Effects of Change in Frequency of Stimulation on Myocardial Depression Produced by Thiamylal and Halothane

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IWATSUKI, N. and IWATSUKI, K. Effects of Change in Frequency of Stimulation on Myocardial Depression Produced by Thiamylal and Halothane. Tohoku J. exp. Med., 1975, 117 (2), 119-124 — The effects of change in the frequency of stimulation on the myocardial contractility depressed by thiamylal and halothane were studied in isolated dog heart muscle. An increase in the frequency of stimulation from 0.1 to 0.6 cps resulted in a progressive increase in net-shortening (±1) and maximum velocity of shortening at 0.4 g preload (V'max), namely a positive staircase. The myocardium previously depressed to a similar degree by thiamylal or halothane still showed a positive staircase. This result indicates that the mechanism to produce the myocardial depression by thiamylal or halothane is not a complete inhibition of Ca++ influx across the cell membrane. The time to reach a steady state of contraction following an increase in the frequency of stimulation was longer in the presence than in the absence of these anesthetics. The degree of recovery from the myocardial depression by increasing the frequency of stimulation was much higher in the presence of thiamylal than in the presence of halothane. This fact suggests that the mechanism to produce the myocardial depression may be different in these two anesthetics.

Isolated heart muscle; myocardial contractility; frequency of stimulation; staircase; thiamylal; halothane

It has been demonstrated that inhalational as well as intravenous anesthetics have a direct negative inotropic effect on the myocardium, but its fundamental mechanisms are not yet fully understood. Krishna and Paradise (1972) have reported that pyruvate administered into the bathing medium reverses the myocardial depression produced by halothane, but it is ineffective for the reversal of depression produced by pentobarbital. Recently we have demonstrated in isolated heart muscle that the myocardial depression produced by thiamylal is reversed readily by dibutyryl cyclic AMP, but the depression produced by halothane resists to the antagonizing effect of this agent (Iwatsuki and Iwatsuki 1974). These two findings suggest that the mechanism of myocardial depression may be different between thiamylal and halothane. Price (1974) has reported the possibilities that halothane restricts the availability of Ca++ to the contractile proteins of the myocardium as well as it interferes with the reaction between Ca++ and

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these proteins. It has been well recognized that an increase in the frequency of stimulation exerts a potent positive inotropic effect on the myocardium (Koch-Weser and Blinks 1963) and this effect depends strongly on Ca**+** exchange during muscle contraction (Grossman and Furchgott 1964; Langer 1965; Teiger and Farah 1967), especially during the systolic phase (McCans et al. 1974; Willerson et al. 1974). Therefore, the investigation of the response of the myocardium to changing the frequency of stimulation under the influence of anesthetics may show the relation of Ca**+** with the myocardial depression produced by the anesthetics. The aim of this study is to determine whether the response to changing the frequency of stimulation is different or not between the myocardium depressed by thiamylal and halothane and to assume the mechanism of myocardial depression produced by these two anesthetics.

**Materials and Methods**

Trabeculae were excised from the right ventricles of healthy mongrel dogs anesthetized with pentobarbital sodium (25 mg/kg; intravenously). Mean muscle length and cross-sectional area of 11 trabeculae used in this study were 5.93±0.43 mm and 0.77±0.11 mm² (mean±s.E.), respectively. The trabecula was suspended in Krebs-Henseleit’s solution which was kept at 27°C and bubbled with a 95% O₂-5% CO₂ gas mixture, and was allowed to contract isotonically with 0.4 g preload by electrical stimulation at a frequency of 6 per min for 90–120 min for stabilization. Electrical stimulation was applied by the platinum field electrodes placed parallel to the long axis of each muscle with a square wave of 5 msec duration and a voltage 20% above threshold. The isotonic lever system, muscle bath, perfusate, electrical stimulator and recording equipments used in this study have been described in the previous paper (Iwatsuki 1973).

The velocity of shortening was measured by a differentiating system (Nihon Kohden S-3056) with the time constant of 5 msec. The frequency of stimulation was changed from 0.1 to 0.3 cps, from 0.3 to 0.6 cps, then from 0.6 to 0.3 cps. At each frequency the maximum velocity of shortening at 0.4 g preload (V’max) and net-shortening (Δl) were measured when contraction reached a steady state. Then, the same series of measurements were repeated in the presence of thiamylal or halothane. Thiamylal, dissolved in distilled water, was added directly to the bathing solution. The concentration of thiamylal used in this study was 3.3 mg%. Halothane was administered by passing its vapor through the bathing solution after vaporized with a Fluotec vaporizer and its concentration was determined by gas chromatography. The concentrations used in this study were 5.8±0.18 mg% (mean±s.E.). All values were expressed as mean±s.E. and analyzed statistically by Student’s t-test.

**Results**

The present study showed that Δl and V’max were increased progressively by increasing the frequency of stimulation. Such a tendency was observed under the influence of thiamylal and halothane (Table 1). The percent values of Δl and V’max at each frequency to the control obtained at 0.1 cps, are shown in Fig. 1. When the frequency of stimulation was changed from 0.1 to 0.3 cps, from 0.3 to 0.6 cps, then from 0.6 to 0.3 cps, Δl was 115±1.4%, 124.4±3.4% and 110±1.1%, and V’max was 126.3±2.6%, 152.8±3.8% and 113.5±2.3% of the control at each frequency, respectively. When the frequency was changed in the same order, Δl previously depressed to 56.6±3.0% of the control by halothane was 71.6
TABLE 1.  Net-shortening (Δl) and maximum velocity of shortening at 0.4 g preload (V'\text{max}) following the change in the frequency of stimulation in the absence and in the presence of thiamylal or halothane

<table>
<thead>
<tr>
<th></th>
<th>0.1 cps</th>
<th>0.3 cps</th>
<th>0.6 cps</th>
<th>0.3 cps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm/muscle length) Halothane</td>
<td>0 mg%</td>
<td>0.176±0.014</td>
<td>0.206±0.015</td>
<td>0.227±0.012 $</td>
</tr>
<tr>
<td></td>
<td>5.8 mg%</td>
<td>0.100±0.010 $</td>
<td>$ 0.126±0.012 $</td>
<td>$ 0.148±0.011 $</td>
</tr>
<tr>
<td>Thiamylal</td>
<td>0 mg%</td>
<td>0.247±0.032</td>
<td>0.281±0.037 $</td>
<td>$ 0.298±0.044 $</td>
</tr>
<tr>
<td></td>
<td>3.3 mg%</td>
<td>0.140±0.014 * 0.187±0.024 $</td>
<td>$ 0.224±0.025 $</td>
<td>$ 0.204±0.025 $</td>
</tr>
<tr>
<td>V'\text{max}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm/sec/muscle length) Halothane</td>
<td>0 mg%</td>
<td>1.11±0.15</td>
<td>1.35±0.18 $</td>
<td>$ 1.61±0.20 $</td>
</tr>
<tr>
<td></td>
<td>5.8 mg%</td>
<td>0.62±0.06*</td>
<td>0.79±0.118 $</td>
<td>$ 0.95±0.133 $</td>
</tr>
<tr>
<td>Thiamylal</td>
<td>0 mg%</td>
<td>2.05±0.25</td>
<td>2.65±0.33 $</td>
<td>$ 3.26±0.423 $</td>
</tr>
<tr>
<td></td>
<td>3.3 mg%</td>
<td>1.13±0.14 $</td>
<td>$ 1.65±0.213 $</td>
<td>$ 2.11±0.24 $</td>
</tr>
</tbody>
</table>

Mean±s.e. * 0.01>p>0.001, † 0.001>p between the values in the absence and in the presence of thiamylal or halothane. $0.05>p>0.01, § 0.01>p>0.001 //0.001>p between the values at the frequencies next to each other.

Fig. 1. The percent values of net-shortening (Δl) and maximum velocity of shortening at 0.4 g preload (V'\text{max}) following the change in the frequency of stimulation in the presence of thiamylal or halothane as compared with the control values at 0.1 cps of frequency.

*, control; †, in the presence of 3.3 mg% of thiamylal; ○, in the presence of 5.8±0.18 mg% of halothane. * 0.05>p>0.02; † 0.02>p>0.01 (halothane vs. thiamylal).

±3.2%, 84.4±5.4% and 72.0±2.4%, and V'\text{max} previously depressed to 56.2±4.5% of the control by halothane was 70.2±5.2%, 84.7±6.6% and 66.2±4.6% at each frequency. Similarly, Δl previously depressed to 57.3±2.9% of the control by thiamylal was 80.5±0.3%, 91.5±3.1% and 83.5±0.3%, and V'\text{max} previously depressed to 55.1±2.1% of the control by thiamylal was 79.2±2.8%, 102.2±3.0% and 78.3±2.2% at each frequency. The percent changes of these
values to the control at 0.1 cps with or without the anesthetics are shown in Fig. 2. When the frequency of stimulation was changed as mentioned above, the percent increases of $dI$ were 15.3±1.4, 24.4±3.4 and 10.1±1.1 and those of $V'_{\text{max}}$ were 26.3±2.6, 52.8±3.8, and 13.5±2.3 at each frequency without the anesthetics. In the presence of halothane the percent increases of $dI$ were 27.0±3.0, 50.0±8.2 and 28.2±3.1 and those of $V'_{\text{max}}$ were 26.0±1.6, 53.0±2.7 and 18.8±1.7 at each frequency. In the presence of thiamylal the percent increases in $dI$ were 40.3±2.5, 59.5±1.9 and 46.2±2.8 and those of $V'_{\text{max}}$ were 46.9±5.3, 87.7±5.6 and 43.3±3.8 at each frequency. The percent increases in $dI$ and $V'_{\text{max}}$ were always great in the presence of thiamylal than in the presence of halothane.

The number of beats necessary for the contraction to reach a steady state after increasing the frequency of stimulation was more in the presence than in the absence of the anesthetics (Table 2). However, when the frequency decreased from 0.6 to 0.3 cps, the number of beats became identical in the presence and in the absence of the anesthetics (Table 2).
DISCUSSION

The effect of the frequency of stimulation on the contraction of isolated heart muscle has been reported in many species (Koch-Weser and Blinks 1963). In the present study $\Delta t$ and $V'_{\text{max}}$ were increased progressively by increasing the frequency of stimulation and these results correspond to those in the previous reports (Koch-Weser and Blinks 1963). Especially, an increase in $V'_{\text{max}}$ indicates that increasing the frequency of stimulation accelerates the myocardial contractility, since $V'_{\text{max}}$ is considered to be the best index of the myocardial contractility (Sonnenblick 1962). A lesser increase in $\Delta t$ than in $V'_{\text{max}}$ observed in this study may be due to a limitation of muscle shortening or due to a decreased duration of "active state" of contraction following an increase in the frequency of stimulation as reported by Sonnenblick (1962). A lesser increase in isometric force than in $V'_{\text{max}}$ reported by Sonnenblick (1962) seems to be an interesting result as compared with ours.

In has been reported that an increase in the frequency of stimulation increases the concentration of Ca++ in the myocardial cells (Grossman and Furchgott 1964; Langer 1965; Teiger and Farah 1967) and when verapamil or D-600, a potent pharmacological inhibitor of Ca++ influx across the cell membrane during the systolic phase of contraction, is used, an increase in the frequency of stimulation rather decreases the contractility of isolated heart muscle (McCans et al. 1974; Willerson et al. 1974). These results indicate that an increase in myocardial contractility by increasing the frequency of stimulation is due to an increased Ca++ influx during the systolic phase of contraction. The present study showed that an increase in the frequency of stimulation resulted in an acceleration of myocardial contractility even in the presence of thiamylal and halothane. This fact suggests that the myocardial depression produced by thiamylal or halothane may not be caused by a complete inhibition of Ca++ influx across the cell membrane by these anesthetics. Recently Price (1974) has reported that increasing Ca++ in the bathing medium antagonized the depressant effect of halothane on the contraction of cat papillary muscle, and the depression is seen even during tetanic contracture, to a lesser degree than during twitches. From these results he had suggested that halothane not only limits the availability of Ca++ to the contractile proteins at the cell membrane, but also interferes with the response of these proteins to Ca++ in the cell. However, as Forman et al. (1972) mentioned, the caffeine added to the medium to obtain tetanic contracture may modify the effect of halothane on the cardiac muscle.

It should be mentioned that in the present study the number of beats necessary for the contraction to reach a steady state after increasing the frequency of stimulation was more in the presence than in the absence of the anesthetics. The rate of Ca++ increase in the myocardial cell to a suitable level for that frequency of stimulation may be slow and/or the response of the contractile proteins to increased Ca++ may be delayed in the presence of these anesthetics.

In the previous paper (Iwatsuki and Iwatsuki 1974) we have reported that the
myocardial depression produced by halothane is harder to be reversed by dibutyryl cyclic AMP than that produced by thiamylal. A similar difference was observed in the antagonizing effect of the frequency of stimulation on the myocardial depression caused by these two anesthetics. Therefore, the mechanism to produce the myocardial depression may be different between thiamylal and halothane.

References