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# Cellular Actions of Halothane on Cat Cerebral Arterial Muscle

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**SUMMARY** The effects of halothane on intracellular membrane potential ( $E_m$ ) and force development in cat MCA were studied. Halothane (0.07–0.14 mM/l) relaxed isolated MCA which had developed myogenic tone. Measurement of  $E_m$  showed that halothane depolarized this preparation in a dose-dependent fashion in the face of vessel relaxation, demonstrating uncoupling of electrical and mechanical activity. Halothane markedly inhibited the contractile effects of histamine and serotonin suggesting that, apart from its direct action on cerebral arterial tone, it also blunts the action of vasoactive agents. When this preparation is partially depolarized from  $-62$  to  $-50$  mV with excess  $K^+$ , halothane, while having only a small (1.2 mV) additional depolarizing effect, consistently elicits contraction rather than relaxation. Thus, the action of this particular volatile anesthetic on cerebral arteries can depend upon the resting level of  $E_m$ . These studies indicate that halothane relaxes myogenic tone in cat MCA by an intracellular mechanism, but that the direction of its effect (i.e., relaxation vs. contraction) may depend upon the prior level of  $E_m$  and muscle cell activation.

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WITH RESPECT TO THE CARDIOVASCULAR SYSTEM, the predominant effects of halothane anesthesia are depressor. Halothane inhibits myocardial functions and decreases peripheral vascular resistance.<sup>1-3</sup> The direct depressor actions of halothane on cardiac muscle appear to involve alterations in intracellular  $Ca^{2+}$  handling and reduction of  $Ca^{2+}$  availability to contractile elements;<sup>4,5</sup> such actions may involve depression of sarcoplasmic reticulum (SR) function.<sup>6</sup> Similarly, in sinoatrial (SA) nodal tissue, halothane exerts negative chronotropic actions which can be partially attributed to reduction of inward current during the action potential and partially due to inhibition of intracellular  $Ca^{2+}$  availability.<sup>7</sup>

Halothane increases cerebral blood flow and can increase intracranial pressure demonstrating its potent cerebral vasodilatory action.<sup>8</sup> However, it is not known whether such effects are direct or result from changes in cerebral metabolism and/or inhibition of normally occurring vasoactive neurotransmitters or hormones. Halothane can also abolish cerebrovascular autoregulatory responses to changing arterial blood pressure.<sup>9,10</sup>

Halothane, like most volatile anesthetics, increases membrane fluidity in a variety of cell types.<sup>11,12</sup> This action of halothane suggests that it can exert direct effects on muscle cells by a mechanism involving changes in membrane potential and in ionic permeabilities. It is the purpose of these studies to determine the

direct action of halothane on mechanical and electrical properties of isolated cat middle cerebral artery (MCA) in order to more clearly define its potent dilatory action on cerebral arteries, and to determine if the predominant actions occur through membrane electrical events or via intracellular mechanisms.

## Methods

Adult mongrel cats (2.5–4.0 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and decapitated. The brain was removed and placed in cold, oxygenated Krebs's solution. MCA were removed and used for study. Three mm segments of artery were mounted in a specially fabricated myograph to record force development. The lumen of the segment was threaded with 2 pieces of 22  $\mu$ m tungsten wire. The wires were stretched over the open jaws of 2 stainless steel rings. One ring was anchored and the other connected to a very sensitive load cell (Kulite Semiconductor Products, Inc.; Ridgefield, N.J.). Under control conditions the preparation was continually suffused with a physiological salt solution containing (in mM):  $Na^+$  141,  $K^+$  4.7,  $Cl^-$  125,  $Ca^{2+}$  2.5,  $Mg^{2+}$  0.76,  $H_2PO_4$  1.7,  $HCO_3^-$  25, glucose 11 and HEPES (N-2-hydroxy-ethylpiperazine-N-2-ethane-sulfonic acid) 5. Solutions were aerated with 95%  $O_2$ /5%  $CO_2$  giving a pH of 7.35–7.42 and  $PCO_2$  of 35–40 torr. Temperature was maintained at 37° C. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane without .01% thymol) was aerated into the solution via a Draeger Vaporizer at 1.5 to 2.5 vol. %. This level of halothane vaporization gave levels of 0.07 to 0.14 mM/l analyzed on a gas chromatograph. Arterial segments were placed under 400 mg passive load and allowed to equilibrate for 90 min prior to study, during which time they developed between 200 and 400 mg of myogenic tone. Histamine ( $10^{-7}$  to  $10^{-3}$  M) was added to the bathing solutions in cumulative doses.

For measurements of  $E_m$ , glass microelectrodes were used according to techniques previously described.<sup>13</sup> They were filled with 3 M KCl and had tip resistances between 50 and 80 megohms, with tip po-

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tentials less than 5 mV. All impalements were made from the adventitial surface of the arterial segments. The criteria for a successful impalement included no change in electrode resistance or tip potential before and after each impalement, a sharp drop in voltage upon entry of the microelectrode into the cell and a sharp return to zero upon exit. The recording amplifier was a Dagan 8100 possessing capacitance neutralization and an internal bridge circuit. Cell impalements were made with the aid of a sliding micromanipulator (Zeiss) and data displayed on a Nicolet digital oscilloscope.

## Results

### Inhibitory Action of Halothane on Middle Cerebral Artery

Middle cerebral arterial segments were mounted in a muscle myograph and equilibrated with a passive load. Ninety percent of the preparations developed myogenic tone ranging from 100 to 400 mg. As can be seen in figure 1, halothane at 2.0 and 2.5 vol. % relaxed these arterial segments. Similar responses were observed in all ten (100%) vessels studied in this manner.

To determine the effects halothane might have on drug induced responses of cat MCA, dose-response curves for histamine-induced mechanical activity were done in the presence and absence of halothane (1.5 vol. % or 0.07 mM/l). A typical experiment in this regard is depicted in figure 2. As can be seen, halothane markedly inhibits both the relaxant (occurring at  $10^{-6}$  and  $3 \times 10^{-6}$  M) and contractile ( $10^{-5}$  to  $10^{-3}$  M) actions of histamine. Quantitation of six such experiments (representing 94% of all preparations studied in this manner) is given in figure 3. Halothane abolishes the relaxant actions of histamine and decreases both sensitivity and contractility to histamine effects.

We examined the effect of halothane on maximum serotonin-induced contractions. As can be seen in figure 4, halothane (1.5 vol. % or 0.07 mM/l) significantly reduced the maximum contraction induced by  $10^{-6}$  M serotonin. Included in figure 4 is the maximum contractile actions of histamine before and after halothane (1.5 vol. %) as compared with the percent maximal activation induced by serotonin.

### Effect of Halothane on the Membrane Potential ( $E_m$ ) of Cat Middle Cerebral Artery

$E_m$  was measured intracellularly with glass microelectrodes from muscle cells of cat MCA. The control  $E_m$  was  $-62 \pm 2.0$  mV. Halothane at 1.5 and 2.0 vol. % (0.07 to 0.14 mM/l) significantly depolarized

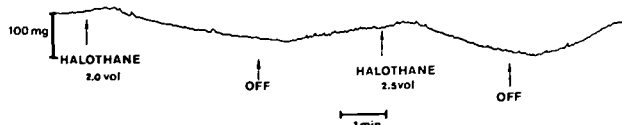


FIGURE 1. Chart recording showing the typical relaxation of tension occurring in a segment of cat middle cerebral artery exposed to two levels of halothane. Note the increase in tone after halothane was turned off.

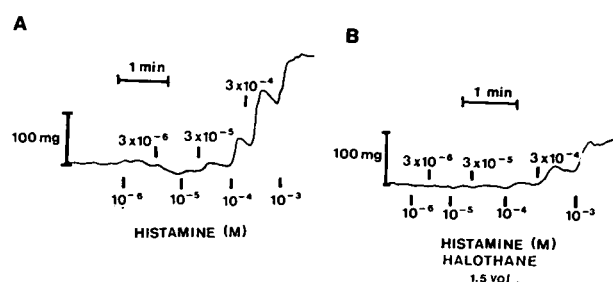


FIGURE 2. Chart recording of a typical dose-dependent curve for histamine induced mechanical activity in a segment of cat middle cerebral artery in the presence and absence of halothane. Note the inhibitory action of halothane on the relaxant action of histamine and the diminution of the magnitude of the contractile response.

all of the 7 preparations studied to a mean value of  $-53 \pm 2.1$  and  $-45 \pm 1.6$  mV respectively (fig 5). This finding demonstrates an uncoupling of electrical and mechanical events by halothane in this preparation in that the vessel relaxation is occurring while the muscle cells are depolarizing. Taken together, such data suggest that halothane, while having direct effects on ion permeabilities resulting in reduction of  $E_m$ , induces relaxation via an intracellular mechanism. If halothane worked via electromechanical coupling in this vessel the membrane depolarization would result in muscle activation, not the observed relaxation.

However, when MCA are partially depolarized and activated by 30 mM  $K^+$  (corresponds to an  $E_m$  of  $-46 \pm 2.0$  mV and 25% maximum activation) halothane

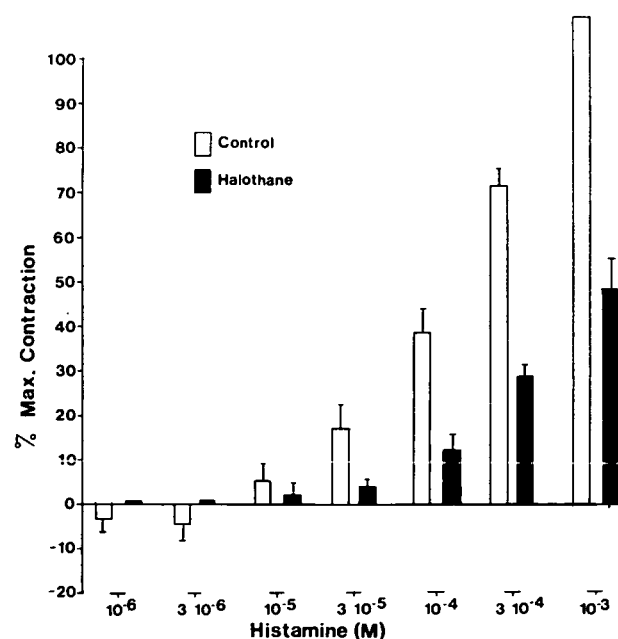


FIGURE 3. Bar graph showing averaged percent of maximum contraction at increasing doses of histamine in segments of cat middle cerebral artery in the presence or absence of halothane. Note that halothane suppressed the usual relaxation to histamine and significantly reduced the maximum contractile response. Lines at the top of each bar represent the standard error of the mean from 6 experiments.

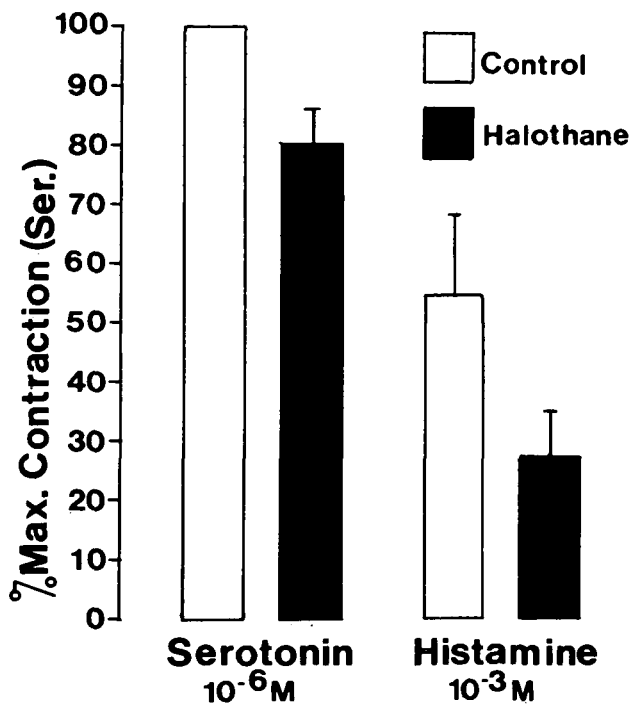


FIGURE 4. Bar graph comparing the effect of halothane on percent maximum contraction to serotonin in serotonin and histamine-treated segments of cat middle cerebral artery. Note that halothane reduced the contractile response to both agents.

induced contraction (fig. 6). Similar findings were observed in 94% of all preparations ( $n = 6$ ) studies in this fashion. Thus, at a normal  $E_m$  of  $-62$  mV halothane relaxes cat MCA, but when the muscle cell membrane is partially depolarized to  $-46$  mV halothane contracts this particular preparation. Similar phenomena were observed in all 5 preparations studied in this manner. Furthermore, when halothane (0.07 mM/l)

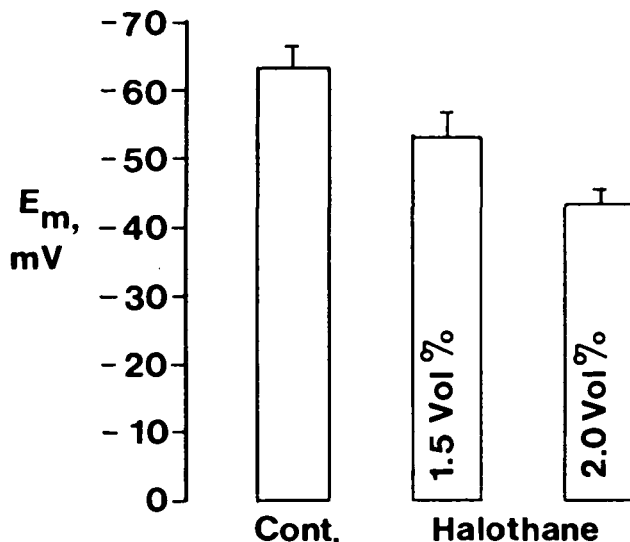


FIGURE 5. Bar graph showing  $E_m$  in segments of cat middle cerebral artery under control conditions and in the presence of two levels of halothane. Note that halothane depolarized the vessel and that depolarization was greater at a higher level of halothane.

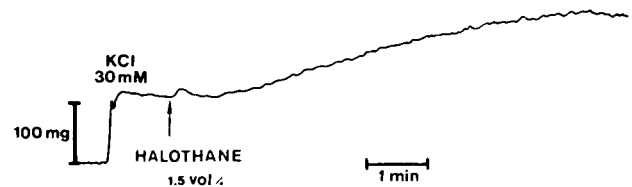


FIGURE 6. Chart recording showing increased contractile activity induced by halothane in segments of cat middle cerebral artery that were previously treated with 30 mM KCl. Similar data were observed in 100% of 6 preparations studied in this manner. The mean force development ( $\pm$  S.E.) to this dose of halothane was  $214 \pm 64$  mg.

was added to preparations depolarized to  $-46$  mV with excess  $K^+$ , only a small additional depolarizing effect was observed in 100% ( $n = 6$ ) of these preparations. The  $E_m$  obtained in the presence of 30 mM  $K^+$  plus halothane was  $-43 \pm 1.7$  mV, a value not significantly different from that recorded in 30 mM  $K^+$  alone, further supporting the concept of electromechanical uncoupling.

#### Discussion

The results of this study demonstrate that halothane inhibits myogenic tone and significantly reduces the contractile effects of histamine and serotonin. The effect of halothane on myogenic tone appears to work via an intracellular mechanism rather than through electromechanical coupling. Uncoupling of electrical and mechanical activity (i.e., depolarization in the face of contraction) is a unique property of halothane in that we are unaware of similar actions mediated by any other pharmacological agents. It would appear that the action of halothane in increasing cerebral blood flow<sup>8</sup> may work through direct inhibitory actions on arterial muscle. The data presented here do not allow direct comparison of the intracellular effects of halothane on  $Ca^{2+}$  availability in cardiac tissue<sup>4,6</sup> with those observed in cerebral arteries, but may prompt future experiments in this regard.

Halothane can destroy autoregulatory behavior in the face of changing arterial blood pressure.<sup>9,10</sup> We have found that myogenic autoregulation of cerebral arteries is dependent upon changes in  $E_m$  of smooth muscle cells (i.e., electromechanical coupling).<sup>14</sup> The finding that halothane uncouples electrical and mechanical events may provide a cellular mechanism through which attenuation of cerebral blood flow autoregulation by halothane occurs.

Our finding that halothane contracts cerebral arteries which are partially depolarized is difficult to reconcile with its inhibitory action on myogenic tone. Depolarization of arterial muscle usually results in activation by increasing  $Ca^{2+}$  conductance through voltage dependent channels.<sup>15-17</sup> It may be that once  $Ca^{2+}$  channels are activated by partial depolarization with excess  $K^+$  halothane can then augment conductance through these sensitive ionic channels. If such a hypothesis is indeed correct, it may be linked to halothane's action of changing membrane fluidity, there-

by, altering ionic permeabilities.<sup>11, 12</sup> An increase in membrane fluidity by halothane may result in the observed depolarization through inhibition of ionic conductances, such as  $K^+$ , by not allowing the channel to remain in a fixed configuration within the membrane. Obviously, more experiments are needed to clarify the direct action of halothane on the plasma membrane.

Since the action of halothane on cerebral arterial muscle appears to be modified by the level of  $E_m$ , its effect on cerebral blood flow may depend upon metabolic and/or humoral conditions which might regulate resting  $E_m$  in this tissue (e.g., cerebral spinal fluid  $K^+$ , adenosine or neural activity). Such a mechanism may help explain the wide variability in cardiovascular responses to halothane anesthesia in humans.

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