Effects of Halothane on Single Atrial, Ventricular, and Purkinje Fibers

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ABSTRACT

The action of halothane on sinoatrial, ventricular, and Purkinje fibers was studied by means of intracellular microelectrodes. Rabbit atrial fibers were not very sensitive to even high (2%) concentrations of halothane. Sheep Purkinje fibers showed a significant hyperpolarization from 82 mv to 87 mv, a marked shortening of the duration of the action potential from 370 msec to 233 msec, and a diminution of the overshoot from 30 mv to 21 mv under the influence of 1% halothane. The rate of rise was not significantly changed with this concentration. In the presence of 2% halothane the resting potential did not differ significantly from the value obtained with 1% halothane. The duration of the action potential and of the refractory period were further diminished to 165 msec and to 109 msec, respectively. The rate of rise was now significantly reduced to 245 v/sec and the overshoot further decreased to 17 mv. Conduction time was increased by about 20% with 1% halothane and more than twofold with 2% halothane. Ventricular fibers of sheep did not show significant changes of the resting potential under the influence of 2% halothane. However, the duration of the action potential was markedly shortened from 338 msec to 231 msec, the effective refractory period was significantly reduced from 295 msec to 208 msec, and the overshoot decreased from 21 mv to 11 mv. Possible mechanisms underlying these changes and which may cause arrhythmias are discussed.

ADDITIONAL KEY WORDS
anesthetic rate of rise arrhythmias membrane potentials refractory period conduction time

Cardiac arrhythmias frequently occur during halothane anesthesia, especially when sympathomimetic amines are administered (1, 2). Although halothane is a widely used general anesthetic agent, little is known about its action on the refractory period and other electrical parameters of single heart cells.

Using extracellular electrodes, Schaer (personal communication) recently found that the maximal driving rate of guinea pig atria was increased under the action of 0.5% to 3.0% halothane. This effect was still present in atria of animals pretreated with reserpine. However, Awalt and Frederickson (3) found with intracellular recordings that halothane did not significantly alter the action potentials of rabbit atria. Hauswirth and Schaer (4) recorded action potentials from single sinoatrial (S-A) nodal fibers of the rabbit under the influence of halothane. Their results showed a significant change of most of the electrical parameters, a decrease of the maximal diastolic potential, and also a decrease of the rate of diastolic depolarization. As a consequence, S-A nodal discharge rate could be decreased but also markedly increased. In addition, Chalazonitis et al. (5-7) measured membrane and action potentials and relative membrane resistance on nerve cells of Helix and Aplysia under the influence of halothane. They found a hyperpolarization of the membrane, a depression of the action potential, and a decrease of relative membrane resistance.

The differing sensitivity of various heart cells to halothane suggested a more systematic investigation of the mechanism by which halothane might promote the tendency toward cardiac arrhythmias. The present experiments
were carried out to show the influence of halothane on single cells of different heart tissues by means of electrophysiological techniques.

**Methods**

Rabbits of unselected breed (300 to 500g) were stunned and their hearts were quickly removed. Right atria were isolated and placed immediately in oxygenated Tyrode's solution. A piece of tissue of about 2 by 2 mm which did not show spontaneous activity was dissected under microscopic observation and then mounted in a perspex chamber. Sheep hearts were obtained from the slaughter house immediately after the sheep had been killed and placed in cooled (4°C) Tyrode's solution. Purkinje strands and thin trabecular muscles were cut out of the left ventricle not later than 45 minutes thereafter and then fixed in the perspex chamber. Tyrode's solution (composition in mM: Na⁺, 148.3; K⁺, 5.4; Ca²⁺, 1.8; Mg²⁺, 0.5; Cl⁻, 146; HCO₃⁻, 12; H₂PO₄⁻, 0.35; glucose 5g/liter), aerated with 95% O₂-5% CO₂ and kept at 37°C, flowed from one of several reservoirs continuously through the chamber. The test solution contained predetermined amounts of halothane. This solution was obtained by leading the gas mixture through a calibrated halothane vaporizer (Draeger, Luebeck) and from there to one of the reservoirs. The equilibration of the test solution with halothane was assumed to be complete after 30 minutes, since longer equilibration times did not alter the effects produced by halothane. Details of this method, the degree of accuracy, and the checking of the actual halothane concentration have been previously described (4). Before administering the test solutions, the preparations were allowed to equilibrate in the bath for at least 1 hour. Intracellular microelectrodes filled with 3M KCl that had a d-c resistance between 10 and 40 megohms were used for recording. The electronic setup consisted of a differential cathode follower, an oscilloscope (Tektronix 502), and a Grass S4-stimulator with a stimulus isolation unit (SIU 4B). The recording system responded to square waves with a time constant of the order of 50 μsec provided that the microelectrodes had d-c resistances of about 10 megohms. Since microelectrodes with d-c resistances up to 40 megohms were used, in some experiments the tracings may have been considerably distorted.

The preparations were driven at a rate of 30/min by applying shocks of supramaximal voltage and 0.5 msec duration through extracellular electrodes. The rate of rise was determined by switching an R-C unit (time constant 20 μsec) between the cathode follower and the amplifier input. The rising phase and the crest of the action potential were brightened by a pulse derived from the amplifier output and fed to the grid of the cathode ray tube. The signals were displayed on the screen of the oscilloscope and photographed with a camera (Robot Royal). In all types of preparations resting and action potentials, rate of rise, and the effective refractory period were measured. In atrial and ventricular muscle it was possible only a few times to record the differentiated upstroke, and therefore no values are given in the appropriate tables. In three Purkinje fibers the conduction time was roughly estimated by differentiating the signal and recording the tracing at high sweep speeds (1 msec/cm). The time lag between the stimulus artifact and the differentiated rising phase was determined. The relative change of conduction time given as a percent of control could therefore be estimated without knowing the exact distance between the stimulating extracellular electrode and the recording microelectrode. Measurements of the effective refractory period were performed by applying double pulses of variable time interval and twice the diastolic threshold (8). The results given in the tables are means ± SEM. Significances of differences were calculated using Student's t-test.

**Results**

Tables 1, 2, and 3 show the changes in electrical measurements of single cells in different cardiac tissues under control conditions and under the influence of halothane. A set of typical action potentials as recorded from different heart tissues under the same conditions is shown in Figure 1. Action potentials of the rabbit S-A node are also shown (A) to allow a quick overall estimate of the action of halothane on heart tissues. Figure 2 shows the influence of halothane on the refractory period of atrium (A) Purkinje fiber (B) and ventricular muscle (C).

**Effects on Electrical Measurements of Rabbit Atrial Fibers**—Although the overshoot was significantly decreased and the 90% repolarization was slightly prolonged, these changes were not very pronounced. This is consistent with previous findings (3). The duration of the 50% repolarization showed a slight shortening (Figs. 1B, 2A, and Table 1). Moreover, these observations show that atrial cells are not very sensitive, even to higher concentration of halothane (2%). In particu-
HALOTHANE AND CARDIAC FIBERS

FIGURE 1
Action potentials under control conditions (left) and under the influence of 2% halothane (right) recorded from rabbit S-A node (A), rabbit atrium (B), sheep Purkinje fiber (C), and sheep ventricular muscle (D). Tyrode's solution, 37°C. Abscissa: time. Ordinate: voltage.

lar, the absence of marked changes in the resting potential or of amplitude of the action potential is in striking contrast to the impressive changes which S-A nodal fibers undergo with 2% halothane (4). At present no explanation can be offered for this difference.

Effects on Electrical Measurements of Sheep Purkinje Fibers.—With halothane all electrical measurements of cardiac Purkinje fibers show significant changes from the control value. With 1% halothane the resting potential was increased and the overshoot and the duration of the action potential were decreased (Table 2). As far as it could be determined within the limits of the recording apparatus, the rate of rise was not markedly changed. With 2% halothane the resting potential was not significantly different from the value recorded with 1% halothane. The overshoot and the duration of the action potential were further decreased. The rate of rise and the effective refractory period now decreased (Figs. 1C, 2B, and Table 2). The conduction time increased to 122% (± 5.7) of control with 1% halothane and to 211% (± 13.2) with 2% halothane.

Effects on Electrical Measurements of Sheep Ventricular Cells.—Since the myocardium proved to be much less sensitive to halothane than Purkinje fibers, only a relatively high concentration (2%) was used to investigate the effect of halothane on these cells. In the presence of halothane, the resting potential was practically unchanged, the overshoot decreased significantly, the duration of the action potential was reduced, and the effective refractory period was shortened.

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TABLE 1
Measurements of Action Potentials of Rabbit Atrial Muscle

<table>
<thead>
<tr>
<th></th>
<th>Resting potential (mv)</th>
<th>Overshoot (mv)</th>
<th>Duration of action potential (msec)</th>
<th>Effective refractory period (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.1 ± 0.8 (20)</td>
<td>16.5 ± 0.9 (21)</td>
<td>99.8 ± 1.6 (21)</td>
<td>55 ± 1.8 (16)</td>
</tr>
<tr>
<td>1% Halothane</td>
<td>74.5 ± 1.0 (22)</td>
<td>14.6 ± 0.9 (24)</td>
<td>101 ± 1.2 (24)</td>
<td>11.3 ± 0.8* (28)</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>75.0 ± 0.7 (27)</td>
<td>11.3 ± 0.8* (28)</td>
<td>116.2 ± 1.5* (30)</td>
<td>52 ± 0.9 (21)</td>
</tr>
</tbody>
</table>

Means ± sem of three successful experiments are given. Numbers in parentheses are the numbers of impalements. *P < 0.01 of the preceding value.

TABLE 2
Measurements of Action Potentials of Sheep Purkinje Fiber

<table>
<thead>
<tr>
<th></th>
<th>Resting potential (mv)</th>
<th>Overshoot (mv)</th>
<th>Duration of action potential (msec)</th>
<th>Rate of rise (v/sec)</th>
<th>Effective refractory period (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.4 ± 1.0 (28)</td>
<td>30 ± 1.3 (28)</td>
<td>370 ± 10.1 (39)</td>
<td>347 ± 10.8 (36)</td>
<td>284 ± 5.5 (16)</td>
</tr>
<tr>
<td>1% Halothane</td>
<td>87 ± 0.9* (15)</td>
<td>21.7 ± 0.9* (16)</td>
<td>233 ± 9.8* (16)</td>
<td>342 ± 16.7 (19)</td>
<td></td>
</tr>
<tr>
<td>2% Halothane</td>
<td>89.4 ± 1.2 (22)</td>
<td>17.2 ± 1.2* (21)</td>
<td>165 ± 5.3* (32)</td>
<td>245 ± 7.1* (37)</td>
<td>109 ± 4.8* (11)</td>
</tr>
</tbody>
</table>

Means ± sem of nine successful experiments are given. Numbers in parentheses are the numbers of impalements. *P < 0.01 of the preceding value. †P < 0.05 of the preceding value.

TABLE 3
Measurements of Action Potentials of Sheep Ventricular Muscle

<table>
<thead>
<tr>
<th></th>
<th>Resting potential (mv)</th>
<th>Overshoot (mv)</th>
<th>Duration of action potential (msec)</th>
<th>Effective refractory period (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.5 ± 0.4 (15)</td>
<td>21.7 ± 0.4 (15)</td>
<td>338 ± 4.2 (15)</td>
<td>295 ± 7.9 (13)</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>77.2 ± 1.7 (19)</td>
<td>11.1 ± 1.8* (20)</td>
<td>231 ± 2.4* (38)</td>
<td>208 ± 1.8* (34)</td>
</tr>
</tbody>
</table>

Means ± sem of three successful experiments are given. Numbers in parentheses are the numbers of impalements. *P < 0.01 of the preceding value.

(Figs. 1D, 2C, and Table 3). Since the movement of the myocardial tissue during excitation easily dislodged the microelectrode, it was seldom possible to determine the upstroke velocity. However, when successfully measured, the rate of rise was about 150 v/sec under control conditions and decreased to about 100 v/sec in the presence of 2% halothane.

In all experiments, a steady state was reached after about 30 minutes, and the changes of the electrical parameters were quickly reversible after washing in normal Tyrode's solution for about 5 minutes.

Discussion
According to Moe and Abildskov (9), the likelihood of development of irregular activity is increased by: (a) abnormal impulse generation which may involve a circus movement (10), ectopic foci (11), or some kind of reentrant mechanism (12); and (b) abnormal conduction which may be caused by a decreased conduction velocity. Also, a shortened refractory period should increase the average number of wavelets and therefore desynchronize cells (12). Under the influence of higher concentrations of halothane (2% to 4%), the S-A nodal discharge rate can rise quite impressively (4). Under these conditions the action potentials of the rabbit S-A node closely resembled an oscillation process that occurs in the plateau range of potentials.
of Purkinje fibers in the absence of potassium (13). The mechanism of oscillatory activity (14) based on voltage clamp measurements of plateau currents in cardiac Purkinje fibers (15, 16) has recently been described. However, there was no indication in atrial, Purkinje, or ventricular fibers of a tendency to develop an ectopic focus.

In a core conductor the velocity of impulse propagation is expected to be proportional to the square root of the electrical conductance of the core (17). Evidence is now available to show that the internal longitudinal resistance of Purkinje fibers markedly increases under halothane and that the input capacitance is significantly reduced (18). The latter effect should balance the increase in internal resistivity to a great extent, although it is not known to which part of the membrane capacitance the reduced input capacitance corresponds. In fact, the conduction time increases much less than would be expected from the marked increase in internal resistance. The shortening of the action potential and the hyperpolarization of the membrane in Purkinje fibers might be explained by assuming that K outward current increases (19). Other possibilities are a decrease of the fully activated sodium current (\(I_{Na}\)) or the rate of electrogenic pumping (20). A reduction of the latter would also produce a hyperpolarization if the pump normally supplied inward current. The decrease of the conduction velocity and of the duration of the action potential may also be explained by the alterations of the cable properties without assuming changes in membrane current; however, membrane current changes are, of course, not excluded.

According to these findings, the shortening of the action potential would be mainly due to a decreased capacitance. Assuming an unchanged membrane current, the (input) capacitance, which is reduced by halothane, would be recharged more quickly. This effect would be greatest toward the end of the plateau when the ionic current is no longer very time-dependent (16). During this phase which may be more than half the total duration of the action potential, the rate of repolarization at each potential should be inversely proportional to the membrane capacitance. Hence, in an action potential lasting up to 1 second a reduction of the membrane capacitance might reduce the duration of the action potential by as much as one half. In shorter action potentials this effect would be expected to be even greater. A decreased capacitance would also explain that the rate of rise is not very much decreased despite a significant decrease of the overshoot. In addition, the resting potential of atrial and Purkinje fibers is increased and this also would counteract a decrease of the rate of rise (21). However, these marked changes in cable properties have only been obtained with 2% halothane. With 1% halothane only a slight increase in internal resistance without any change in capacitance was found (Hauswirth, unpublished observation). Moreover, it should be emphasized that there is no evidence that other widely used general anesthetics might not have similar side effects in even lower concentrations, and the doses of halothane used here may well correspond to clinical overdosage since in vivo a large fraction of halothane is attached to plasma proteins and body tissues, mainly fat (22, 23). The shortening of the refractory period is thought to increase the probability of nonuniformity (12). An additional tendency toward arrhythmias may arise from the fact that the refractory period of Purkinje and ventricular fibers are not shortened to the same extent. Moreover, the exposure of the heart to the action of adrenaline (24, 25) or isoproterenol (26) during halothane treatment contributes even more to abnormal activity(1, 2). It may therefore be quite safely concluded that with halothane, especially in the presence of sympathomimetic amines, arrhythmias can occur for the following reasons: (a) a decreased conduction velocity, which is mainly due to an increased internal resistance, (b) a marked shortening of the refractory period, and (c) a pronounced disparity of the refractory period between Purkinje and ventricular fibers.
Acknowledgments

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