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Halothane and Isometric Contractions of Isolated Pregnant Rat Myometrium

Nicholas J. Naftalin, M.B., Ch.B., W. P. C. Phear, B.Sc.,† Alan H. Goldberg, M.D., Ph.D.‡*

The effects of halothane on isometric contractions of isolated pregnant uterine muscle strips were evaluated in tissue obtained from 13 midpregnant rats. Peak developed tension was depressed in a dose-related manner at halothane concentrations above 0.8 vol per cent, but was not affected at lower halothane concentrations. Time to peak tension was reduced 10–20 per cent, and relaxation time, 10 per cent, by halothane concentrations ranging up to 2.2 per cent. Total resting tension consisted of a passive component and a calcium-dependent component. In concentrations above 0.8 per cent, halothane rapidly removed 100 per cent of the calcium-dependent resting tension. At lower concentrations, halothane reduced it 50 per cent. The passive component of resting tension was unaffected by halothane. These actions of halothane can prevent postpartum hemostasis. They occur even with very low anesthetic concentrations and can be detected soon after introduction of anesthetic into the muscle bath. This indicates that the hemostatic hazards associated with the use of halothane for delivery may not be prevented by limiting the concentration of halothane or the duration of anesthetic exposure. (Key words: Anesthetics, volatile, halothane; Uterus, halothane; Pregnancy, halothane; Anesthesia, obstetric, halothane.)

NUMEROUS REPORTS have indicated that peripartum use of halothane anesthesia may cause uterine atonicity followed by intra- and postpartum hemorrhage.[1–6] Although some reports have indicated that restriction of the duration[7] or depth[8,9] of anesthesia may alleviate the severity of this complication, there is a lack of data relating changes in myometrial contractility to halothane concentration. In addition, the fact that activity of uterine muscle alters markedly during the process of parturition must be considered: stages of quiescence, profound activity, and tonic contracture follow one upon the other in quick succession. Unfortunately, these changes in themselves are poorly understood.

In the present study, the effects of halothane on several characteristics of the mechanical performance of mid-pregnant rat myometrium have been observed. The results indicate that halothane alters different components of the contraction process to various extents, and suggest a possible mechanism for the extreme sensitivity of the parturient uterus to halothane.

Methods

Thirteen mid-pregnant (7–14 day) white Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Massachusetts), weighing between 250 and 300 g, were used. After sacrifice, a strip of uterine muscle was obtained from the ante-mesometrial border and suspended between two clamps in a muscle bath, as previously described.[10] The muscle was induced to contract once each minute with a 15-volt r.m.s. 60-Hz a.c. stimulus of 5-second duration. The muscle bath contained 12 ml Krebs-bicarbonate solution, the composition of which, in mEq/l, was:

ABBREVIATIONS

RT = time to 90 per cent relaxation
T_{pd} = peak developed tension
TPT = time to peak tension
Tr_{ca} = calcium-dependent resting tension
Tr_p = passive resting tension
TrT = total resting tension

* Research Fellow in Anesthesia, Harvard Medical School and Boston City Hospital.

† Associate in Anesthesia, Harvard Medical School.

‡ Associate Professor of Anesthesia, Harvard Medical School; Visiting Physician for Anesthesiology and Director, Anesthesia Research Laboratory, Boston City Hospital.

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Address reprint requests to Dr. Goldberg, Anesthesia Research Laboratory, Boston City Hospital, Boston, Massachusetts 02118.

Na^+, 146; K^+, 5.0; Ca^{2+}, 4.5; Mg^{2+}, 2.5; Cl^-, 130; HCO_3^-, 25; HPO_4^{2-}, 1.4; SO_4^{2-}, 2.5; plus 11.1 mM glucose and 0.026 mM EDTA. The bath, which was constantly perfused with 95 per cent O_2/5 per cent CO_2 with or without halothane, was maintained at 20 ± 1 C and *p*H 7.4, and was replaced every 20 minutes.

Halothane was vaporized with 95 per cent O_2/5 per cent CO_2 delivered at 4 l/min through a Fluotec vaporizer. The gas mixture was then directed into the muscle bath and/or an equilibration vessel through sintered glass discs. All solutions were pre-equilibrated for 20 minutes for gas tension and temperature prior to being placed in the muscle bath. When the experimental protocol dictated a change in the composition of the solution, the bath was rapidly emptied and refilled with the appropriate pre-equilibrated mixture.

The rate of gas flowing to the equilibration vessel and to the muscle bath was limited to 10 ml/min by flowmeters situated just proximal to these chambers. Excess gas was discharged into a vent. Gas samples were obtained in the region of high gas flow, just proximal to the flowmeters. Three samples were obtained at each gas concentration. These were analyzed with a dual-flame gas chromatograph calibrated with commercially obtained standards (Precision Gas Products, Linden, N.J.), and measured directly in vol per cent. Peak heights of individual gas samples varied by less than 5 per cent.

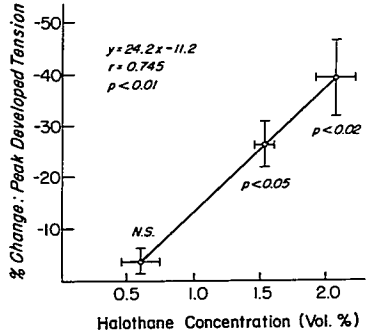

FIG. 1. Effect of halothane on isometric developed tension in pregnant rat myometrium. Brackets indicate standard errors of the mean. *P* values refer to changes in developed tension from control.

Each muscle was allowed to equilibrate for 30 minutes with a small resting tension (0.25–0.50 g). A length–tension curve was then obtained, and the muscle was studied thereafter at the length where developed tension (T_{pd}) had reached an optimal value and total resting tension (TrT) had just begun to increase steeply. The preparation was allowed to equilibrate at this length for an additional 30 minutes, by which time stress relaxation had lowered TrT to an asymptotic

TABLE 1. Effect of Halothane Concentration on Myometrial Contractility (Means ± SE)*

	Control	0.6 Per Cent Halothane	Control	1.5 Per Cent Halothane	Control	2.1 Per Cent Halothane
Peak developed tension (T_{pd}) (g/cm² × 10^{-3})	1.90 ± 0.35	1.81 ± 0.23	2.00 ± 0.42	1.43 ± 0.27	2.70 ± 0.76	1.59 ± 0.50
Significance of change	NS		$P < 0.05$		$P < 0.02$	
Time to peak tension (TPT) (sec)	7.80 ± 0.59	7.00 ± 0.50	8.10 ± 0.65	6.12 ± 1.09	7.60 ± 0.64	6.33 ± 0.36
Significance of change	$P < 0.01$		$P < 0.01$		$P < 0.05$	
Time to 90 per cent relaxation (RT) (sec)	28.00 ± 4.80	25.45 ± 3.71	31.00 ± 5.30	27.18 ± 3.88	32.00 ± 5.20	28.73 ± 4.32
Significance of change	$P < 0.05$		$P < 0.05$		NS	

NS = not significant.
* All data from Group I (n = 7).

TABLE 2. Total, Passive, and Calcium-dependent Resting Tensions in the Presence and Absence of Halothane (Means ± SE)*

	Control	Halothane Concentration (Mean)		
		0.6 Per Cent	1.6 Per Cent	2.2 Per Cent
Resting tension, total (TrT) (g/cm² × 10^{-3})	1.63 ± 0.14	1.16 ± 0.27	0.99 ± 0.25	0.89 ± 0.31
Resting tension, passive (Tr_P) (g/cm² × 10^{-3})	0.88 ± 0.14	0.76 ± 0.25	0.99 ± 0.25	0.89 ± 0.31
Resting tension, calcium-dependent (Tr_{Ca}) (g/cm² × 10^{-3})	0.75 ± 0.14	0.40 ± 0.07	0	0

* All data from Group II (n = 6).

value. A control contraction was then recorded.

Seven muscles (Group I) were then exposed to three concentrations of halothane administered in random order for 20-minute periods, alternating with similar control periods without halothane. One contraction was recorded at the end of each exposure to halothane and O_2/CO_2. In each myogram, in addition to T_{pd}, the time to peak tension (TPT: the time from the onset of the stimulus to T_{pd}), and time to 90 per cent relaxation (RT: the time for T_{pd} to decline by 90 per cent were also measured. The average halothane concentrations (vol per cent) were (means ± SD): 0.61 ± 0.37, 1.54 ± 0.19, and 2.09 ± 0.15 per cent.

Six muscles (Group II) were exposed to three halothane concentrations (means ± SD): 0.58 ± 0.27, 1.61 ± 0.22, and 2.18 ± 0.51 per cent. In these muscles, at the end of each 20-minute equilibration period with the anesthetic, the bathing medium was replaced with a solution which contained the same halothane concentration as had been present in the bath, and 1 mM EGTA, but did not contain any calcium or EDTA. After resting tension was recorded under these conditions, the bath solution was replaced with one which was equivalent to the last, but contained no anesthetic.

This organization of the Group II experiments allowed us to measure resting tension in the presence and absence of both calcium and halothane. Thus, the effects of halothane on calcium-dependent tone (Tr_{Ca}) and non-calcium-dependent or passive tone (Tr_P) could be evaluated. Tr_P was taken as total resting tension (TrT) minus Tr_{Ca}.

The Group II muscles, in the calcium-free solution, were then re-exposed to halothane for an additional 15 minutes and the effects on resting tension were observed.

At the end of each experiment, muscle length was read from a micrometer. Average cross-sectional area for all muscles, calculated on the basis of a specific gravity of 1.054,[11] was 0.919 mm² ± 0.14 (SD). All tensions were corrected for cross-sectional area.

The statistical analyses used were Student's t test and least squares regression, with $P < 0.05$ taken as the level of significance.

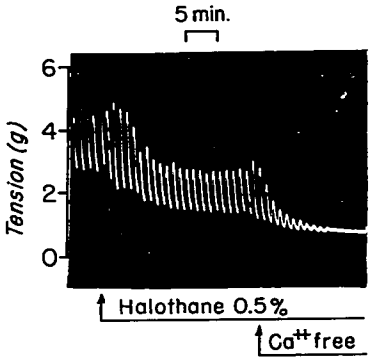

FIG. 2. Effects of 0.5 per cent halothane and removal of calcium on resting tension. A decrease of approximately 50 per cent in TrT occurred following exposure to halothane and an additional 20 per cent reduction occurred in the calcium-free medium.

Results

Group I

T_{pd} was not significantly affected at the lowest halothane concentration, but was reduced by an average of 26 per cent with 1.54 per cent halothane and 39 per cent with 2.09 per cent halothane (table 1). The percentage change in T_{pd} *vs.* halothane concentration fit a statistically significant least-squares regression (fig. 1).

TPT was reduced 10–20 per cent by halothane, irrespective of concentration (table 1). This change was consistently significant, but no linear relationship between TPT and halothane concentration could be demonstrated.

RT was shortened by 10 per cent (table 1) at all halothane concentrations. This change was significant at the two lower concentrations, but not at 2.09 per cent halothane.

Group II

In the standard Krebs–bicarbonate solution, TrT was reduced an average of 32, 46, and 51 per cent by 0.6, 1.6 and 2.2 per cent halothane, respectively (table 2, figs. 2 and 3).

After equilibration with 0.6 per cent halothane, the removal of calcium reduced resting tension an additional 35 per cent (from 1.16 g/cm^2 × 10^{-3} to 0.76 g/cm^2 × 10^{-3}; table 2, fig. 2). At the higher halothane concentrations, the removal of calcium elicited no further alteration in resting tension (table 2, fig. 3).

In the calcium-free solution, resting tension remained unchanged during exposure to any concentration of halothane.

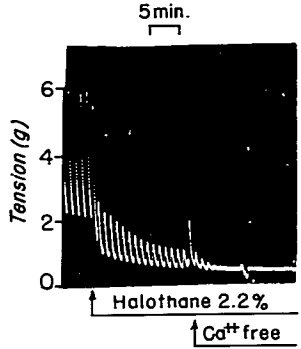

FIG. 3. Effects of 2.2 per cent halothane and removal of calcium on resting tension. At this anesthetic concentration, removal of calcium elicited no alteration in TrT.

Discussion

Previous authors have attempted to show depressed performance of isolated human myometrial strips with halothane. However, in these reports, interpretation of halothane effects is made difficult by the use of small numbers of spontaneously contracting muscles and definitions of contractility that are imprecise[12,13] or complicated by varying frequencies of contractions.[14]

The present study, which contains precise definitions of all measurements, was performed on rat muscle at 20 C because at this temperature a steady experimental state can be achieved and any change can be precisely quantitated without interference from spontaneous muscle activity.[15,16] Cyclic mechanical changes in myometrial activity have been demonstrated at 20 C, indicating that muscle retains its sensitivity to ovarian and placental hormones at this temperature.[10]

The changes in uterine muscle that occur during labor and delivery are dependent on the development of retraction, *i.e.*, a progressive shortening of the resting length of the upper segment of myometrium. Maximal shortening is prevented during labor because of the presence of the products of conception *in utero* and the resistance to stretch of the lower uterine segment and cervix. After delivery, when these restrictions on shortening are removed, the uterus retracts fully, and in so doing exerts a tourniquet-like action on vessels in the placental bed. This is the hemostatic mechanism of the intra- and immediate postpartum period. It is apparent that any agent that interferes with the development of retraction could be responsible for hemorrhage at the time of delivery.

Initial favorable reports on the use of halothane at the time of delivery[5-17] were modified when it became apparent that significant loss of uterine "tonicity" occurred

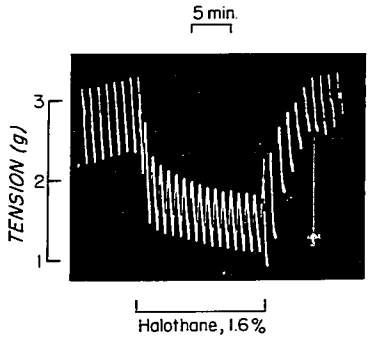

FIG. 4. Effect of halothane on uterine contractility. The change in TrT was immediate. There was a 2-minute delay before the effect on T_{pd} was apparent.

with its use.[1-6] Nevertheless, halothane is still used in obstetric practice,[18] and the uterine relaxation it causes may be of benefit under certain circumstances (*e.g.*, internal podalic version or mid-forceps rotation when the uterus has clamped down on the fetus).

The muscle shortening that occurs in the *in-vivo* phenomenon of retraction would be expected to appear in the present isometric experiments as the calcium-dependent component of resting tension. The major action of halothane even at low concentrations is on this manifestation of retraction. It appears that it is through this mechanism that halothane prevents the normal hemostatic mechanisms from occurring.

We found total resting tension to be composed of two components: a calcium-dependent component, and a second, purely passive component. The major effect of halothane, irrespective of concentration, was upon that portion of TrT which is calcium dependent (Tr_{Ca}). Halothane, in concentrations of 1.5 per cent or more, removed 100 per cent of Tr_{Ca}, and even in low concentrations exerted a large depressant effect on this variable. No halothane concentration had any effect on the purely passive component of resting tension (Tr_P).

Developed tension in this study correlates clinically with contractions of the uterus during labor. It was unaffected by the lowest concentrations of halothane. Higher concentrations did exert a depressant effect on T_{pd} and would be expected to have a similar effect on contraction in the intact uterus.

This reduction in isometric developed tension is related to the shortened time to peak tension. However, this does not completely explain the change in T_{pd}, since the shortened TPT is constant at all halothane concentrations studied, whereas the effect on T_{pd} is progressive with increased halothane concentration and is of much greater magnitude. Presumably, some other factor, such as a direct effect on contractile proteins or a reduction in availability of intracellular calcium, is also operative.

The latter hypothesis could also explain the shortened relaxation time. Since the relaxation process involves translocation of myoplasmic calcium into intracellular organelles, if less calcium is to be sequestered, less time is needed for this process.

The site of action of halothane on pregnant uterine muscle remains to be identified. The rapidity of halothane's effect on resting tension (fig. 4) indicates that a superficial site, such as the cell membrane, may be involved. A separate mechanism may be associated with halothane's more delayed effect on developed tension.

The effects of anesthetics on the cell membrane have been reviewed recently.[19] Halothane causes an expansion of the volume of the cell membrane in erythrocyte ghosts.[20] This change, which is ten times greater than the expected expansion due to the volume of anesthetic itself, is associated with increased fluidity in the lipid fraction of membrane. This in turn may alter membrane protein function and calcium-dependent ATPase activity.[21] Such actions of halothane may be important in producing the rapid loss of calcium-dependent tone observed in the present experiments. This effect may be compounded by the depressant action of halothane on myofibrillar ATPase activity[22] and by conformational changes in the contractile proteins themselves.[23]

Since the experiments were performed *in vitro* at 20 C, the relationship of the findings of this investigation to processes occurring *in vivo* at 37 C remains uncertain.

Nevertheless, the present study clearly demonstrates that calcium-dependent resting tension in strips of pregnant rat myometrium is profoundly reduced by halothane concentrations between 0.4 and 0.8 vol per cent and is completely obliterated by halothane concentrations above 1.5 vol per cent. We believe this to be the most important effect of halothane on this pregnant mammalian myometrium.

Developed tension was not affected by halothane concentrations less than 0.8 vol per cent. At concentrations greater than 1.5 vol per cent, developed tension was depressed in a dose-related manner.

As the major depressant effects of halothane are evident at low concentrations and occur rapidly after introduction of the anesthetic into the muscle bath, we conclude that the hazards associated with the use of this agent for delivery may not be prevented by limitation of either the duration of exposure or the concentration of the anesthetic, at concentrations of 0.6 vol per cent or more.

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