

Mechanically induced action potential changes and arrhythmia in isolated and in situ canine hearts

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ABSTRACT Stretch of excised myocardial tissue causes electrophysiological and potentially arrhythmogenic changes in transmembrane action potentials but corresponding data of the intact mammalian heart are lacking. The effects of increases in ventricular volume and pressure on epicardial monophasic action potentials were therefore investigated in isolated, cross circulated and in situ canine hearts. In seven isolated hearts, increases in ventricular volume and pressure resulted in (1) a linearly related decrease in action potential amplitude ($r = 0.988$; slope = 0.41% amplitude·ml⁻¹; volume intercept = 17.6 ml), mainly due to a decrease in maximum diastolic potential; (2) a decrease in action potential plateau duration (at 20% repolarisation) by 19 (SD 8)%; and (3) appearance of early afterdepolarisations, reaching up to 18% of total action potential amplitude. Afterdepolarisations occurred only when ventricular outflow was obstructed at end diastole but not at end systole. In eight in situ hearts, increase in left intraventricular pressure produced by transient occlusions of the ascending aorta was also accompanied by decrease in maximum diastolic potential and action potential plateau duration, and by appearance of early afterdepolarisations. In both isolated and in situ intact ventricles, the loading induced electrophysiological changes were associated with occurrence of ectopic ventricular beats. These data show that mechanical overload produces significant electrophysiological changes in the intact canine ventricle which may lead to arrhythmia.

Ventricular arrhythmias are frequently encountered in cardiac diseases associated with volume or pressure overload, such as aortic valve disease¹⁻³ or dilated cardiomyopathy.⁴ It has been suggested that some of these arrhythmias might be due to myocardial stretch which may alter cellular electrophysiological properties.⁵ In isolated muscle preparations and frog ventricle, stretch shortens the action potential,⁶⁻⁸ decreases the resting potential,⁹⁻¹¹ and induces afterdepolarisations and extrasystoles.^{7 8 12} For the intact mammalian heart, however, experimental evidence for such stretch induced electrophysiological

and potentially arrhythmogenic changes has not been provided. This lack of data may be due to the fact that electrophysiological effects of myocardial stretch are not conspicuous in the surface electrocardiogram, and that transmembrane action potential recordings are very difficult to obtain from a vigorously beating ventricle. Recently, a contact electrode technique has been developed which can record monophasic action potentials (MAPs) from the surface of the beating heart and which appears less sensitive for motion artifacts than intracellular recordings.^{13 14} We therefore felt that this contact electrode technique is well suited for studying the influences of mechanical loading on the electrophysiological properties of intact mammalian ventricles.

The purpose of this study was first to evaluate the electrophysiological effects of pressure and volume loading in the isolated canine heart; MAPs were recorded from the left ventricular epicardium during varying ventricular loading conditions and quantitatively analysed for changes in duration,

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resting and action potential amplitude, and occurrence of afterdepolarisations. Second, we investigated the association between the occurrence of these mechanically induced electrophysiological changes and ventricular arrhythmias in the in situ canine heart.

Methods

ISOLATED HEARTS

We studied seven isolated, blood perfused canine hearts. The method of isolating, supporting and imposing a physiological load on canine hearts has been described in detail by Sunagawa *et al.*¹⁵ Figure 1A schematically depicts the volume and impedance loading servo system and MAP recording probe. Blood from the femoral arteries of a support dog was used to perfuse the isolated heart at a coronary arterial pressure maintained constant at 80 mm Hg by a servo pump. A water filled balloon was placed in the left ventricle (LV), and the balloon volume was controlled and measured by a servo system. Ventricular pressure was measured with a high fidelity semiconductor pressure transducer (Millar, model 380), placed in the balloon (fig 1A). The command signal for the servo pump was provided by a digital computer programmed to simulate a physiological aortic input impedance which could be varied by the computer.¹⁶ This computer based system allowed control and rapid modification of diastolic and/or systolic loading conditions of the ventricle, including sudden changes between isovolumetric and ejecting contraction modes.

MAPs were recorded from the left ventricular (LV) epicardial surface of the isolated heart with the contact

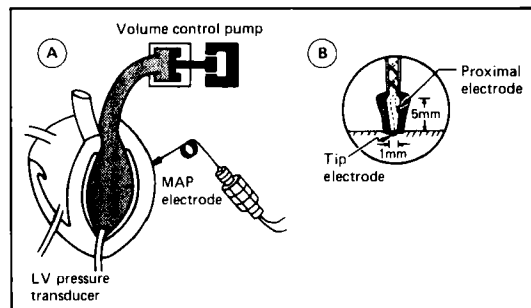


FIG 1 A, Schematic diagram of the isolated heart preparation which allowed precise control and measurement of ventricular volume and pressure by a volume servo system and impedance loading system (see text and refs^{16 17} for details). B, Magnified view of distal end of contact electrode probe with tip and proximal electrode, surrounded by a piece of foam rubber. Stable contact between the tip electrode and the epicardium of the beating heart is maintained by a spring loading mechanism (A).

electrode recording technique.^{13 14} Recordings with this extracellular electrode technique have been shown to indicate accurately the duration and configuration as well as relative changes in transmembrane resting and action potential amplitude of muscle cells adjacent to the electrode.¹⁷ The recording device has two non-polarisable electrodes made from sintered silver-silver chloride pellets; one electrode is kept in intimate contact with the epicardium by a spring loaded mechanism, the other electrode is positioned 5 mm away from the tip and has electrical contact to the epicardium through a small saline soaked piece of foam rubber (fig 1B). MAP recordings were preamplified with high input impedance, direct current (DC) coupled differential amplifiers. Using this contact electrode technique, rather than suction electrodes, MAPs of stable amplitude, smooth contour and isopotential diastolic baselines could be recorded continuously over periods of several hours. This allowed recordings to be made from a single epicardial site throughout the experimental protocol. A cardiac surface electrogram was recorded simultaneously with another pair of electrodes, one electrode placed on the left, the other on the right ventricular free wall. The purpose of measuring the electrogram was to provide a means of identifying aberrantly conducted and ventricular escape beats.

Pacing of the isolated hearts was performed with two wire electrodes sewn to the apex of the LV or to the remaining atrial tissue, using twice diastolic threshold stimuli of 2 ms duration. The stimulus cycle length was kept constant at 500 ms throughout all experiments.

IN SITU HEARTS

Eight pentobarbitone-anaesthetised mongrel dogs, ventilated with room air through a cuffed endotracheal tube by a Harvard ventilator, underwent a left lateral thoracotomy. The pericardium was incised and distended so as to suspend the exposed heart. Hearts were allowed to beat at their spontaneous rate without pacing. Baseline sinus rate did not change more than 5 per cent during the interventions.

MAPs were recorded from the exposed anterior surface of the left ventricle with the contact electrode described above. In addition, epicardial unipolar electrograms were recorded by connecting the proximal (foam rubber) electrode of the MAP recording probe to the positive input, and a distant reference electrode (sewn onto the aortic root) to the negative input of a separate DC coupled amplifier. Using this method, local unipolar DC coupled epicardial electrograms could be recorded simultaneously and from the same sites that the epicardial MAP recordings were being made, permitting a direct comparison of the two signals

during the interventions. Because the foam rubber electrode was anchored to the epicardium by the spring loaded MAP tip electrode, unipolar electrograms were of exceptional stability. The purpose of the simultaneously recorded unipolar electrogram was to observe whether changes in the MAP were associated with changes in the local QRS, T wave and U wave. One or two standard electrocardiographic leads were also recorded to differentiate sinus beats from ectopic atrial or ventricular beats.

LV pressure and aortic pressure were measured simultaneously using a double tip manometer catheter with one transducer on either side of the aortic valve. In order to assess changes in wall strain near the site of the MAP recording, a pair of sonomicrometer crystals were plunged into the subepicardial layer one to two cm apart from each other so that the MAP recording electrode could be placed between them. The signal from the ultrasonic transducers was processed as described previously¹⁸ and displayed along with the other measurements on a multichannel chart recorder. In three experiments, an electromagnetic flow probe was placed on the left main coronary artery to assess the effect on myocardial perfusion of obstructing the ascending aorta.

PROTOCOLS

Isolated hearts — MAPs were recorded continuously from an LV epicardial site, while the following interventions were carried out: (1) the LV was constrained to contract isovolumetrically, and volume was increased gradually from 10 ml to 45 ml, maintained at the higher level for 60 s, and then again gradually decreased to the baseline volume of 10 ml; (2) with isovolumetric contractions, LV volume was suddenly increased and decreased in steps of approximately 10 ml, maintaining each step for 30 s; (3) the LV was ejected against the computer generated impedance afterload, and LV volume was clamped at end systole and end diastole.

In situ hearts — While recording MAPs continuously from the epicardium of the anterior free wall of the left ventricle, a large pair of rubber coated haemostats was used to clamp the aorta proximal to the brachiocephalic trunk. This resulted in a sudden and considerable increase in LV pressure. The clamp was released after 3-6 s.

DATA ANALYSIS

The amplitude of the MAP was defined as the difference in mV between the diastolic baseline and the crest of the plateau (phase 4).¹³ Because the MAP amplitude does not represent the absolute transmembrane voltage,¹⁷ we expressed amplitude changes during an intervention as a percentage of the preceding control value. Relative changes in the

maximum systolic and maximum diastolic potential were also assessed individually and expressed in percent of total amplitude. The duration of the MAP was analysed quantitatively at levels of 20% (MAPD₂₀) and 90% (MAPD₉₀) repolarisation, respectively. The amplitude of afterdepolarisations was defined as the difference in mV between the end diastolic baseline and the afterdepolarisation, measured 10 ms after the deflection point at which the afterdepolarisation deviated from phase 3 of the MAP, and expressed in per cent of total MAP amplitude. Data acceptable for MAP analysis were derived only from stable recordings, which were judged on the basis of: (1) constant amplitude, morphology, and stable resting potential before and after control; (2) isoelectric phase 4 with no suggestion of afterdepolarisations during control; (3) stable amplitude of phase 2 exceeding 20 mV. Correlations between volume changes and electrophysiological effects were determined by standard linear regression analysis.

Arrhythmias frequently occurred during various loading interventions in both the isolated and in situ hearts. In the in situ hearts, arrhythmia occurrence (one or more ectopic ventricular depolarisations) during clamping of the aorta was compared with that before and after the loading interventions. Arrhythmias of the isolated ventricle were not correlated with the loading interventions because it would be difficult to exclude the possibilities that these arrhythmias were caused by friction between the balloon and the endocardium or tethering of the mitral annulus by the balloon mounting apparatus. In the isolated hearts, therefore, the volume loading interventions were used for quantitative analysis of MAP changes only, and only arrhythmia free volume loading interventions were analysed.

ANIMAL CARE

All studies were carried out in concordance with specific recommendations on the ethical principles of animal experimentation, as approved by the Administrative Panel on Laboratory Animal Care of the Johns Hopkins University.

Results

ISOLATED HEARTS

Original experimental recordings of LV pressure, LV epicardial MAPs, and LV volume of the isolated, isovolumetrically beating heart are presented in fig 2. These recordings from two representative experiments illustrate the effect of a gradual change in LV volume on LV pressure and MAP recordings. Initially, LV volume and diastolic and systolic pressures were at low levels (10 ml; 0 and 20 mm Hg respectively in this

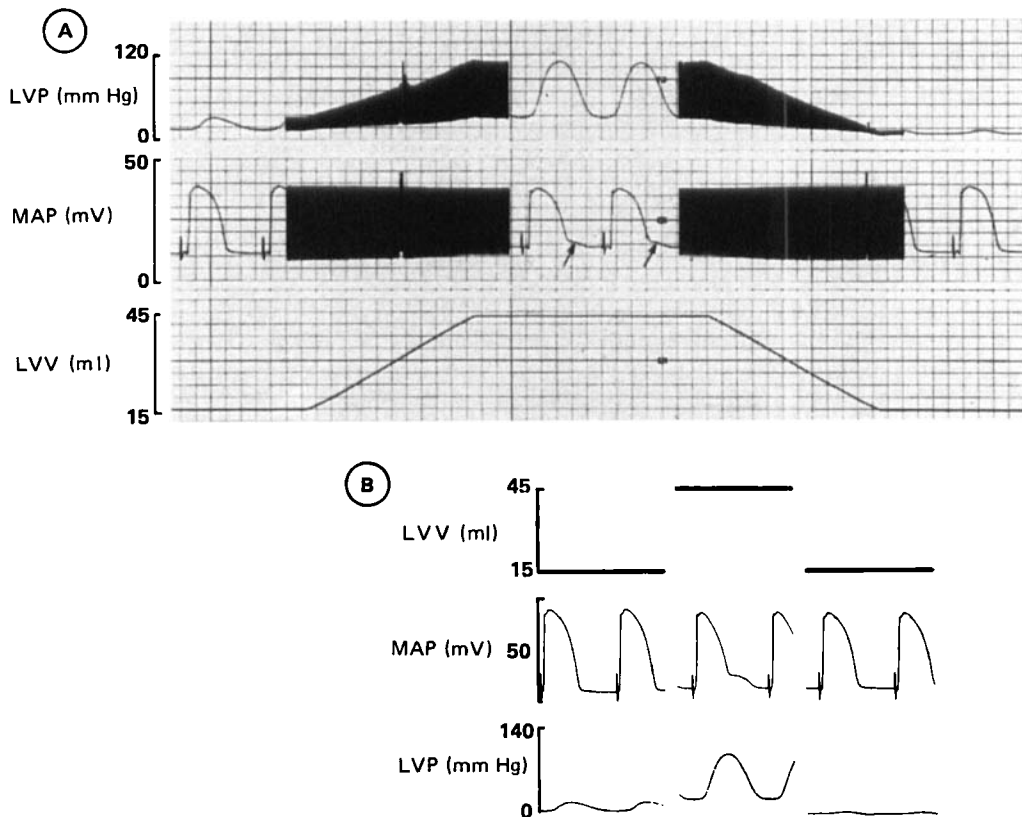


FIG 2 Original recordings of left ventricular volume (LVV), LV pressure (LVP), and monophasic action potentials (MAP) of an isovolumetrically beating ventricle during gradual volume loading and unloading. Panel A shows decrease in MAP amplitude and occurrence of small afterdepolarisations (arrows) with volume increase. Panel B shows less decrease in MAP amplitude, but more pronounced afterdepolarisations at high volume. Time calibration can be derived from the interval between two successive MAP recordings which is 500 ms in both panels.

example), and the MAP recordings demonstrate near isoelectric diastolic potentials between successive responses. The volume was then gradually increased over 60 s to a maximum volume of 45 ml, resulting in a gradual increase in diastolic filling pressure and systolic developed pressure. Simultaneous with these changes in LV dimensions and pressure, the voltage of the MAP (both diastolic and systolic) decreased and small ‘‘humps’’ or early afterdepolarisations occurred at the end of phase 3 repolarisation (arrows in fig 2A). When, after 1 min, the ventricular volume was again gradually lowered to baseline, MAP amplitude returned to control and the afterdepolarisations disappeared. Figure 2B shows another example in which volume loading produced prominent afterdepolarisations but less diastolic depolarisation. This recording also shows marked shortening of MAP plateau duration with the greater volume (see below).

The volume induced pressure increase and

electrophysiological changes occurred with a parallel time course, suggesting that the electrophysiological effects of loading developed on a beat-to-beat basis, without appreciable delay. This was further tested by increasing and decreasing LV volume in discrete steps of 10 ml, each step being maintained for 30 s. In all five preparations submitted to this protocol, the changes in MAP amplitude occurred within 1-2 beats after a volume increment or decrement and remained constant at that volume for the 30 s observation periods.

Figure 3 summarises the effects on total MAP amplitude of eight transient volume ramps in seven isolated hearts. There appeared to be a ‘‘threshold volume’’ above which MAP amplitude began to decrease. Volume changes within a moderate range from 10 to approximately 20 ml had little or no effect on the MAP. With further increases in volume (the tested maximum was 45 ml) both systolic and diastolic

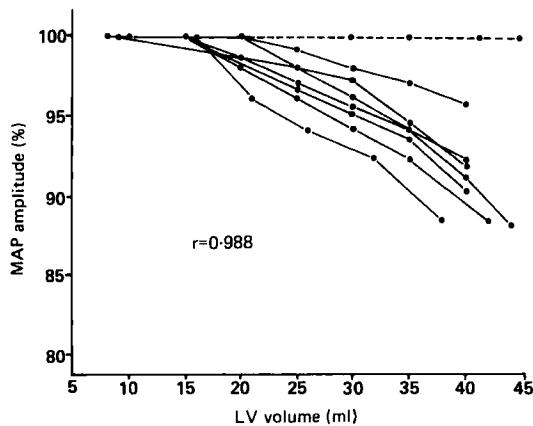


FIG 3 Decrease in MAP amplitude during increase in the volume of isovolumetrically beating ventricles. Values are expressed in percent of MAP amplitude at slack volume. In one ventricle, volume increase did not significantly alter MAP amplitude (dotted line). The average linear regression was calculated only from the seven loading interventions in which significant MAP amplitude changes occurred (solid lines).

potential decreased in a linear fashion; decrease in diastolic potential accounted for 82 (6)% of the decrease in total MAP amplitude. This relationship was observed during seven of eight volume loading experiments and could be fitted by linear regression; on average, total MAP amplitude decreased by 0.41 % per ml LV volume (volume intercept 17.6 ml; $r = 0.988$). The volume intercept denotes the threshold volume below which the MAP had a constant amplitude.

The relationship between ventricular volume and afterdepolarisations in the isolated isovolumetrically beating heart is quantitatively depicted in fig 4. Under isovolumetric conditions, afterdepolarisations generally were present even at relatively low ventricular volumes, and increased in amplitude with volume. At a given volume, the amplitude of the afterdepolarisations varied considerably between different ventricles, while at a given site in the same ventricle a linear relation between volume and size of afterdepolarisations was found. In one ventricle no afterdepolarisations were observed up to a volume of 40 ml.

The quantitative relationship between ventricular volume and the duration of the MAP at two different levels of repolarisation is shown in fig 5. The MAP duration at 20% repolarisation (plateau duration) decreased fairly linearly with ventricular volume up to a tested maximum of 40 ml. The MAP duration at 90% repolarisation (MAPD₉₀), however, increased with volume. As is illustrated in the inset of fig 5, the increase in MAPD₉₀ was due to the development of

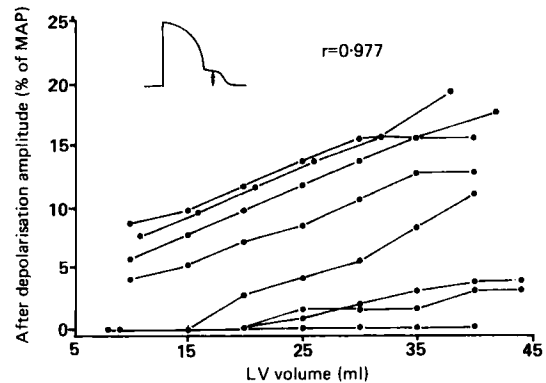


FIG 4 Increase in magnitude of afterdepolarisations during increase in volume of isovolumetrically beating ventricles. The average r value was computed for the seven out of eight loading interventions during which afterdepolarisations were recorded.

afterdepolarisations. The volume increase not only increased the amplitude of the afterdepolarisations (fig 4) but also their duration.

To examine whether the changes seen in the MAP were due to the increase in ventricular volume or to the circumstance that the ventricle was made to contract isovolumetrically, or to a combination of both, we examined how switching the mode of contraction from ejecting to isovolumetric influenced the contour of the MAP. Figure 6 shows a result typical for three such experiments. As shown in the left panel, MAPs with no afterdepolarisations were recorded when the

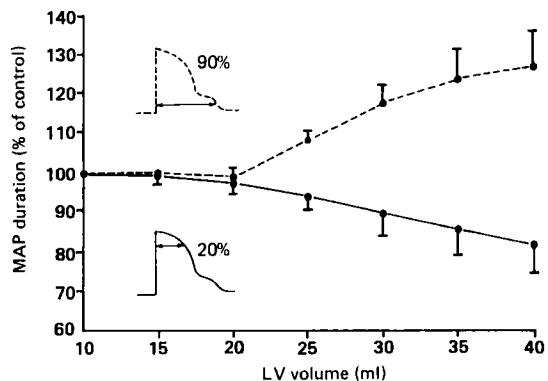


FIG 5 Effect of LV volume on MAP duration at repolarisation levels of 20% (MAPD₂₀, solid line) and 90% (MAPD₉₀, dotted line). Average of seven volume loading interventions in six isovolumetrically beating ventricles are presented. Bars = SD. MAPD₂₀ (plateau duration) decreases linearly with volume. MAPD₉₀ shows a shortening trend only during a very low range of volume increase. At volumes above 20 ml, MAPD₉₀ increases, due to an increasing magnitude and duration of afterdepolarisations.

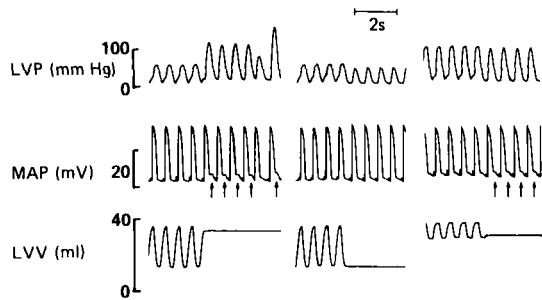


FIG 6 Relations between ventricular preload, afterload, and changes in monophasic action potential. *Left panel: Normal MAPs were recorded when the ventricle was allowed to eject freely (low outflow impedance). When ventricular volume was suddenly clamped at end diastole, afterdepolarisations immediately occurred (arrows). Middle panel: Clamping ventricular volume at end systole did not produce afterdepolarisations in the MAP recording. Right panel: Small afterdepolarisations were recorded when the ventricular contractions started at high diastolic volume and also had to eject against increased afterload. These afterdepolarisations increased when the volume was clamped at end systole.*

ventricle was allowed to eject, even from high end diastolic volumes. When ejection was suddenly prevented at end diastole while the ventricular volume was high, afterdepolarisations appeared on the first beat following the switch. In contrast, no afterdepolarisations were recorded when the ventricle was clamped at the low, end systolic volume (middle panel). With high diastolic volume a decrease in diastolic and systolic MAP amplitude could again be seen (right panel).

IN SITU HEARTS

Under control conditions, MAPs recorded from the epicardium of the left or right ventricle had normal configurations with smooth, stable diastolic baselines in all eight in situ hearts studied. Volume and pressure loading in this preparation was accomplished by transiently clamping the aorta. Figure 7 shows an aortic clamp of 5 s duration, during which time the aortic pressure distal to the clamp decreased pulselessly. LV volume was not measured in the in situ hearts, but an increase in ventricular dimensions could be derived from the signal of sonomicrometer crystals which were implanted in the anterior wall of the LV near the MAP recording site, and demonstrated a marked increase in mean segment length during the aortic clamping. The example shown in fig 7 is atypical in that arrhythmia did not occur during the aortic occlusion. This arrhythmia free example was selected to illustrate the loading induced MAP changes without modification by arrhythmia. As fig 7 shows, the increase in mean segment length produced by the

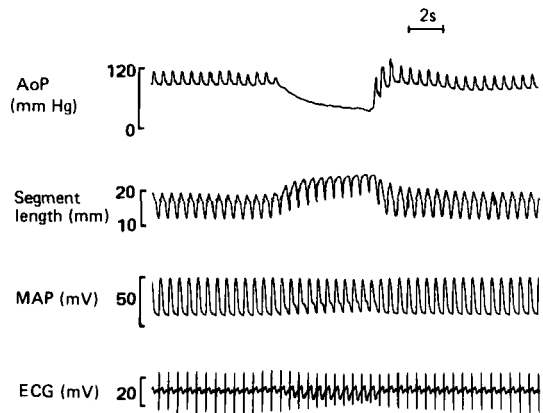


FIG 7 Effect of transient aortic clamp on LV segment length, epicardial MAP recordings and epicardial unipolar electrograms in the in situ heart. *The onset of the aortic clamp is indicated by the sudden fall in aortic pressure (AoP). The MAP recording shows loss in maximum diastolic potential and occurrence of afterdepolarisations. These changes are accompanied by ST changes in the local unipolar electrogram.*

aortic occlusion was associated with several changes in the epicardial MAP: (1) a decrease in the maximum diastolic potential; (2) a shortening of plateau duration; and (3) early afterdepolarisations. The local unipolar electrogram, recorded in the immediate vicinity of the MAP recording site, showed reduction in QRS amplitude, increase in positive T wave amplitude, and development of negative U waves. All of these electrographic changes paralleled the changes in the adjacent MAP recording. Release of the aortic clamp resulted in rapid recovery of aortic pressure, decrease in LV segment length, and normalisation of the MAP and unipolar electrogram.

An original recording in which clamping of the aorta resulted in arrhythmia is shown in fig 8. Aortic occlusion, indicated by the loss of aortic pressure, resulted in increase of LV pressure and segment length (segment length not shown in fig 8). Within one or two beats of the onset of the aortic clamp and rise of LV pressure, afterdepolarisations appeared in the MAP recordings. A few beats later, as LV pressure rose further, a loss in maximum diastolic potential of the MAP also became apparent. During the second half of the aortic occlusion period, ectopic ventricular beats occurred. Release of the aortic clamp again resulted in normalisation of all recordings.

Figure 9 shows another example of aortic clamping with accompanying ventricular arrhythmias. The first beat after the onset of the clamp is followed by a premature depolarisation which generates relatively little pressure. This beat is followed by a compensatory pause and a post-extrasystolic beat with

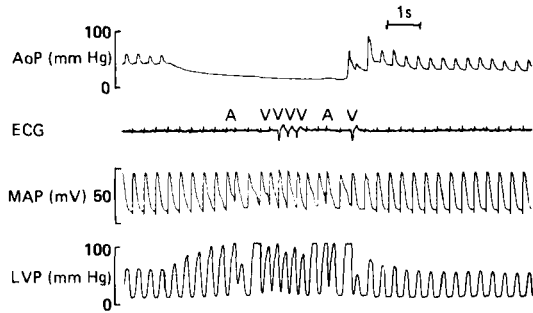


FIG 8 Development of afterdepolarisations and reduction in maximum diastolic potential of LV epicardial MAP recordings during transient aortic occlusion (see fall in aortic pressure [AoP] distal to clamp). The ECG demonstrates two atrial (A) and four ventricular (V) ectopic beats during the aortic clamp. MAP afterdepolarisations were greater during beats with high pressure and absent during low pressure.

a markedly increased pressure response. The MAP of this potentiated, post-extrasystolic beat exhibits an afterdepolarisation and is followed by another premature depolarisation. This premature response is again accompanied by a very low systolic pressure response (which fuses with the falling pressure limb of the preceding beat) and does not have an afterdepolarisation. This sequence of a weak premature beat without afterdepolarisation and a potentiated post-extrasystolic beat with afterdepolarisation (and subsequent premature depolarisation) repeats itself for two more cycles, until release of the aortic clamp. As seen in the simultaneously recorded surface ECG, this alternation of electrical and mechanical events produces an electrocardiographic pattern of ventricular bigeminy. This phenomenon was observed during three transient aortic occlusions.

While arrhythmia was absent before and after the period of aortic occlusion in all eight in situ hearts studied, ventricular ectopic beats occurred during 18 out of 23 aortic occlusions (85%). Afterdepolarisations were recorded in 82% of occlusions during which arrhythmia occurred and were absent in 13% of occlusions despite occurrence of arrhythmia.

Discussion

This study demonstrates that increases in mechanical load of the canine left ventricle, which is contracting isovolumetrically or switched from ejecting to isovolumetric contractions, produce several significant changes in epicardial monophasic action potentials. First, increase in preload volume and developed pressure produced decreases in the

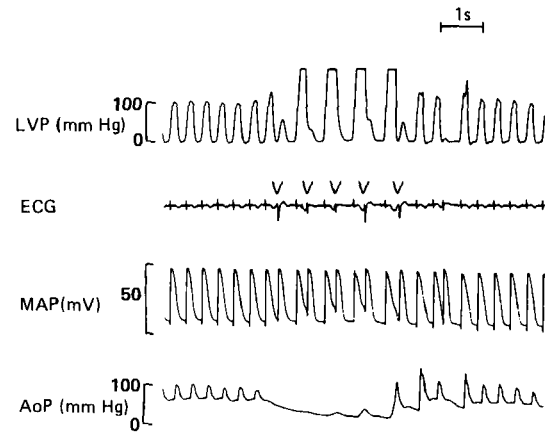


FIG 9 Afterdepolarisations and arrhythmia with bigeminal pattern during transient aortic occlusion. Further details in text.

amplitude of the MAP, mainly due to a decrease in maximum diastolic potential and to a lesser degree in systolic potential. Second, increase in preload and pressure shortened the MAP, particularly at the plateau level. Third, augmented ventricular load produced early afterdepolarisations. These load induced electrophysiological changes coincided with the occurrence of arrhythmia. Here, we shall focus on three aspects of the results: (a) possible limitations to the interpretation of monophasic action potentials; (b) comparison of our findings to previous studies linking changes in mechanical load to those in action potential configuration; and (c) relationship between load induced action potential changes and arrhythmia.

VALIDITY OF MAP RECORDINGS

In this study, electrophysiological effects of altered ventricular loading conditions were assessed by MAP recordings obtained with a special contact electrode pressed against the ventricular epicardium. We have previously demonstrated in isolated rabbit septum preparations that such recordings, although of smaller amplitude than intracellular measurements, accurately indicate the time course of membrane repolarisation and relative changes in membrane resting and action potential amplitude.¹⁷

In an early comparison of intracellular action potentials and suction electrode MAPs in an isolated rabbit heart, Hoffman *et al*¹⁹ noticed afterdepolarisations in the suction electrode record but not in the microelectrode record. Based on this observation they dismissed the afterdepolarisation as a movement artifact. Lepeschkin, one of their co-authors, later challenged this interpretation and advanced the theory that afterdepolarisations, including those induced by stretch, might be

responsible for the U wave in the surface ECG.²⁰ Lipeschkin reasoned that the MAP records activity from deeper layers "which are subjected to much more mechanical stress than the superficial layers from which transmembrane potentials are usually taken." Similar conclusions were drawn by Lab^{5 8} from experiments in isolated frog ventricle.

Unfortunately, direct validation of the contact electrode recordings in this study of whole canine ventricles is not possible due to the vigorous ventricular wall motions that make undistorted microelectrode recordings extremely difficult. For the following reasons, however, we believe that the recorded MAP changes and the occurrence of afterdepolarisations reflect true transmembrane electrophysiological events and not movement artifacts: (1) We never recorded afterdepolarisations from ventricles beating under physiological preload and allowed to eject against normal afterloads, despite vigorous movement. (2) Isovolumetric beats exhibit less wall motion than ejecting beats, yet afterdepolarisations were greater in isovolumetric than in ejecting beats. (3) Clamping at high volume but not at low volume was a *sine qua non* for the occurrence of afterdepolarisations, although ventricular wall motion was increased (fig 6). (4) Decrease in MAP amplitude and occurrence of afterdepolarisations was associated with loss in R wave amplitude and development of negative U waves in the adjacent unipolar electrogram. The unipolar electrogram was recorded with an independent electrical circuit (fig 7) unlikely to reflect similar motion artifacts; yet the changes seen in both the MAP and the unipolar electrogram compliment each other.²¹ (5) The occurrence of afterdepolarisations was often followed by the occurrence of premature, ectopic beats whose action potentials had a reduced amplitude as is expected when the membrane is partially depolarised. (6) Finally, as discussed below, our findings in the intact canine ventricle are consistent with previous observations on mechano-electrical feedback, recorded by microelectrode or insulated gap techniques in excised ventricular muscle preparations. It is important, however, that until final proof can be obtained, recordings of afterdepolarisations in the intact heart should be interpreted with caution.

COMPARISON WITH PREVIOUS STUDIES ON MECHANO-ELECTRIC FEEDBACK

Our findings on mechano-electric feedback in intact isolated and *in situ* canine hearts confirm and extend the implications of previous experiments on isolated amphibian hearts and excised mammalian myocardium which showed that myocardial strain leads to a decrease in resting and action potential magnitude,^{10 11} shortening of action potential

duration,^{7 8 20 21} and occurrence of afterdepolarisations.^{7 8 20 21}

Decrease in resting and action potential amplitude during stretch has been noted previously in excised myocardial preparations using microelectrode techniques.⁹⁻¹¹ The mechanism underlying this stretch effect is not understood. It may be speculated that stretch of myocardial cells, and their membranes, changes ion channel conductances or the surrounding ionic milieu such as to produce the observed potential changes. Lekven *et al* noted reduction in canine electrocardiographic R wave amplitude during increases in ventricular volume and suggested that this is due to a change in ventricular geometry or intraventricular conductance.²² Stretch induced reduction of membrane potentials, as shown in this and previous studies, would provide an alternative, or additional, explanation that also might pertain to R wave reduction in dilated cardiomyopathies.

Shortening of action potential duration during volume increase, also reported previously in isolated and *in situ* myocardium,^{7 8 20 21} was most notable at the plateau level (phase 2) in our study. The effect of volume increase on the total action potential duration could not always be assessed reliably because of superimposed afterdepolarisations; these produced a prolongation of the apparent action potential duration at 90% repolarisation. In an earlier study of canine ventricles which reported shortening of action potential duration at 90% repolarisation with increased volume,²³ data were obtained in a generally lower range of volumes than in the present experiments, and diastolic baseline potentials were often taken as the average diastolic potential, thereby minimising the contribution of subtle afterdepolarisations. One of the mechanisms proposed to explain length dependent shortening of action potential plateau duration is an increase in intracellular activator calcium concentration at longer muscle length²⁴ that may hasten inactivation of calcium channels,²⁵ or increase calcium activated potassium outward conductance.^{26 27} Further possible mechanisms of mechanically mediated shortening of action potential duration have been discussed.^{5 28}

The afterdepolarisations that occurred during high loading conditions in this study are morphologically similar to those defined by Cranefield²⁹ as early afterdepolarisations which interrupt or delay normal repolarisation of the cardiac action potential. Such early afterdepolarisations, distinct from delayed afterdepolarisations associated with calcium overloaded sarcoplasmic reticulum,³⁰ may reflect the inhibition of outward currents so that membrane potential persists at the plateau level.³¹ They have been observed in isolated cardiac tissues under a variety of conditions, including exposure to

catecholamines, reduced potassium concentrations, slow pacing, hypoxia, and high PCO_2 (see Wit and Rosen³² for review). None of the above listed conditions, which have in common prolongation of action potential plateau duration, appeared to be present in this intact heart study.

We therefore favour two alternative explanations. First, afterdepolarisations may result from direct effects of myocardial cell stress or stretch on electrical properties of the cell membrane. Stretch activated single ion channels have recently been identified in skeletal muscle under patch clamp,³³ and it seems reasonable to expect that stretch of cardiac tissue may also alter specific or non-specific membrane currents, or distort the intra- or extracellular ion milieu such that changes in membrane potential ensue. The second hypothesis is that abrupt changes in myofilament length and therefore calcium affinity³⁴ could produce rapid changes in intracellular calcium concentration that modulate the gating of a non-specific cation conductance.³⁵

Whatever the cellular mechanism may be, systolic stress seems to be an extremely important factor for the electrophysiological changes. This is testified in our intact ventricle preparation by the fact that switching from ejecting to isovolumetric beats at the same end diastolic volume caused a remarkable increase in afterpotentials (fig 6).

Although acute increase in ventricular wall stress in systole may compromise myocardial perfusion, this is unlikely to be the cause of the electrophysiological changes seen in our experiments. Decreases in action potential amplitude occurred within a single or just a few beats following the increase in ventricular pressure. We have shown previously that severe ischaemia, produced by coronary artery occlusion in the working heart, requires at least 30 s to produce significant changes in amplitude and duration of the action potential.¹⁴ Also, in the *in situ* hearts, clamping of the ascending aorta distal to the coronary artery orifices resulted in increased coronary perfusion (verified in three experiments by left main coronary artery flow measurements), which would be expected to counteract ischaemia.

CAUSAL RELATION BETWEEN MECHANICALLY INDUCED ELECTROPHYSIOLOGICAL CHANGES AND ARRHYTHMIA?

The ectopic ventricular beats associated with mechanical loading of ventricles could derive from any one or all of the volume induced electrophysiological changes observed (action potential shortening, decreased diastolic potential, and afterdepolarisations). Shortening of the action potential plateau duration may increase excitability by shifting the strength-interval curve to the left.^{22 36}

Decrease in maximum diastolic potential and presence of afterdepolarisations may produce arrhythmia by bringing the resting membrane potential closer to threshold.³² It has also been reported that transient aortic occlusion in the intact canine heart prolongs the effective refractory period in the segment which demonstrates decreased segmental shortening.³⁷ During isovolumetric contraction, shortening of one segment necessitates lengthening of another, and this may explain the different extent, according to site, of loading induced afterdepolarisation observed in this study, and the divergent effects of mechanical loading on refractoriness in previous studies.^{22 37 38} Further, these early afterdepolarisations (which also might be considered delays in repolarisation) may also augment heterogeneity by locally enhancing or inhibiting excitation, depending upon whether the predominant effect of delayed repolarisation is to drive potentials closer to threshold or to delay recovery from refractoriness.³⁸ Heterogeneous electrophysiological characteristics in the ventricle have long been suspected to facilitate arrhythmia.³⁹ Finally, early afterdepolarisations occurring in some parts of the ventricle but not in others would create a voltage gradient that might produce ectopic depolarisation.⁴⁰ Given these additional dimensions of potential arrhythmogenesis, further clarification of the origin of premature ventricular activation and its relation to afterdepolarisation may require simultaneous determination of refractoriness and action potential contour⁴¹ at multiple sites including the endocardium.

Stretch induced electrophysiological changes may also explain how extrasystoles may precipitate and perpetuate further arrhythmia, even when preload and afterload remain constant. An extrasystole entails the occurrence of a subsequent post-extrasystole that develops greater contractile force than steady state or premature responses.⁴² Based on our observations, this increased pressure might give rise to an afterdepolarisation that could, in turn, produce another premature depolarisation, thereby perpetuating an ongoing cycle (eg, fig 9). A similar pattern of alternating afterdepolarisations and ventricular bigeminy has been described previously in the frog⁸ and canine⁴³ ventricle.

CONCLUSION

We have observed alterations in epicardial monophasic action potentials induced by modification of ventricular preload and afterload in the isolated canine heart. In general, increased load was associated with the occurrence of early afterdepolarisations, shortening of the MAP plateau duration, and a decrease in the maximum diastolic potential. In *in situ* hearts, the same associations between load and MAP alterations were observed. Furthermore, in these in

situ hearts, there was a strong correlation between the occurrence of afterdepolarisations and arrhythmias. Although only a correlation, a causal relationship between load induced electrophysiological changes and arrhythmias described above provides an attractive explanation for the high prevalence of arrhythmias found in patients with ventricular pressure and/or volume overload.¹⁻⁴

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