Influence of Halothane, Chloroform and Methoxyflurane on Potassium Content of Rat Atria

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An electronic device was developed which allows passage of anesthetic gas or vapor into a medium containing isolated contractile tissue such that any degree of depression of the force of contraction of the tissue can be achieved and maintained. The isolated rat atrial preparation was used in this investigation. After a one-hour equilibration period halothane, chloroform, or methoxyflurane was introduced for a two-hour period in sufficient quantity to produce and maintain a 50 per cent decrease in the force of contraction. The atria were then analyzed for potassium and compared to control atria treated as above but without anesthetic. Control atria showed mean values of 79.7 mEq. K'/kg. wet tissue and 353 mEq. K'/kg. dry tissue. None of the anesthetics tested produced significant alterations in these values.

Inhalation anesthetics are known to depress the force of contraction of isolated cardiac muscle. The mechanism of this depression, however, is not understood. We considered the possibility that the myocardial depression is caused by inhibition of the mechanism for maintaining ionic gradients across cell membranes. Such a mechanism could be in the formation or utilization of adenosine triphosphate (ATP), a substance known to be necessary for provision of the energy for muscular contraction and maintenance of ionic gradients across cell membranes. Metabolic inhibitors such as anoxia, which interfere with ATP production, are known to elicit marked losses in tissue potassium as well as a reduction in the contractile force of isolated rat atria. If anesthetics were to seriously interfere with formation or utilization of ATP, as suggested by the work of Jowett and Quastel, one would expect the force of contraction to be decreased and tissue potassium levels to decrease. The present study was devised, therefore, to determine whether anesthetic-induced changes in the force of contraction are related to changes in the potassium content of rat auricular tissue.

Methods

Research involving the effects of inhalation anesthetics on isolated contractile tissues has been hampered by the lack of a suitable method of metering into the bathing medium quantities of anesthetics which will affect the tissue in a constant and reproducible manner. An electronic device which will accomplish this function has been developed recently in our laboratory. With this device, the Anesthetistat, any reasonable control level can be obtained by the adjustment of a potentiometer.

In our system, the tissue, which is tied to an electrode holder, is suspended by a thread from a strain gauge in an open, well-oxygenated constant temperature bath. It is stimulated at a constant rate and the force of contraction is measured by a strain gauge. The signal from the strain gauge is recorded on an Offner Dynograph. An amplified signal from the strain gauge is obtained from the Dynograph and is used as the input signal for the Anesthetistat. The Anesthetistat is connected to a solenoid valve which can select either a 95 per cent oxygen-5 per cent carbon dioxide mixture or an oxygen-carbon dioxide-anesthetic vapor mixture. This gas mixture is then fed into the tissue bath through a fritted glass bubbler. When the force of contraction is greater than the preset value, the
solenoid valve allows the anesthetic vapor mixture to pass into the bath. Correspondingly, when the force of contraction is lower than the preset value, presumably caused by too much anesthetic, the valve allows only the passage of the oxygen mixture into the bath and the excess anesthetic agent evaporates from the bath. Thus, by this simple servo-mechanism, we can preset and continuously control the effect of the anesthetic on the force of contraction of the tissue.

To this same preparation, we have attached a device which automatically maintains the resting (diastolic) tension at a preset level. The strain gauge is mounted on a rack and pinion which is connected to a small servomotor through a suitable gear train. The motor is connected to a servo-amplifier and resting tension detector which uses the same signal that is fed into the Anesthetistat. The system is such that when the resting tension decreases, the motor lifts the strain gauge, stretching the tissue and thus increasing the resting tension to the predetermined level. When the resting tension increases, the motor correspondingly lowers the strain gauge. This servo-mechanism will follow and correct for the slow changes in resting tension, normal for isolated tissue preparations, with much greater ease and accuracy than manual adjustments.

Isolated rat auricles were suspended in a medium containing 120 mM NaCl, 6 mM KCl, 1.22 mM CaCl₂, 1.34 mM MgSO₄, 1.21 mM NaH₂PO₄, 25.3 mM NaHCO₃, and 5.5 mM glucose and stimulated 200 times per minute at 29°C. The muscles were contracting against a 0.7 g. load. After a one hour equilibration period anesthetic was introduced into the bathing solution for a two hour period to produce and maintain a 50 per cent decrease in the force of contraction. The muscles were then removed, blotted, weighed, dried overnight at 95°C, dissolved in 0.5 ml concentrated nitric acid, diluted to 25 ml with water and analyzed for potassium with an Evans Electrooselenium Limited flame photometer. The results were compared with muscles stimulated for three hours in the absence of anesthetic.

Results
The degree of control of the force of contraction achieved by the Anesthetistat can be seen in figure 1. The force of contraction was measured every fifteen minutes in each experiment. Eighteen auricles not exposed to anes-
Table 1. Effects of Inhalation Anesthetics on Potassium and Water Contents of Isolated Rat Atria

<table>
<thead>
<tr>
<th></th>
<th>Atria</th>
<th>mEq. K⁺/kg Wet Tissue</th>
<th>mEq. K⁺/kg Dry Tissue</th>
<th>% Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>79.7 ± 5.1*</td>
<td>353 ± 40.9</td>
<td>77.3 ± 1.8</td>
</tr>
<tr>
<td>Halothane</td>
<td>10</td>
<td>79.0 ± 5.2</td>
<td>320 ± 48.2</td>
<td>77.1 ± 2.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>12</td>
<td>75.3 ± 6.8</td>
<td>336 ± 38</td>
<td>77.4 ± 5.3</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>9</td>
<td>81.7 ± 1.6</td>
<td>356 ± 15.9</td>
<td>76.9 ± 1.1</td>
</tr>
</tbody>
</table>

* Standard deviation.

Anesthetic demonstrated a slight gradual reduction in force of contraction over the two hour period. Auricles exposed to anesthetic demonstrated a mean decrease in force of contraction of approximately 50 per cent which was maintained reasonably well over the experimental period.

Analyses of the muscles for potassium and water are shown in table 1. According to the t test none of the results were different from the control values at the 5 per cent level of significance.

Discussion

Quantities of halothane, chloroform, and methoxyflurane which depressed the force of contraction of isolated rat auricles 50 per cent for two hours were without significant effect on tissue potassium and water contents. This may indicate that the mechanism of the anesthetic-induced depression of contractility is either different from or more sensitive than that responsible for maintaining tissue potassium concentrations. If depression of contractility by these anesthetics was due to an interference with ATP production or utilization, then one might also expect an interference with the operation of the “sodium-potassium pump” which is supposedly dependent on a supply of ATP. Inhibition of ATP production or utilization should, therefore, result in accumulation of sodium and depletion of potassium. It may be, however, that the mechanisms involved in muscle contraction are more sensitive to changes in ATP levels than those responsible for proper operation of the “sodium-potassium pump.” Experiments to test this possibility are currently underway.

It is possible that changes in tissue potassium occurred, but were so small as to be undetected by our methods. Hajdu has calculated that potassium leaves the frog heart at a rate of 0.127 mM per liter of fiber water per beat. This amount is then readmitted between beats. If the rat heart behaved like the frog heart and if the mechanism for readmitting potassium were completely inhibited in our experiments by anesthetics while the rate of efflux were not changed, our preparations would have been completely depleted of potassium in about five minutes. Since no detectable changes occurred over a two hour period it seems likely that this mechanism has not been seriously interfered with.

It is possible that in these experiments the general anesthetics were acting on the actomyosin protein system directly. The theories of Pauling, Miller, and Featherstone et al predict an interaction of general anesthetics with protein in such a way as to interfere with their function. Our data do not permit speculation on this point.

Summary

An electronic device has been developed which enabled us to meter quantities of volatile anesthetics into a medium containing isolated contractile rat auricles such that the tissues were affected in a constant and reproducible manner. With this device the force of contraction was depressed to approximately 50 per cent of the equilibrium value for two hours with halothane, chloroform and methoxyflurane. None of these agents significantly affected the potassium or water contents when compared to control-auricles not exposed to anesthetics.

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References


AMNIOTIC FLUID EMBOLUS Predisposing factors toward amniotic fluid embolus are precipitous or tumultuous labor, especially when oxytocic stimulation has been used, and traumatic delivery. Massive amniotic fluid embolus or infusion leads to rapid death with cardiorespiratory failure. If the infusion occurs more slowly, the patient may survive the initial respiratory and circulatory insult, only to develop hypofibrinogenemia and severe hemorrhage. The varying picture of respiratory distress, cyanosis, hypotension, central nervous system disturbances, and excessive bleeding can be mistaken for cardiac failure, anesthetic accidents of various sorts, and trauma to the reproductive tract. Early recognition of this maternal complication occurring during labor and delivery is a must, as treatment is urgent and varies with the particular problem at hand. (Phillips, O. C., and others: Amniotic Fluid Embolus, Obstet. Gynec. 24: 431 (Sept.) 1964.)

DELIVERY AND BRAIN TUMOR Pregnancy is not uncommonly associated with brain tumors. Patients with brain tumors may be endangered by sudden changes in the cerebrospinal fluid pressure, such as may occur during labor. Cerebrospinal fluid pressure fluctuations during labor result from maternal straining in response to pain and are not due to the activity of the uterine musculature. Regional anesthesia for labor and delivery should not affect the infant nor the cerebrospinal pressure if pain is relieved. Continuous peridural techniques are recommended. Vaginal delivery by forceps is urged if obstetrically feasible. Cesarean section may be carried out under carefully managed regional or general anesthesia, and may even be followed with safety by immediate craniotomy. There is no necessity for routine abdominal delivery in gravidae harboring brain tumors. (Marx, G. F., and others: Anesthetic Management of the Parturient with Intracranial Tumor, Obstet. Gynec. 24: 122 (July) 1964.)