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Venomotor Changes Caused by Halothane Acting on the Sympathetic Nerves

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Experiments were performed to determine whether depression of venomotor responses with halothane results from interference with sympathetic activation or from an effect on venous smooth muscle cells. Changes in isometric tension of isolated canine saphenous-vein strips were recorded. Adrenergic activation was achieved by transmural electrical stimulation, by addition of tyramine, and by addition of norepinephrine. Halothane (0.5 to 3 per cent) did not significantly alter basal tension. It lessened the reaction of the veins to electrical stimulation but not their response to norepinephrine; it increased the response to tyramine. Since the responses to norepinephrine and tyramine were not decreased, halothane appears to act on the nerve terminal to prevent release of neurotransmitter associated with nerve-terminal depolarization. Thus, halothane causes inhibition of electrically induced vasoconstriction in cutaneous veins, probably by interfering with the release of norepinephrine from nerve terminals rather than by an inhibitory effect on the smooth-muscle cells. (Key words: Anesthetics, volatile, halothane; Veins, halothane; Sympathetic nervous system, norepinephrine.)

HALOTHANE appears to dilate peripheral veins, and has been shown to depress their reactivity to reflex stimuli.[1,2] The basis of these actions is not known, but two possibilities suggested by studies in the intact organism are that halothane attenuates or abolishes sympathetic control of peripheral venous tone[3,4] or that it has a direct depressant action on vascular smooth muscle cells.[5] The first of these hypotheses appears the more likely, since—in the unanesthetized state—sympathetic outflow is the main regulator of peripheral venomotor tone.[6] However, halothane has been demonstrated to have a depressant effect on uterine and aortic smooth muscle cells.[7,8]

A convenient way to learn which of these two mechanisms predominates is to examine the action of halothane on isolated veins. The saphenous vein of the dog was used because it is an adrenergic nerve–muscle preparation in which the effects of halothane on the smooth muscle cells and those on the nerve endings can be separated.

Methods

Saphenous-Vein Strips: Source and Preparation

The experiments were performed on isolated lateral saphenous veins from mongrel dogs (15 to 25 kg) that had been anesthetized with intravenous pentobarbital (30 mg/kg). Vein segments approximately 8 cm long were excised and a helical strip, 2 to 3 mm wide and approximately 4 cm long, was prepared from each. The wet weights of single strips were 80 to 120 mg. These preparations were suspended in a jacketed organ bath (fig. 1) containing 20 ml of Krebs–Ringer's bicarbonate solution. The temperature was maintained at 37 C. The ionic concentration of the solution (in millimoles per liter) was: NaCl 118.2, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2,

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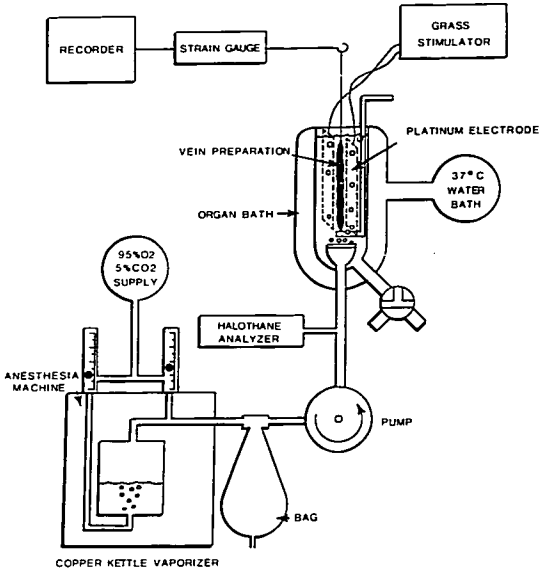

FIG. 1. Halothane system. Diagram of arrangements for mounting vein strips in organ bath and for delivering controlled concentration of halothane in mixture of 95 per cent oxygen and 5 per cent carbon dioxide.

$CaCl_2$ 2.5, $NaHCO_3$ 25, calcium disodium ethylenediamine tetraacetate (EDTA) 0.026, and glucose 11.1. The solution was aerated continuously with a mixture of 95 per cent O_2 and 5 per cent CO_2 flowing through a fritted glass disk at the bottom of the bath.

One end of the vessel strip was secured to a narrow horizontal supporting bar at the bottom of the tissue bath and the other end was connected by a silk thread to a force-displacement transducer (Grass FT03). Changes in isometric tension were recorded on a multichannel recorder (Gould Brush 220). To permit comparisons between strips, each was placed at the optimal point of its length–tension curve,[9] determined from use of a standard electrical stimulus (square-wave 9 V, 2 msec, 15 Hz for 10 sec).[10] The strips were allowed to equilibrate at the optimal length for 1½ to 2 hours prior to experimentation.

HALOTHANE

Halothane was delivered from a Copper Kettle vaporizer to give concentrations of 0.5, 1, 2, and 3 per cent in the O_2–CO_2 mixture aerating the Krebs–Ringer's solution. The concentration in the resulting gas mixture was monitored continuously by an infrared halothane analyzer (LB11) calibrated daily with a halothane mixture. In preliminary experiments, it was found that equilibration of halothane in Krebs–Ringer's bicarbonate solution was complete within 5 minutes and that stable bath concentrations were achieved if the halothane–O_2–CO_2 mixture was pumped at a constant rate (> 300 ml/min) through the fritted disk at the bottom of the bath. Delivered in this manner, the measured halothane concentrations agreed with predicted values (based on 0.70 as the partition coefficient of halothane in Krebs–Ringer's solution[11]).

To determine how long the vein strips must be exposed to halothane before the experiments begin, one must know the time required for equilibration of halothane in the venous tissue. The method used for predicting equilibration in Krebs–Ringer's solution could not be employed, since the partition coefficient between venous smooth muscle and halothane in that solution is unknown. Therefore, an indirect approach was employed. Seven vein strips taken from different dogs were suspended in the control solution and contraction was induced by 2-Hz electrical stimulation (as described below). Then the strips were incubated with 1 and 2.5 per cent halothane in the organ bath for periods of 15, 30, 45, 60, and 90 minutes, with the electrical stimulation repeated after each exposure. Equilibrium was assumed to have been attained at 30 minutes because the effect obtained at that time with each halothane concentration remained virtually unchanged for a further 60-minute period (fig. 2). Therefore, in the main series of experiments exposure to halothane was maintained for 30 minutes.

For each experiment the concentration of halothane in the bath solution was measured by a gas chromatographic method previously described.[12] Briefly, samples from the bath

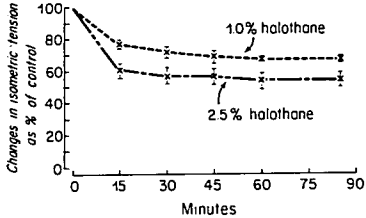

FIG. 2. Effects of 1 and 2.5 per cent halothane on responses of vein strips from seven dogs (means ± SE) to electrical stimulation (9 V, 2 msec, 2 Hz). Response without halothane (3.88 ± 0.80 g) is taken as control, and isometric tension is plotted against time as percentage of it (*scale, left*). Both curves differ significantly from control ($P < 0.01$) at each time. Beyond 30 minutes, the depressant effect levels off.

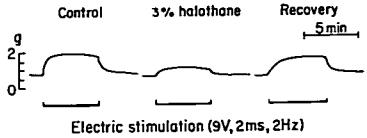

FIG. 3. Effect of halothane on contractions caused by electric stimulation in the saphenous vein. Tracings show that the response of a single vein strip to electrical stimulation was depressed by 3 per cent halothane but recovered in its absence. Interval between tracings: 30 minutes.

were aspirated and placed in airtight vials, and halothane was extracted with *n*-heptane. Aliquots of the extract were analyzed with a Barber-Coleman 5000 gas chromatograph equipped with a 6-foot column of Carbowax 400/Porasil C, 100 to 120 mesh, operating at 105 C with a ^{63}Ni electron-capture detector.[12] Prior to analysis of the samples, a calibration curve for this instrument was constructed with use of known concentrations of halothane.

ELECTRICAL STIMULATION

Two straight platinum electrodes were placed parallel to the vein strips. Supramaximal rectangular impulses of 9 V amplitude and 2 msec duration, at various frequencies between 0.5 and 10 Hz, were applied to the electrodes by a direct current power supply and a switching transistor (RCA 2N 3034) triggered by a Grass stimulator (Model S4).[9,10] Electrical stimulation was maintained for 5 minutes in each instance.

DRUG STIMULATION

Single-dose responses and dose-response curves were obtained with *l*-norepinephrine bitartrate (Winthrop Laboratories) and tyramine hydrochloride (Abbott Laboratories). Each dose of either drug was contained in 0.1 ml of Krebs–Ringer's solution added to the organ bath. The dose of tyramine is expressed as final bath concentrations of the salt, but the dose of norepinephrine is given in terms of the free base. The drugs were removed from the bath solution

TABLE 1. Effect of Halothane on Responses of Six Saphenous-vein Strips to 2 Hz Electrical Stimulation (Means ± SE)

Halothane		Response:Tension Increase (g)
In Aerating mixture	In Organ Bath (mg/100 ml)	
None	—	2.90 ± 0.20
0.5 per cent	2.70 ± 0.20	2.47 ± 0.64*
1 per cent	5.38 ± 0.28	2.30 ± 0.81*
2 per cent	10.81 ± 0.58	2.00 ± 0.74*
3 per cent	15.97 ± 0.32	1.70 ± 0.71†

* Differences from control and from response to next higher concentration significant ($P < 0.05$).
† Difference from control significant ($P < 0.001$).

by overflowing the preparations with aerated Krebs–Ringer's solution at 37 C.

General Rules

For both the electrically stimulated and the drug-induced contractile responses, the effects of different concentrations of halothane on each vein strip were compared with the control response obtained when the strips were being oxygenated with the O_2–CO_2 mixture. To confirm return to control levels, contractile responses were repeated after halothane was washed out.

In those experiments that tested the effects of halothane on responses to more than one stimulation frequency, the bath fluid was replaced after each stimulation with solution already equilibrated with halothane. Intervals of 10 minutes elapsed between stimulations, which allowed for complete relaxation of the strips. In each group of experiments, the doses of the drugs and the frequencies of electrical stimulation were randomized. The number of strips reported in the results section is the number of dogs used. Mean values are given, with standard error of the mean. For statistical evaluation of the data, Student's t test for paired samples was used. P values less than 0.05 were considered significant.

Results

Basal Tension

In the concentrations used (0.5, 1, 2, and 3 per cent), halothane did not alter basal tension significantly. However, in some individual strips a slight decrease of tension was found with exposure to the higher concentrations.

Contractile Responses

To Electrical Stimulation. Electrical stimulation (2 Hz) caused an increase of tension;

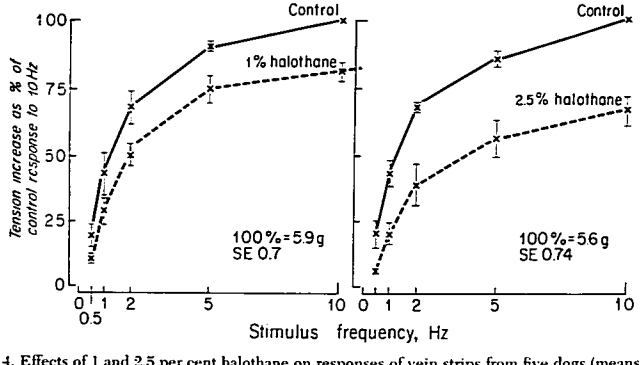

FIG. 4. Effects of 1 and 2.5 per cent halothane on responses of vein strips from five dogs (means ± SE) to electrical stimulation at five frequencies. Solid line = response without halothane. Response to 10 Hz stimulation without halothane is taken as maximum, and other data are plotted as percentages of it.

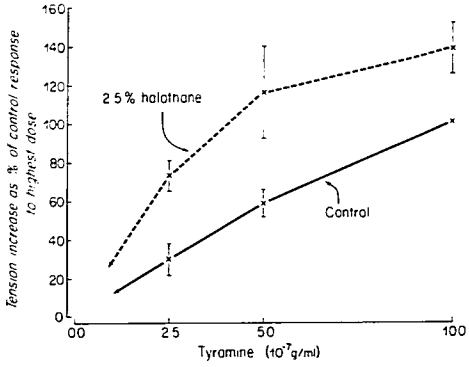

FIG. 5. Effects of 2.5 per cent halothane on responses of vein strips from five dogs (means ± SE) to increasing doses of tyramine. Solid line = response without halothane. Response to 10×10^{-7} g/ml tyramine (2.5 ± 0.7 g) is taken as 100 per cent (*scale, left*), and the other data are plotted in relation to it.

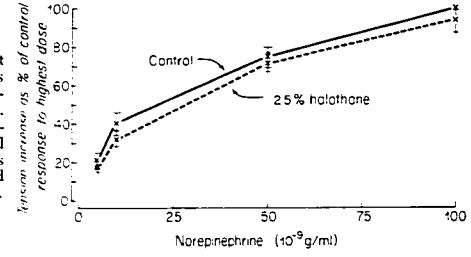

FIG. 6. Effects of 2.5 per cent halothane on responses of vein strips from five dogs (means ± SE) to increasing doses of norepinephrine. Solid line = response without halothane. Response to 100×10^{-9} g/ml norepinephrine (3.2 ± 0.9 g) is taken as 100 per cent (*scale, left*), and other data are plotted in relation to it.

this response was lessened consistently by exposure to 3 per cent halothane (fig. 3). In eight preparations from different dogs, the mean response to electrical stimulation (2.74 g ± 0.84) was depressed by 48 ± 10 per cent ($P < 0.001$). The bath concentrations of halothane averaged 15.89 ± 0.32 mg/100 ml. In a separate series of experiments, concentrations of halothane from 0.5 per cent also significantly reduced the response to 2-Hz stimulation (table 1). The effect of halothane was abolished by washing out the halothane with fresh buffer and aerating the preparation with 95 per cent O_2 and 5 per cent CO_2.

Contractions were induced in five strips by electrical stimulation at different frequencies (0.5 to 10 Hz). With halothane concentrations of 1 and 2.5 per cent, the contractile responses to all frequencies were significantly ($P < 0.05$) less than control (fig. 4). The effect of 2.5 per cent halothane on responses to the lower-frequency stimulation (0.5 to 1 Hz) was significantly greater than the effect on the response to 10 Hz ($P < 0.05$), but its effects on responses to 2 and 5 Hz were not.

To Tyramine. In five strips the contractions caused by 5×10^{-7} g/ml tyramine were increased from 2.7 ± 0.5 to 3.5 ± 0.5 g ($P < 0.05$) by 2.5 per cent halothane; at 1 per cent a similar augmentation was found. Figure 5 shows, for a separate series of experiments, the shift of the dose-response curve obtained with 2.5, 5, and 10×10^{-7} g/ml and the effect of 2.5 per cent halothane. The augmentation of the response to tyramine was reversible.

In six strips the response to tyramine (5×10^{-7} g/ml) was tested in control solution;

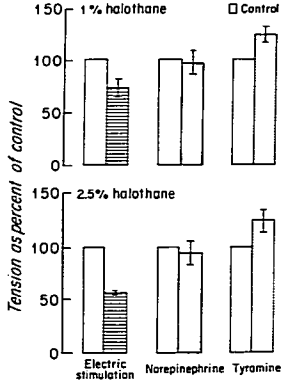

FIG. 7. Effects of halothane on matched responses of vein strips from six dogs (means ± SE) to electrical stimulation, tyramine, and norepinephrine. In each instance, response without halothane is taken as control (100 per cent, *scale, left*).

in the presence of the monoamine oxidase inhibitor pargyline (3×10^{-5} g/ml); in the presence of both pargyline and halothane; and after washing out the halothane. In these circumstances halothane did not significantly potentiate the contractions caused by tyramine (table 2).

To Norepinephrine. Contractions were obtained in six strips by addition of norepinephrine (5×10^{-8} or 1×10^{-8} g/ml). With halothane (1 or 2.5 per cent) the responses were essentially unchanged. In five other strips, 2.5 per cent halothane did not alter significantly the contractile responses to increasing doses of norepinephrine (5, 10, 50 and 100×10^{-9} g/ml) (fig. 6).

To Electrical Stimulation, Norepinephrine, and Tyramine. In six other strips contractions were obtained with electrical stimulation, norepinephrine, and tyramine. The frequency of electrical stimulation and the dose of each agent were chosen to produce contractions of comparable amplitude. With 1 per cent halothane (fig. 7, *upper*), the increases of tension caused by norepinephrine (2.22 ± 0.41 g) and by tyramine (2.84 ± 0.60 g) were 97.2 and 125 per cent of their control values, respectively; the contraction caused by electrical stimulation (2.83 ± 0.98 g) was decreased to 73.4 ± 8.3 per cent of control. Addition of 2.5 per cent halothane did not further influence the responses to norepinephrine and tyramine, but produced a significant further decrease in the response to electrical stimulation (to 57.3 ± 3.1 per cent).

Comment

The purpose of the experiments was to see whether halothane achieves its effects on peripheral veins by interfering with sympathetic nerve activity or by inhibiting the venous smooth muscle cells.

Essential to interpretation of the results is the evidence that transmural electrical stimulation excites adrenergic postganglionic nerve fibers to release catecholamines, which then activate the smooth muscle cells. Contractions of isolated vein preparations in response to such stimulation are abolished by blockade of postganglionic sympathetic transmission with bretylium tosylate and tetrodotoxin, by reserpine pretreatment, by surgical denervation, and by alpha-adrenergic blockade.[10,13,14,15] Hence, the response to electrical impulses is due to the release of neurotransmitter from the sympathetic nerve endings.

The results show that halothane, in concentrations that did not significantly alter resting tension, caused depression of the response to transmural electrical stimulation. This depression occurred with all concentrations and at all frequencies tested. In contrast to this, halothane did not significantly lessen

TABLE 2. Effects of Halothane on Responses to Tyramine in Presence of MAO Inhibitor

Control*	2.1 ± 0.3
Pargyline (3×10^{-5} g/ml)	3.7 ± 0.4
Pargyline and halothane (2.5 per cent)	4.0 ± 0.4
Pargyline (after halothane washout)	4.1 ± 0.4

* Increase in tension (g; mean ± SE) caused by 5×10^{-7} g/ml tyramine.

the contractile response to norepinephrine, and it increased that to tyramine. Since the latter causes contraction of isolated cutaneous veins by mobilization of endogenous norepinephrine, halothane does not inhibit the response of venous smooth muscle to exogenous or endogenous catecholamines.

The effects of halothane on contractions of isolated veins caused by electrical stimulation and by tyramine resemble the effect of acetylcholine, which inhibits the release of norepinephrine from nerve endings during electrical stimulation but not during addition of tyramine.[16] This suggests that halothane, like acetylcholine, acts on the nerve terminal to inhibit release of norepinephrine associated with nerve-cell depolarization. Although electrical stimulation and tyramine both liberate norepinephrine, the release by nerve stimulation depends on the presence of extracellular Ca^{++}, while that by tyramine does not.[17,18] Reduced influx of Ca^{++} may be the mechanism underlying the effect of halothane.[19,20]

In the dog's cutaneous veins the presence of the enzyme monoamine oxidase has been demonstrated.[21] Since halothane increases the response to tyramine but not that to exogenous norepinephrine, it may inhibit intraneuronal monoamine oxidase. The observation that in the presence of pargyline halothane does not potentiate the response to tyramine supports this interpretation.

Analysis of the influence of halothane on the peripheral vasculature, to date, has been based on digital and forearm plethysmographic studies in man.[1,3,4,22] Those studies showed arteriolar dilatation and increased venous compliance, which is consistent with the clinical observation that the superficial veins are dilated during halothane anesthesia. The present experiments demonstrate that venous responses to sympathetic activation are depressed in the presence of halothane, which explains the observations that the peripheral vascular effects of the agent are attenuated by sympathetic blockade.[3,4] These results are consistent with the idea that venomotor changes associated with halothane are due mainly to the action of this agent on the sympathetic nerve endings in the venous wall.

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Obstetric Anesthesia

PARACERVICAL BLOCK Following a standardized paracervical block (PCB), serial fetal and maternal blood concentrations of lidocaine were determined in ten patients. The mean maternal and fetal lidocaine concentrations reached maximum values between 9 and 10 minutes. Post-PCB fetal bradycardia was encountered, once associated with elevated fetal lidocaine concentrations and once associated with low or normal concentrations. Post-PCB fetal bradycardia was not seen in the one instance when fetal lidocaine concentration exceeded the concomitant maternal concentration. Fetal acidosis was seen in one case of post-PCB fetal bradycardia. Apgar scores were uniformly good at 5 minutes. Preliminary studies indicate that the lidocaine metabolite (monoethylglycinexylidide)/lidocaine ratio is greater in the fetus than in the mother. *(Petrie, R. H., and others: Placental Transfer of Lidocaine Following Paracervical Block, Am J Obstet Gynecol 120:791–801, 1974.)*

TRANSCUTANEOUS MONITORING OF P_{O_2} DURING LABOR Alterations of fetal heart rate and decrease of *p*H in fetal capillary blood serve as criteria of fetal hypoxia during delivery. Heretofore, *p*H monitoring has been on an intermittent basis, whereas continuous surveillance, together with continuous registration of fetal arterial P_{O_2}, would have been preferable. A Clark P_{O_2} electrode that can be attached by suction to the fetal scalp during labor has been developed. Continuous transcutaneous measurement of fetal P_{O_2} can thus be accomplished, as the scalp capillaries are arterialized by local hyperthermia. The small electrode (thickness 5.5 mm, diameter 25 mm) is attached to the fetal scalp during the second half of the second stage of labor and can remain in place for several hours, that is, until delivery is completed. The P_{O_2} of maternal blood was monitored transcutaneously as well, by application of a heated electrode to the sternum. Fetal heart rate and uterine contraction pressure were also monitored. During a uterine contraction synchronous decreases of fetal blood P_{O_2} and fetal heart rate appeared on the monitor. Maternal blood P_{O_2} rose secondary to increased ventilation during a contraction. Leaving such a P_{O_2} electrode in place throughout delivery and beyond allows continuous observation of oxygenation during the transition from placental to pulmonary gas exchange. *(Huch, A., and others: Initial Experiences with Continuous Transcutaneous P_{O_2} Monitoring of Mother and Child during Delivery, Geburtshilfe Frauenheilkd 33:856–858, 1973.)*