The Effect of Methoxyflurane on the Inotropic State of Myocardial Muscle

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The direct effect of methoxyflurane upon the inotropic state of the myocardium was studied in an isolated cat papillary heart muscle preparation. Methoxyflurane decreased muscle ability to develop force and shorten and the velocity of shortening for a given load (power). The decrease in the inotropic state of muscle was dose-dependent, as evidenced by progressive decreases in maximum velocity of myocardial muscle shortening (V_{max}), power, and work. Methoxyflurane also caused changes in the active state of the myocardium (force-generating processes), as shown by decreases in maximum rate of force development (dF/dt) and time-to-peak isometric force (TTF_m). The results suggest that methoxyflurane exerts a negative inotropic effect on the intrinsic contractile state of cardiac muscle owing to alteration in the mechanical energy derived from chemical reactions within the contractile system.

IT HAS BEEN CLAIMED that methoxyflurane has a direct negative inotropic influence upon the myocardium, as indicated by the decreased ventricular work performance as a function of the ventricular filling pressure[1] and by decreased myocardial contractile force developed by relatively isometric segments of ventricular wall.[2] However, recent studies indicate that the work performance of the left ventricle is readily altered by changes in the resistance to ejection (afterload) without necessarily involving a change in the inotropic state.[3,4] Changes in myocardial contractile force reflect only changes in force generation processes, but do not reflect the velocity of myocardial muscle shortening.[1,5] Recently, we reported that methoxyflurane exerts a negative inotropic effect upon the contractile state of the intact heart, as determined by force–velocity relations.[6] Changes in the inotropic state of the myocardium due to a direct negative inotropic influence of the anesthetic agent may, however, be complicated by changes in nervous, humoral and metabolic influences *in vivo*.[1]

Accordingly, the present study was designed to determine the direct effect of methoxyflurane on the inotropic state as measured in terms of mechanics of contraction, separated from the extrinsic cardiac control mechanism, in the isolated cat papillary muscle, using methods previously described.

Definition of Terms

Muscle mechanics: the study of force and motion of heart muscle using the principles of physics.

Model of muscular contraction: the mechanical analogue of muscle. According to Hill, force is generated by a contractile element (CE) arranged in series with an elastic element (SE)[8] (fig. 1).

Force–velocity relation: the muscle's ability to develop force and shorten. The reciprocal relation of force and velocity expresses the initial velocity of isotonic shortening of CE (V_{ce}) as a function of developed force (F) in heart muscle contracting isotonically against an afterload.[9] The developed force (F) is equal to the load the muscle carries (afterload) during shortening. Hence, in the afterloaded isotonic contraction, load is synonymous with developed force (F).

Maximal velocity (V_{max}): the initial velocity of isotonic shortening when the muscle carries no load. Since the smallest preload is necessary to establish the initial resting muscle length, V_{max} is measured indirectly by extrapolation of the force–velocity curve to zero load.

Peak force (F_m): in grams, the maximum active force developed by heart muscle following stimulation during isometric contraction.

dF/dt: in g/sec, the first derivative of the course of force development relative to time, measured as the slope of the force–time curve.

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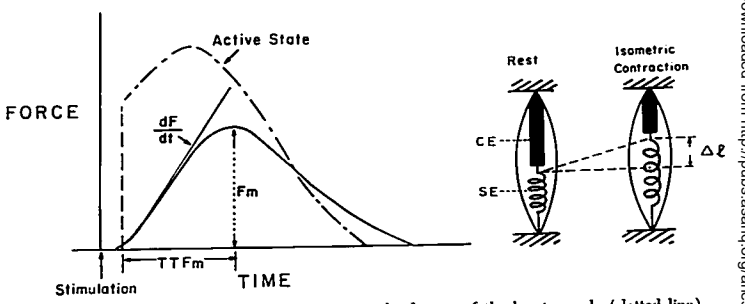

FIG. 1. Courses of active state of the contractile element of the heart muscle (dotted line) and recorded isometric force (solid line) are shown relative to time. Initial maximal rate of force development is denoted by the slope of a straight line. The time interval between the onset of contraction and the peak force is shown as TTF_m. On the right, Hill's model of muscular contraction, consisting of the contractile element (CE) and the series elastic element (SE) at rest (left) and during isometric contraction (right) is shown. The third elastic element, not shown in the figure, is parallel with both CE and SE. Upon activation, chemical energy is converted into mechanical energy. During isometric contraction, both ends of the muscle are fixed by external constraints; thus, CE stretches SE (Δl) until developed force equals the maximal force (F_m) of which the CE is capable. The rate of force development (dF/dt) is a function of not only the instantaneous velocity of shortening (dl/dt) of CE but also modulus of elasticity (stiffness; dF/dl) of SE. Both dl/dt and dF/dl are functions of developed force (F); thus:

$$dF/dt = (dF/dl) \cdot (dl/dt)$$

TTF_m: in msec, the time from the beginning of force development to peak force (F_m) during isometric contraction.

Active state: "the force development at constant contractile element length,"[10-12] a mechanical measurement of the chemical processes that take place within the contractile machinery of the activated muscle.

Intensity of the active state: reflects the degree to which muscle is activated, expressed in terms of capacity to develop force or the rate of shortening (velocity).

Duration of the active state: indicates how long the active state persists during contraction, assessed in terms of the length of time during which force is generated.

Figure 1 illustrates the course of active state and force development relative to time in myocardium contracting isometrically.

Materials and Methods

Right ventricular papillary muscles were excised from 12 normal cats (weighing 1.5 to 2.4 kg) anesthetized with chloralose intraperitoneally (80 to 100 mg/kg). Each served as its own control. The methods used to measure force–velocity relations and the velocity of shortening for a given load (power) were identical to those reported previously,[7] with the following exceptions.

Change in the intensity of the active state was assessed by determining rate of force development (dF/dt). Changes in duration of the active state were determined by the length of time during which force developed (TTF_m).[13] The temperature of the bath was maintained at 22 C in six studies and at 37 C in five. This was done to determine whether change in temperature affects the mechanics of contraction of the muscle exposed to methoxyflurane, since it has been shown that changes in temperature alter the time course of the active state and, consequently, force–velocity relations.[13] In one experiment, the effect of duration of exposure to methoxyflurane at a constant concentration was studied at 27 C.

Following control measurements of force–velocity relations and intensity and duration of the active state, methoxyflurane was administered to the muscle via the bathing solution (Krebs-Henseleit) bubbled with a gas mixture (95 per cent O_2 and 5 per cent CO_2) containing the anesthetic. The concentration of methoxyflurane in the bathing solution was mea-

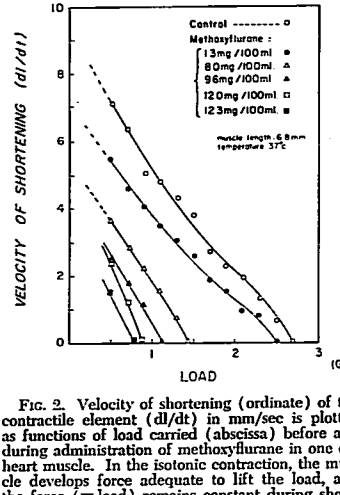

FIG. 2. Velocity of shortening (ordinate) of the contractile element (dl/dt) in mm/sec is plotted as functions of load carried (abscissa) before and during administration of methoxyflurane in one cat heart muscle. In the isotonic contraction, the muscle develops force adequate to lift the load, and the force (= load) remains constant during shortening. When the velocity is extrapolated to zero load, maximal velocity of shortening (V_{max}) is obtained. Peak isometric force (F_m) is recorded when the load is increased in a step-wise fashion until no shortening occurs. The initial muscle length is constant (6.8 mm) with preload 0.5 g, rate 12/min, and temperature 22 C.

sured by gas chromatography[14] before and after the completion of each force–velocity study and expressed as an average value. Data were analyzed statistically by Fisher's t test and by the paired t test.[15] Values were averaged and expressed as mean ± SEM. Differences between groups were considered statistically significant when $P < 0.01$ and probably significant when $0.01 < P < 0.05$.

Results

Analysis of isotonic and isometric contractions were made on 47 occasions in 12 cat papillary heart muscles before and during administration of methoxyflurane. Average values of muscle length and blotted weight were 7.3 ± 0.5 mm and 12.3 ± 1.0 mg, respectively. In general, values of V_{max} of the muscles studied at 22 C were lower than those at 37 C. In contrast, values of F_m of the heart muscles studied at 22 C were higher than those at 37 C. However, the direction and magnitude of changes in V_{max}, F_m, power, work, dF/dt and TTF_m at 22 C were similar to those observed at 37 C. Hence, the data obtained at 22 C and 37 C were analyzed as one group.

Isotonic Contraction

The administration of methoxyflurane (mean concentration: 12 ± 1.6 mg/100 ml) caused decreases in both V_{max} and F_m in all 12 experiments (six at 22 C, one at 27 C and five at 37 C). Force–velocity curves were shifted to the left when the heart muscle was exposed to methoxyflurane, and the degree of leftward shift was dose-dependent. At any given loading condition (afterload), both power and work were reduced. Figures 2 and 3 represent findings in one heart muscle exposed to a step-wise increase in concentrations ranging from 1.3 to 12.3 mg/100 ml.

Maximum velocity of isotonic shortening (V_{max}), maximum isometric force (F_m), and maximum power and work varied directly with anesthetic concentration (fig. 4). Correlation

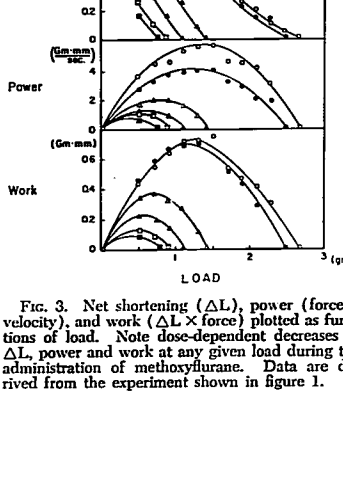

FIG. 3. Net shortening (ΔL), power (force × velocity), and work (ΔL × force) plotted as functions of load. Note dose-dependent decreases in ΔL, power and work at any given load during the administration of methoxyflurane. Data are derived from the experiment shown in figure 1.

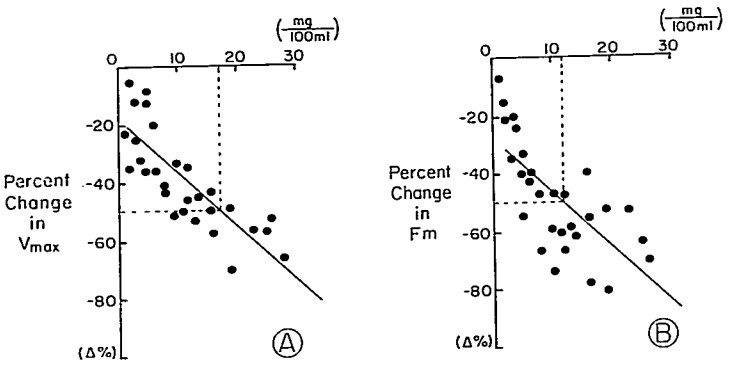

FIG. 4. The effects of methoxyflurane on the maximal velocity (V_{max}) and peak isometric force (F_m) in 11 cat papillary heart muscles studied at 22 and 37 C. Abscissa: concentration of methoxyflurane in mg/100 ml of Krebs-Henseleit solution; ordinate: Percentage change in V_{max} (left) and F_m (right) during the administration of methoxyflurane: the equations of the regression lines are $Y = -1.8\ x - 19.2$ (for V_{max}) and $Y = -1.9\ x - 28.1$ (for F_m).

coefficients relating percentage changes in V_{max}, F_m, power and work to methoxyflurane concentration were −0.79, −0.71, −0.67, and −0.67, respectively. The average values of methoxyflurane that produced 50 per cent depression in 11 muscles at 22 and 37 C were 17 mg/100 ml for V_{max} and 11.5 mg/100 ml for F_m, respectively.

Figure 5 shows the effect of duration of exposure to the anesthetic on force–velocity relations in one muscle studied at 27 C. The force–velocity curve obtained after the one-hour recovery period following a four-hour exposure to the anesthetic was virtually the same as that obtained during the control period.

Isometric Contraction and Active State

Percentage changes from the control values of dF/dt and F_m in 11 isometrically-contracting muscles exposed to methoxyflurane were directly related to anesthetic concentration (fig. 6). Correlation coefficients relating percentage changes in dF/dt and F_m to methoxyflurane concentration were −0.68 and −0.71, respectively.

TTF_m averaged 301 ± 24 msec in 11 muscles exposed to methoxyflurane, and was lower ($P < 0.05$) than that obtained during the control state (399 ± 45 msec). When values of TTF_m were paired with those obtained during the control state, decreases in TTF_m during administration of methoxyflurane were significant ($P < 0.01$). However, percentage changes in TTF_m in muscles exposed to methoxyflurane did not correlate with concentration (correlation coefficient: −0.30).

Modulus of Elasticity

Figure 7 illustrates the modulus of elasticity of series elastic element (dF/dl) given as a function of load (F) before and during administration of methoxyflurane in one muscle. The slope (k) of the straight line equation ($dF/dl = kF$) equals the modulus of elasticity. Values for k averaged 3.26 ± 0.11 (not normalized for muscle length) in eight muscles during the control state did not differ significantly (3.27 ± 0.13) from those obtained during administration of methoxyflurane ($P > 0.5$).

Discussion

The major finding of the present study is that methoxyflurane exerts a direct negative inotropic effect on the intrinsic contractile state of the myocardium, as evidenced by the decreased ability of myocardial fibers to develop force and shorten. Reduction of the maximal velocity (V_{max}) or the rate of myocardial

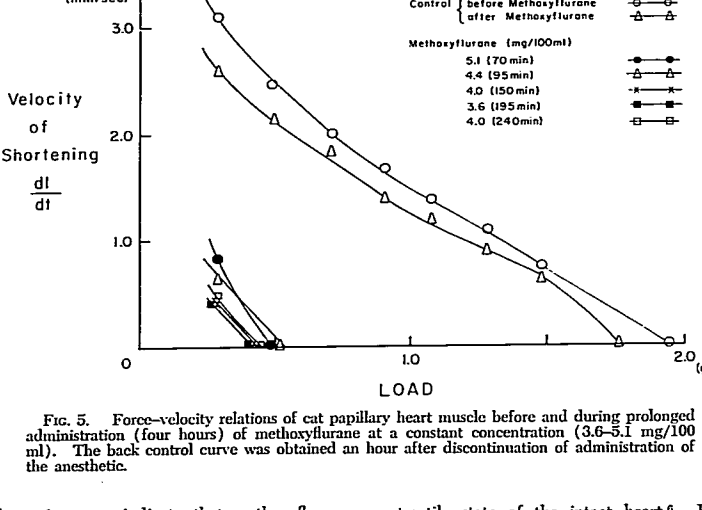

FIG. 5. Force–velocity relations of cat papillary heart muscle before and during prolonged administration (four hours) of methoxyflurane at a constant concentration (3.6–5.1 mg/100 ml). The back control curve was obtained an hour after discontinuation of administration of the anesthetic.

shortening may indicate that methoxyflurane depressed the contractile state of the muscle directly, since decrease in V_{max} has been related to alteration in the rate of force development at each contractile site.[16] Recent studies show that the changes in V_{max} are independent of changes in force development due to changes in length of cardiac muscle prior to contraction (preload), and uniquely determine changes in the inotropic state of the myocardium (*i.e.*, contractility).[17] Therefore, the reduction in V_{max} due to methoxyflurane indicates that this anesthetic depressed the contractility of isolated heart muscle. The changes are comparable to those in the intact heart, since aortic pressure may be equated with the afterload of the intact left ventricle, which ejects blood by development of force with shortening of myocardial muscle.[4] Changes in the force–velocity relation in the isolated muscle are, therefore, analogous in a degree to those of intraventricular pressure development (force) and the rate of ventricular ejection (velocity) in the intact heart. Recent studies in our laboratory reveal that methoxyflurane also has a negative inotropic effect upon the contractile state of the intact heart.[6] Evidently, in relation to the direct inotropic effect on heart muscle mechanics, this agent has a negative inotropic effect on the myocardium of both isolated muscle and intact heart.

An interesting finding of the present study was that the altered contractile state of heart muscle, as reflected by the dose-dependent change in V_{max} during isotonic contraction (fig. 4), was accompanied by a change in the rate of force development (dF/dt) in isometric contraction (fig. 6). At any given loading condition (afterload), there was a decrease in the velocity of myocardial muscle shortening in all muscles exposed to methoxyflurane, resulting in a reduction of power and work. The concomitant decreases in maximal power and work given as a function of load (fig. 3) further substantiate the negative inotropic effect of this agent. These findings are also in accordance with those observed in the intact heart during methoxyflurane anesthesia.

It should be pointed out that the actual performance of the CE is characterized by the active state.[9–11] The term "active state" may be defined as a mechanical measure of the chemi-

cal processes in the CE and characterized in terms of four parameters; force, velocity, instantaneous muscle length, and time.[13] In skeletal muscle, the concept of active state was introduced to explain the ability of muscle to bear maximum force at the onset of the contraction when "quick stretch" was applied.[18] Under these conditions, the resulting force reflects CE alone, independent of the SE connections, since the latter is prestretched. Difficulty in assessing the active state in cardiac muscle has been related to the slow onset and decay of the active state.[12]

The rate of force development (dF/dt) of the CE during isometric contraction is a function of the product of the stress/strain characteristics (modulus of elasticity or stiffness) of the SE and the velocity of shortening of the CE.[9] This relation is expressed in the differential equation: $dF/dt = (dF/dl) \cdot (dl/dt)$. Data in the present study indicate that methoxyflurane did not alter stiffness of the SE. These findings are consonant with previous studies showing that stiffness of the SE is not altered during the administration of halothane[7] or various inotropic interventions.[20] Therefore, it is reasonable to state that the changes in dF/dt observed in the heart muscle exposed to methoxyflurane parallel those in the velocity of shortening of the CE. These findings in the isolated heart muscle suggest that measure-

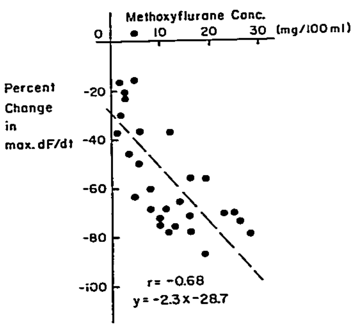

FIG. 6. Percentage changes in F_m of 11 isometrically contracting heart muscles plotted against the methoxyflurane concentration in mg/100 ml. The regression line: $Y = -2.3\ x - 28.7$.

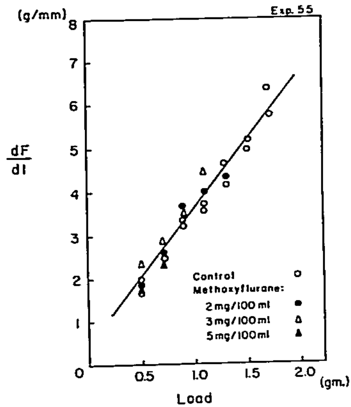

FIG. 7. Modulus of elasticity (stiffness: dF/dl) of SE, where $dF/dl = (dF/dt)/(dl/dt)$ is a linear function of load ($dF/dl = k \cdot F$) and is unaltered during administration of methoxyflurane.

ment of myocardial contractile force by means of a Walton strain gauge *in vivo* during methoxyflurane and halothane anesthesia should provide information relative to velocity of shortening of the CE, if one analyzes the rate of change in force development.

It was of interest to determine whether the induced change in intrinsic contractile state of the myocardium was the result of prolonged exposure to methoxyflurane at a constant concentration. Changes in force–velocity curves measured at 70, 95, 150, 195 and 240 min during the administration of methoxyflurane were essentially the same (fig. 5). Thus, it seems apparent that tachyphylaxis does not occur in the heart muscle during prolonged exposure to methoxyflurane.

Of particular interest is the observation that methoxyflurane caused a significant reduction of time-to-peak force (TTF_m) with decreased rate of force development (dF/dt). In previous studies, we reported that halothane causes decreases in rate of force development, as does methoxyflurane, but prolongs the duration of contraction as reflected by increased TTF_m. Changes in the manner of shift of the force–velocity relation observed in muscle exposed

to halothane were similar to those observed in the present study. Decreased peak force and rate of force development by the myocardium exposed to halothane were related to decreased intensity of the active state.[7] Similarly, methoxyflurane decreased the intensity of the active state of the heart muscle as measured by the leftward shift in the force–velocity curve (fig. 2) and by the decreased dF/dt (fig. 6). However, in the case of methoxyflurane, the duration of the active state was significantly decreased, as reflected by decreased TTF_m. These findings suggest that the negative inotropic effect of methoxyflurane may be related to not only decreases in intensity, but decreases in the duration of active state.

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Erratum

In the editorial on scientific measurement in the February issue (ANESTHESIOLOGY 30: 125, 1969), "Celsius" was misspelled.