, the reduction in SCC by inhalation anesthetic agents is not by means of adrenergic mechanisms.

Halogenated anesthetic agents generally exert their cellular action through effects on ion channels. In the resting squid giant axon, anesthetics block a voltage-independent K+ channel that appears to be specific for these agents. However, other investigators have reported an excitation of voltage-gated Na+ and K+ currents by inhalation anesthetic agents. In human red blood cells, halothane inhibits hyperpolarization and reversibly inhibits calcium-sensitive potassium channels. Cellular mechanisms responsible for the halothane-induced reduction in transmembrane electrical measurements across the isolated I-CB require further study.

Key words: halothane, enflurane, isoflurane, ciliary body, short-circuit current (SCC), aqueous humor production, rabbit

References