Effect of Cyclopropane on Myocardial Contraction and Membrane Potentials

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It has been demonstrated both experimentally in animals and clinically that among the prominent pharmacologic actions of cyclopropane are its effects on rhythmicity, excitability, and contractility of cardiac muscle.[1-3] Considerable evidence has been published relating to the possible role the autonomic and central nervous systems, as well as hypoxia and hypercarbia, may have in the production of these cardiac effects. While such studies have indicated some of the important factors that may contribute and modify the action of cyclopropane on the heart, knowledge as to whether this agent directly affects the basic cellular events underlying the electrical and mechanical activity of cardiac tissue is still incomplete.

A study, therefore, was undertaken to investigate the effects of various concentrations of cyclopropane on transmembrane potentials of isolated rabbit atria perfused *in vitro*. These membrane effects have been related to simultaneously-measured contractile tension, electrical excitability, and perfusate concentrations of cyclopropane and oxygen.

Experiments on dogs were also performed to determine the effects of cyclopropane on ventricular membrane potentials in the intact animal and to relate these effects to simultaneously-measured blood levels of cyclopropane.

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Methods

Atria for *in vitro* studies were obtained from white New Zealand rabbits. A rectangular strip of muscle about 5 by 15 mm. was dissected from either the right or left atrial appendage. The atrial strip was suspended horizontally in a 115-ml. muscle chamber by two pair of hooks. One pair, attached to one wall of the muscle chamber, was connected to the output of a square-wave generator used for stimulating the preparation. The other pair of hooks holding the tissue was attached to the cantilever beam of a Grass strain gauge transducer which permitted the recording of nearly isometric contractile tension. In most of the experiments, the muscle was driven at a constant rate of 75 per minute, using a stimulus pulse duration of 5 msec. at twice the threshold voltage. The electrical excitability of the atria was determined by noting the threshold voltage needed to elicit contractile response at stimulus pulse durations of 0.2, 0.5, 1.0, 2.0, 8.0 and 12.0 msec.

Transmembrane potentials were recorded with 3 *M* KCl-filled glass microelectrodes[4] using a cathode follower-amplifier circuit. Transmembrane potentials and contractile tension were recorded simultaneously on a direct writing oscillograph.

The chamber in which the atria were suspended was continuously perfused with a modified Tyrode's solution. An oxygen (95 per cent)–carbon dioxide (5 per cent) gas mixture was passed directly into the muscle chamber through a sintered tube. The perfusion reservoir was similarly aerated. The effects of cyclopropane were studied by introducing the gas directly into the muscle chamber via a second sintered tube. Rate of flow of the gases was monitored by use of a rotameter.

The concentration of cyclopropane and oxygen in the muscle chamber perfusate was determined by the method of Orcutt and Waters.[6] Calculated factors for the solubility of cyclopropane were taken from values given by Robbins.[7] Sampling of the perfusate was made by withdrawing a quantity of perfusate anaerobically from the muscle chamber with a syringe. Particular care was taken to exclude any gas bubbles from the sample.

The usual experimental procedure was to allow the preparation to stabilize for about 60–120 minutes after mounting in the muscle chamber. At a bath temperature of 23–25° C., contractile tension usually increased during this period. Once a constant contractile tension value was achieved, control recordings of transmembrane potentials, excitability data, and perfusate concentrations of oxygen were obtained. Cyclopropane was then introduced into the muscle chamber. Periodic recordings of transmembrane potentials and simultaneously measured contractile tensions were made. At the end of ten minutes, atrial excitability was again measured, transmembrane potentials recorded, and another sample of the bath perfusate obtained for the determination of gas concentrations. During the administration of the cyclopropane, a continuous and constant flow of the oxygen (95 per cent)–carbon dioxide (5 per cent) mixture was maintained. Unless otherwise noted, the cyclopropane was turned off at the end of ten minutes and the preparation allowed to recover. During this recovery period, periodic recordings of transmembrane potentials were obtained. As soon as contractile tension had returned to the pre-cyclopropane value, excitability data, as well as perfusate concentrations of oxygen and any residual cyclopropane, were obtained.

In the experiments on intact dogs, the animals were anesthetized with cyclopropane and maintained on artificial respiration, using a Palmer pump. The chest was opened through an intercostal incision and the heart exposed and supported by a pericardial cradle, according to the method of Hoffman and Suckling.[8] Standard lead 2 electrocardiograms were recorded using needle electrodes. Transmembrane potentials from single cells of right or left ventricle were obtained with the same apparatus and techniques used in the *in vitro* studies, except that the indifferent electrode for the transmembrane recording circuit was placed on the epicardium.

Measurement and analysis of the transmembrane potential recordings were made according to the scheme and configurational designations used by Cranefield and Hoffman.[9] These designations are shown on the control record of figure 1. Phase 0 of the action potential is the rapid membrane depolarization associated with a change of potential of approximately 115 mv. Phase 1 is the rapid portion of membrane repolarization preceding the period of slow repolarization, phase 2 (sometimes referred to as the plateau phase). The terminal phase of repolarization is designated as phase 3, and the diastolic portion is phase 4 ("membrane resting potential"). In atrial fibers, the plateau phase of repolarization is usually less prominent than the plateau phase seen in ventricular tissue (fig. 4).

Results

Isolated Atria Studies. The introduction of cyclopropane into the perfusate produced a decrease in contractile tension of atrial muscle. This negative inotropic effect was associated with an increase in the rate of repolarization of the first and second phases of the membrane action potential. Action-potential changes usually appeared before a decrease in contractions was noted. Total action-potential duration was not significantly changed in those experiments where the preparation was driven at a constant rate of 75 per minute. Membrane action-potential amplitude and resting potentials were not markedly changed. However, with prolonged administration of cyclopropane (beyond 20 minutes), action-potential overshoot was decreased. When the administration of cyclopropane was discontinued, contractile tension and action-potential returned to their control values and configuration. A typical experiment illustrating these effects is shown in figure 1. Table 1 summarizes resting-potential and action-potential values before and during the administration of several concentrations of cyclopropane in six different right atrial preparations.

The degree of depression of contractile tension accompanying the administration of cyclopropane to right atrial preparations was

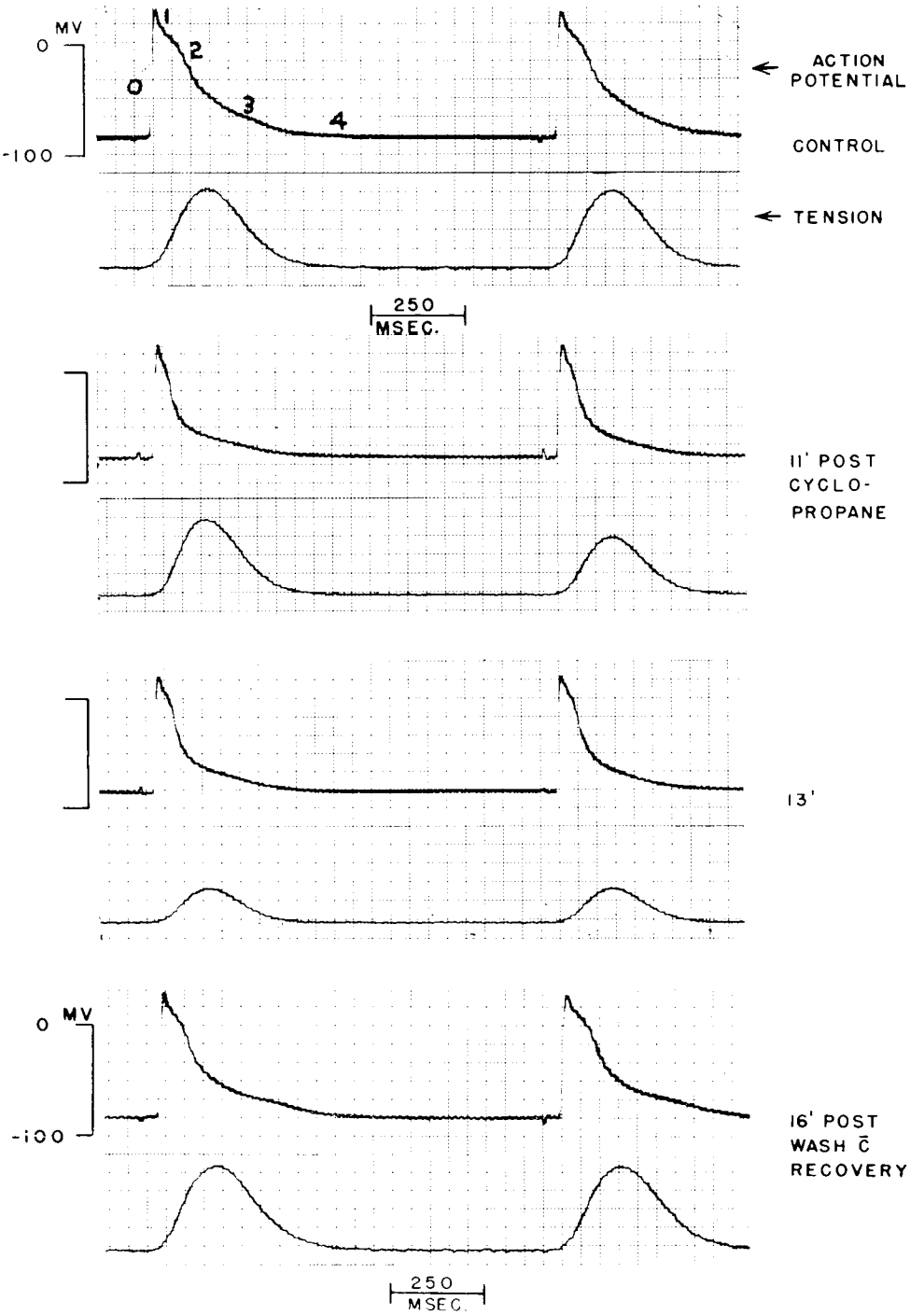


FIG. 1. Simultaneously recorded transmembrane potentials and contraction tension from driven, isolated rabbit right atrial preparation. Designations for various phases of the transmembrane potential are shown on the control record. Effects of cyclopropane (perfusate concentration 6–7 ml./100 ml.) are shown in middle records. Reversibility of cyclopropane action is shown in bottom recording, taken 16 minutes after discontinuing cyclopropane. Stimulus artifact is visible on transmembrane record just prior to depolarization (phase 0).

TABLE 1. Effect of Various Concentrations of Cyclopropane on Right Atrial Membrane Resting-Potential (MRP) and Action-Potential (AP) Values

Expt.	Control			Post C3H6 (10 Minutes)			
	O2	MRP	AP	O2	C3H6	MRP	AP
	(ml./100 ml.)	(mV.)		(ml./100 ml.)		(mV.)	
A	1.74	80.1±1.4 (5)	95.6±1.4 (5)	1.68	7.73	83.5±1.4 (5)	84.0±1.4 (5)
B	1.79	77.8±.9 (9)	88.3±.9 (9)	1.43	5.98	72.8±.7 (9)	79.1±.7 (9)
C	1.82	70.1±1.8 (9)	79.0±1.9 (9)	1.78	1.22	71.2±1.1 (9)	81.2±1.3 (9)
D	1.10	77.8±2.3 (6)	96.9±2.3 (6)	0.88	1.00	77.2±6.3 (6)	97.1±6.6 (6)
E	1.88	60.0±.3 (3)	80.0±.3 (3)	1.31	3.18	65.2±5.2 (6)	85.2±5.2 (6)
F	2.18	64.3±1.0 (12)	82.0±1.2 (12)	1.23	1.06	72.8±.5 (6)	86.1±.5 (6)

Figures in parentheses refer to the number of observations from which the mean MRP and AP were calculated.

found to be directly related to the amount of cyclopropane saturation of the perfusate. This relationship is illustrated in figure 2, where the percentage decrease in contractile tension for right atrial tissue is plotted versus the measured perfusate concentration of cyclopropane.

Since increasing cyclopropane concentration was accompanied by a concomitant decrease in the oxygen saturation of the perfusate (table 1), the possibility that relative hypoxia was contributing to the changes in contraction and action potential had to be considered. Hypoxia alone has been shown to produce changes in contraction and action potential qualitatively similar to those seen in this study.[5] Experiments, therefore, were performed in which hypoxia was produced by gassing the perfusate with nitrogen in an amount that produced a 50 per cent reduction in contractile tension in ten minutes. Results showed that this effect was associated with a

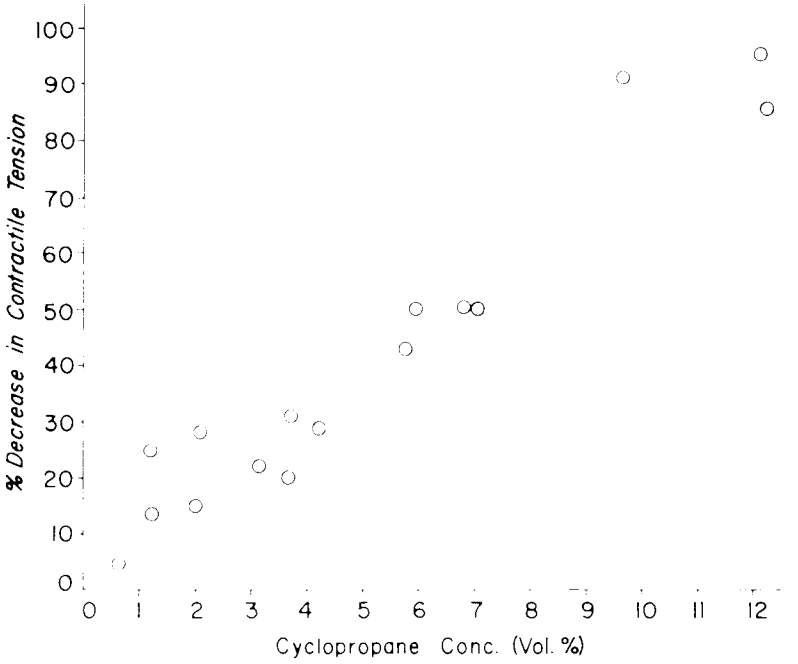


FIG. 2. Relationship between cyclopropane concentration in both perfusate and percentage decrease in contractile tension of isolated rabbit atrial preparations. Each point represents the change occurring in single preparations exposed once to a given concentration of cyclopropane for a duration of ten minutes.

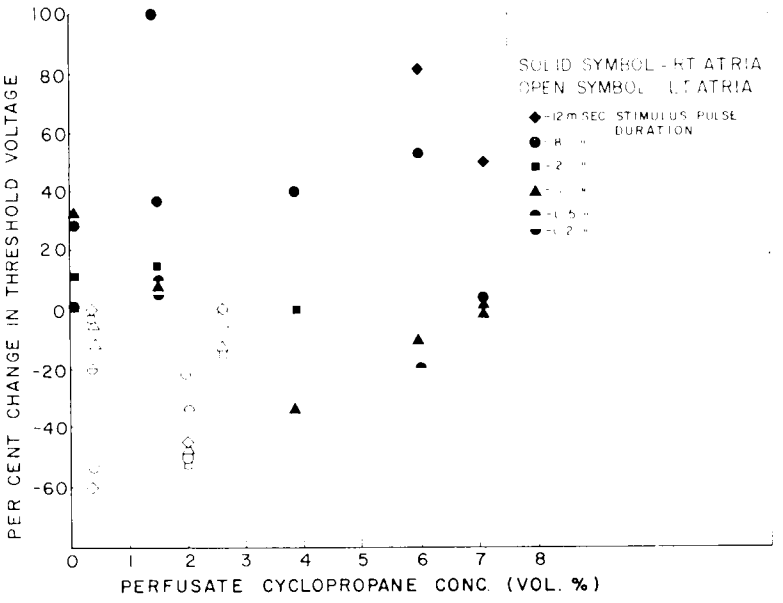


FIG. 3. Excitability changes in right and left atrial preparations produced by various concentrations of cyclopropane. Excitability is plotted on the ordinate as percentage changes in threshold voltage from pre-cyclopropane value. Positive change represents a decreased electrical excitability. Note that for all pulse durations used in left atrial preparations, there was an increase in excitability (decreased threshold voltage) noted in the presence of cyclopropane.

33–80 per cent reduction in perfusate oxygen saturation from control values. Membrane action-potential amplitude, duration, and resting potential were also decreased. Table 2 shows typical action-potential and resting-potential values during nitrogen-induced hypoxia.

In those cyclopropane experiments where the contractile tension had been reduced to 50 per cent of the control value in ten minutes, oxygen saturation was decreased by only 5–35 per cent from the control value.

The negative inotropic effect and increase in the rate of repolarization of the atrial action-potential produced by cyclopropane were accompanied generally by a decrease in the electrical excitability (increased threshold voltage) in six out of seven right atrial preparations in which excitability was measured. In all four left atrial preparations in which excitability data were obtained, cyclopropane increased the electrical excitability (decreased threshold voltage). Figure 3 summarizes the changes in electrical excitability at different cyclopropane concentrations for right and left atrial muscle.

Intact Dog Studies. The question may be raised as to whether the changes seen in the isolated rabbit atrial preparations *in vitro* are unique for this preparation, and whether any transmembrane effects of cyclopropane would be seen in intact spontaneously beating myocardium *in situ*. Therefore, four experiments were performed on open-chest dog preparations, recording transmembrane action-poten-

TABLE 2. Effect of Nitrogen-Induced Hypoxia on Atrial Contraction and Membrane Potentials

Expt.	Control			Post N$_2$ (10 Minutes)			
	O_2	MRP	AP	O_2	% Decrease Contraction	MRP	AP
	(ml./100 ml.)	(mv.)		(ml./100 ml.)		(mv.)	
G	2.15	77.1±1.6 (6)	86.5±1.6 (6)	0.43	63	71.1±1.2 (6)	81.8±1.2 (6)
H	2.20	88.6±.8 (5)	100.0±.8 (5)	1.48	49	76.2±4.3 (6)	73.5±1.3 (6)
I	2.63	87.2±1.0 (5)	105.2±1.0 (5)	1.29	42	76.2±3.1 (6)	82.5±3.5 (6)

MRP = membrane resting potential; AP = action potential. Figures in parentheses refer to the number of observations from which the mean MRP and AP values were calculated.

tial changes in ventricular single cells and relating any changes to measured venous blood concentrations of cyclopropane. Figure 4 shows a representative record of left ventricular transmembrane action-potential and lead 2 electrocardiographic changes occurring in a dog continuously receiving cyclopropane and oxygen until ventricular fibrillation occurred. Measured blood cyclopropane concentration varied between 16.8 and 23.4 ml./100 ml. during the recordings.

Results of these open-chest dog experiments indicate that, with concentrations of cyclopropane adequate to achieve venous blood levels of the agent as high as 21 ml. 100 ml., left ventricular resting potentials and action potentials were not changed from those values recorded at anesthetic levels associated with a stage III, plane 1, level of anesthesia.

The effects on action-potential duration and plateau phase of repolarization were variable owing to the changes in spontaneous rate of beating of the heart. Thus, at slowed heart rates, the rate of repolarization of the plateau phase of the action potential was slowed. At faster heart rates, rate of repolarization of the plateau phase of the action potential was increased. With sustained elevation of blood levels of cyclopropane (21–23 ml. 100 ml.), irregularities in the electrocardiogram appeared (coving of the T wave), and an A-V nodal rhythm emerged with an increase in action-potential duration and prolongation of the plateau phase. Finally, ventricular fibrillation occurred, and action-potential amplitude and duration decreased.

Discussion

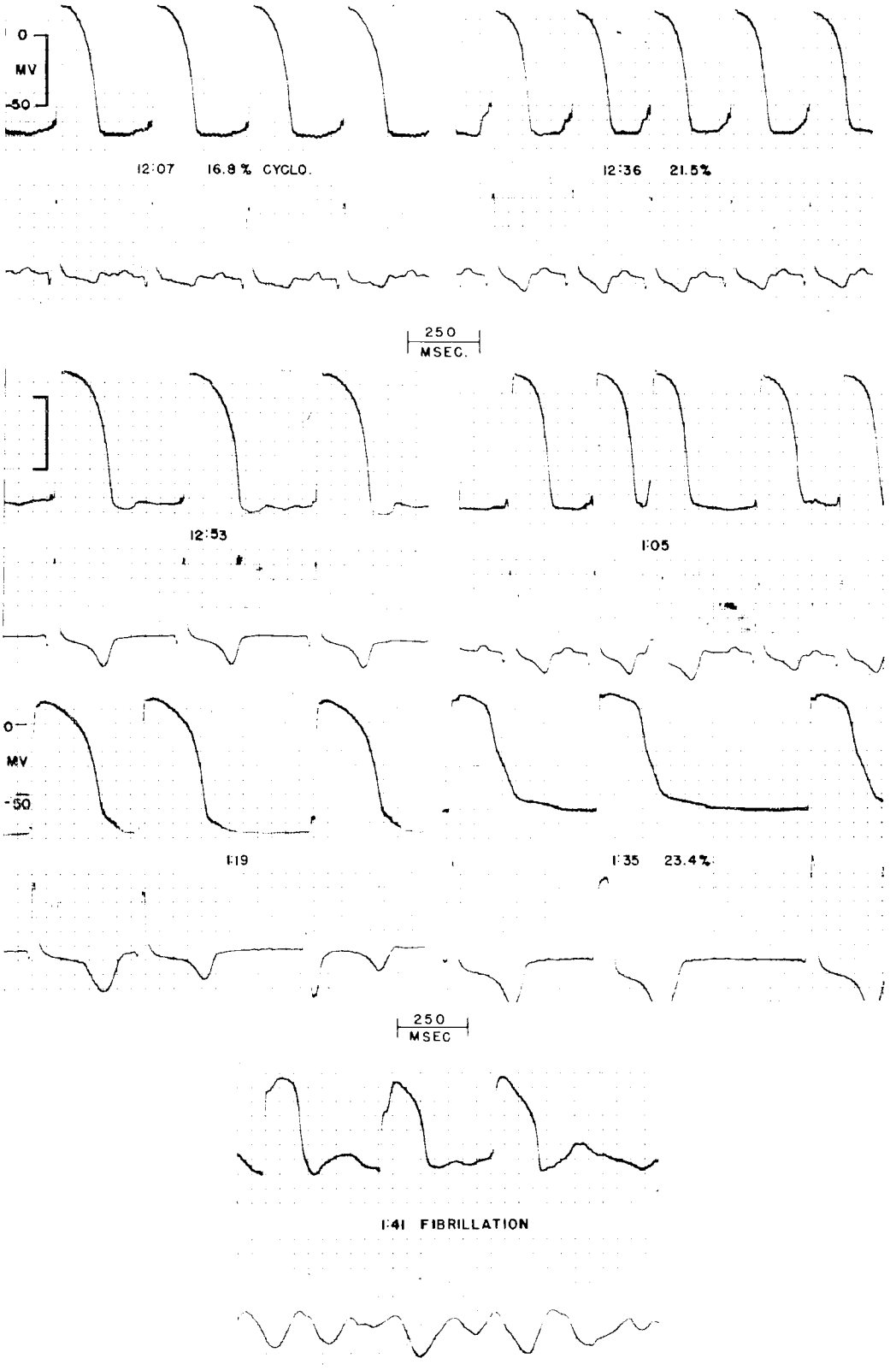
The consistent observation that the administration of cyclopropane, in the concentrations tested, was accompanied by a negative inotropic effect in isolated atrial tissue, is in agreement with the results obtained by others utilizing isolated cat auricle[7] and turtle and frog ventricle[10, 11] preparations. A depression of ventricular contraction force with cyclopropane in intact dog heart *in situ* has also been reported.[12, 13]

The results of measurement of oxygen saturation of the perfusate in the present study points to the possibility that the decrease in atrial contractile tension accompanying the administration of cyclopropane may be due, in part, to the production of the relative tissue hypoxia. The fact that the perfusate oxygen saturation had to be reduced 33–80 per cent with nitrogen to achieve a 50 per cent decrease in contractile tension, whereas only a 5–35 per cent reduction of perfusate oxygen saturation in the presence of cyclopropane was sufficient to produce equal or greater reduction in contraction for the same interval, suggests that the drug *per se* possesses a negative inotropic action. Whether this hypoxic effect contributed to the results of the earlier investigations already mentioned cannot be answered since the perfusate or blood oxygen saturation was not reported. However, from the gas flow rates and cyclopropane concentrations used, relative hypoxia probably contributed to the recorded effects on contraction.

The most prominent and consistent effect on transmembrane potentials accompanying the administration of cyclopropane to isolated electrically-driven preparations was the acceleration of rate of repolarization of the plateau phase of the action potential. This effect was associated with a decrease in contractile tension. This consistent relationship between the membrane action-potential and contraction changes suggested that the characteristic myocardial depressant action of cyclopropane might be the result of this agent producing changes in myocardial membrane permeability and ion-transport mechanisms known to be involved in determining and regulating the rate of cardiac membrane repolarization.[8]

However, there are many examples of dissociation of force of contraction from the configuration or duration of the membrane action

FIG. 4. Transmembrane potentials from single cells of left ventricle of dog's heart, *in situ*. Simultaneously recorded lead 2 ECG is shown on lower portion of each record. Measured blood cyclopropane concentrations (ml./100 ml.) are also shown on three records. Note lack of effect of cyclopropane on membrane resting potential or action potential amplitude despite appearance of T-wave changes and nodal rhythm. Only with the onset of ventricular fibrillation is amplitude and resting potential decreased. Repolarization changes are associated with rate changes.



potential which argue against this generalization.[5, 11] While the data obtained in the present study using cyclopropane suggest a general association between the increase in rate of membrane repolarization and depression of contraction, the results by no means prove or disprove a causal relationship between these two parameters.

It is conceivable that both the diminished contraction and changes in membrane action-potential repolarization seen with cyclopropane may be results of separate actions of the drug on membrane and contractile properties. This view has some support in the results of preliminary experiments where pretreating the atria with atropine decreased or prevented the membrane action-potential changes normally seen with cyclopropane, although the characteristic decrease in contractile tension still occurred. Confirmation and extension of these findings would offer support for the concept that cyclopropane *per se* has a direct depressant effect on myocardial contractile mechanisms, and this action is not necessarily mediated through changes in membrane electrical events. Interestingly, in dogs, atropine treatment also has been shown to be ineffective in preventing the decrease in ventricular contraction force characteristically produced by cyclopropane.[12]

The interpretation of the membrane potential changes in the intact, innervated, spontaneously beating dog heart is complicated by the presence of changes in pacemakers, ectopic beats, and arrhythmias. Under these variable conditions, it is not clear whether there are any significant qualitative differences in the membrane potential changes produced by cyclopropane in ventricular tissue *in situ* and isolated atrial tissue. The general lack of effect of cyclopropane on resting-potential and action-potential amplitude in both isolated atrial and intact ventricular preparations is worthy of mention, however. Action potentials obtained during ventricular fibrillation in the intact dog studies are qualitatively similar to fibrillation potentials produced by other means,[5, 11] and are not considered characteristic of cyclopropane alone. The fact that ventricular contraction force is depressed *in situ*,[12] as well as *in vitro*,[10, 11] supports the view that atrial and ventricular myocardium are qualitatively similar in their reaction to cyclopropane. On the other hand, changes in the electrical excitability produced by cyclopropane in atrial muscle do point to the possibility that right and left atrial myocardium react differently to various concentrations of cyclopropane. Significant differences in ionic and biochemical composition of right and left atrial tissue have been shown,[13, 15] and these differences may offer a clue to the different effect cyclopropane has on the excitability of these two tissues.

Summary

The effects of various concentrations of cyclopropane on atrial and ventricular myocardial membrane potentials have been studied in isolated rabbit-atrial preparations and intact dogs. Atrial membrane potential changes have been related to simultaneously measured contractile force, excitability and perfusate oxygen saturation.

In all concentrations studied, cyclopropane produced a negative inotropic effect on isolated, electrically driven atrial myocardium. This negative inotropic effect in right atrial tissue was found to be directly related to the measured cyclopropane concentration in the perfusate. The production of relative hypoxia accompanying the administration of cyclopropane probably contributes to this negative inotropic effect, as shown by control studies where hypoxia was produced with nitrogen-gassed perfusate.

Measurement of atrial transmembrane potentials using the microelectrode technique showed that the negative inotropic effect of cyclopropane was accompanied by an acceleration of the rate of repolarization of the first and second phases of the atrial membrane action potential. Membrane resting and action-potential amplitude were only slightly affected. Action-potential overshoot was decreased, however, with prolonged administration of the drug.

The negative inotropic effect and increase in the rate of repolarization of the atrial action potentials produced by cyclopropane were found to be generally associated with a decrease in the electrical excitability of right atrial tissue, but an increase in excitability in left atrial preparations.

Recordings of ventricular transmembrane potentials in open-chest dogs during cyclopropane anesthesia showed that membrane resting and action-potential amplitude were not markedly changed until blood levels of cyclopropane were obtained which produced ventricular fibrillation. Action-potential repolarization was variably affected with blood levels of cyclopropane ranging between 16–24 ml./100 ml. This variable effect on ventricular action potentials was associated with changes in spontaneous rate, ectopic beats and arrhythmias.

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ENDOSCOPY The substitution of promethazine for a barbiturate in the combination barbiturate-narcotic-atropine (or scopolamine) improved the premedication for endoscopy under topical lidocaine anesthesia. There were fewer failures, less anxious patients and less salivation. Hypotension did not occur. (*Findlay, C. W.: The Value of Promethazine Hydrochloride in Preparing Patients for Peroral Endoscopy. Amer. Rev. Resp. Dis. 86: 272 (Aug.) 1962.*)