High-density epicardial mapping during current injection and ventricular activation in rat hearts

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Macchi, Emilio, Massimo Cavalieri, Donatella Stilli, Ezio Musso, Silvana Baruffi, Giorgio Olivetti, Philip R. Ershler, Robert L. Lux, and Bruno Taccardi. High-density epicardial mapping during current injection and ventricular activation in rat hearts. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1886–H1897, 1998.—The purpose of this study is to report new methods for manufacturing precision electrode arrays for recording high-resolution potential distributions from epicardial surfaces of small-animal hearts. Electrode arrays of 64 leads (8 × 8) and 121 leads (11 × 11) were constructed with a tulle substrate to which insulated, fine silver wires (60-µm diameter) were attached by knots at mesh node intervals of 540 × 720 µm. Insulation was removed at the tips of the knots. Potential distributions and waveforms were recorded from saline solutions and rat heart epicardium during ventricular paced beats and during passive current injection in the diastolic interval. Electrical responses obtained from rat epicardium compared favorably with those observed in studies of larger-animal hearts, which used arrays having greater electrode spacing, and revealed the effects of myocardial anisotropy. Epicardial potentials measured early after stimulation in the region surrounding the pacing site were interpreted in terms of potentials generated by an equivalent quadrupolar source. We conclude that electrode arrays for epicardial mapping of small hearts can be constructed with sufficient ease and precision to allow detailed study of fiber structure and electrophysiology in these hearts in normal and pathological conditions.

knowledge of the relationship between myocardial fiber architecture, spread of excitation, and the associated extracellular potential is necessary for correctly interpreting ECG signals. This relationship has generally been studied by recording epicardial potentials during pacing in normal dog hearts (28, 33), in hearts that underwent remodeling, with nontransmural necrosis, or in the presence of healed infarcts (16, 35). This same relationship has hardly been studied in the heart of small animals despite their widespread use for evaluating cardiac function in normal and pathological conditions and for therapeutic purposes because of the need for high-resolution electrode arrays to explore in detail a significant portion of the limited epicardial surface.

The aim of the present study was to perform in vivo measurement of epicardial potentials in the rat heart during current injection and ventricular activity, with a view toward identifying fiber orientation and architecture (19, 21) and defining patterns of ventricular excitation in normal and pathological hearts. The rat was utilized in our investigation in consideration of the finding that it has been used more than any other animal for electrophysiological studies in vitro and for ECG analysis in various heart conditions (3, 4, 12, 31, 32). A preliminary study (2) utilizing 2-mm interelectrode distance epicardial arrays over the entire ventricular surface revealed some limitation in the resolution of potential distribution details that could be observed on the ventricular surface of rat hearts during normal and paced activity. To improve the resolution of detail on the limited surface of rat epicardium, epicardial electrode arrays with interelectrode distance as small as 540 µm were constructed. These electrode arrays require a higher degree of accuracy in manufacturing than do lower-resolution arrays because epicardial potential distributions are affected to a greater extent by nonuniformities of array geometry, electrode contact area, and electrode polarization when the interelectrode distance is reduced.

We describe 1) the technique used to manufacture high spatial resolution electrode arrays for recording good quality extracellular potentials over limited epicardial areas, 2) in vitro tests of electrode arrays by recording the potential distribution in response to a current injection in saline solution, and 3) the epicardial potential distribution during unipolar current injection and ventricular activity in the rat heart in vivo.

The results indicate that high-resolution mapping arrays can be successfully used on the hearts of small animals and that the features observed in maps during...
current injection and ventricular activity are similar to those observed in larger hearts. Thus the technique may be exploited to estimate the passive and active electric properties of the myocardium in normal hearts and in the presence of myocardial structure altered as a consequence of various heart conditions such as hypertrophy, ischemia, and infarction.

METHODS

Construction of Electrode Array

In a first attempt, electrode arrays for recording epicardial potentials in rat hearts were fabricated by fastening the uninsulated ends of 60-µm-diameter silver wires to a patch of nylon sock at interelectrode distances of 500 µm as previously described for lower-resolution arrays in dog hearts (1). Silver wire was produced by Metalli Preziosi (Paderno Dugnano, Italy), and an insulating layer of acetalic polyvinyl was applied by INVEX (Quattordio, Italy). These high-density arrays exhibited two defects: irregular geometry and large electrode contact surface area. Irregular array geometry, as evidenced by uneven interelectrode distance, resulted from nonuniform tension applied to elastic filaments of the sock during manual fastening of electrodes. On the other hand, a large electrode contact surface area affected the distribution of current injected at the epicardium, which flowed preferentially through the low-resistance epicardial liquid layer, and only a small fraction of the current passed through the myocytes.

To overcome these difficulties, the previously used technique was modified by adopting tulle as the stiff substrate for electrodes in place of the nylon sock. Tulle is a sheer machine-made net with hexagonal, rectangular, or rhombic mesh. It is made of cotton or nylon and is used chiefly for veils and evening dresses and also for wrapping nougats. The type of tulle we used has 20-denier nylon filaments corresponding to 50-µm-diameter, 540 x 360-µm rectangular mesh openings, with single filaments along one direction, and pairs of closely apposed filaments in the orthogonal direction (Manifattura Beccalli, Bosisio Parini, Italy) (Fig. 1A). The relative stiffness of tulle net allows the construction of a uniform electrode array with sufficient flexibility to conform to the rounded epicardial surface. Uniform size knots with a loop diameter of 250 µm (Fig. 1B) were obtained at regular positions on the mesh (Fig. 1, A and C) according to the following procedure. The end of the insulated silver wire was lightly lubricated with petrolatum and fastened to the inelastic nylon substrate under a dissecting microscope by pulling both ends of the knot with moderate tension. The short end of the knot was subsequently twisted around the long end and cut close to the loop. Thus the short end of the knot was constrained away from the array to prevent damage of the myocardial cells during epicardial recording. After all the knots were fastened, the electrode array was inverted, and the insulation was removed from a limited area of each loop by means of a stripping paste (dichloromethane and methanol; Baldini Vernici, Porcari, Italy). Thus each electrode consisted of a double buffering technique, and data were stored sequentially.

Electrode Chloriding

Silver electrodes were chlorided to obtain uniform impedance values for all array electrodes. The small surface area of the electrodes makes empirical methods of chloriding difficult to use. Optimal chloriding current density and time were determined by measuring electrode impedance-frequency characteristics for different amounts of chloriding currents expressed in milliamperes times seconds per square centimeter (10, 20). Thus electrode chloriding consisted of the following steps. First, each electrode was briefly chlorided, and its resistance was monitored on the oscilloscope to verify that its value was in the expected range. Subsequently, all electrodes were simultaneously chlorided with optimal current density and time, and electrode impedance was reduced and made stable in the 30-kΩ range (for details see APPENDIX A). After preparing >500 electrodes, as described in Construction of Electrode Array, we were able to obtain electrodes of reproducible size, shape, and impedance.

Mapping System

Electrodes were connected to AC-coupled, variable-gain differential amplifiers of a 256-channel mapping system (8). The reference electrode was positioned at the insulating boundary of a cylindrical saline bath for in-vitro measurements or on the root of the aorta in animal experiments. Data were recorded at a bandwidth of 0.03–500 Hz, input impedance of 10^12 Ω, and sampling rate of 1 kHz/channel. No additional filtering was used to minimize waveform distortion. The continuous flow of data from the experiment, at the overall rate of 256 kHz with 12-bit resolution, was handled by a double buffering technique, and data were stored sequen-
tially on a Quadra 800 Macintosh hard disk in real time. Recording length could be selected between 1 s and the time interval corresponding to maximum available contiguous disk space. The entire set of waveforms was continuously monitored on the oscilloscope screen during recording sessions. Calibration factors were computed by feeding a reference triangular signal to all amplifier inputs and correcting for equal mean square value on all output signals. The triangular signal had a peak value spanning the amplifier output range for the selected gain, 70-s period, and 10-s duration to obtain significant values of the calibration factors.

Data Analysis

Data were displayed off-line as waveforms and equipotential contour lines on the graphic terminal and on a laser printer for quality control. Contour lines were computed with a custom-written program. The electrode array was triangulated by dividing the rectangular mesh with lower left-upper right diagonals. Contour lines were drawn in each triangle with linear interpolation. The choice of the diagonal may affect the results of the interpolation locally in the presence of steep spatial potential gradients. However, this simple representation allows rapid detection of electrodes having poor contact. Contour lines were not plotted in the six triangles having the current injection electrode as the vertex, which was disconnected from the corresponding amplifier during stimulation.

In Vitro Tests

The electrode array was immersed in a 0.9% NaCl solution. Unipolar biphasic current pulses, 5-ms duration and 50- to 100-µA intensity, were injected through one electrode, and potentials were recorded from all other electrodes. Current injection and potential recording were sequentially repeated for each array electrode. Data recorded in the saline solution were displayed as waveforms and potential distributions to evaluate electrode array performance. Waveform distortion on all electrodes revealed polarization of the current injection electrode, whereas waveform distortion at a single electrode indicated a high impedance value of that electrode. On the other hand, potential distributions revealed geometry irregularities within the array as deviations from circular equipotential lines, with the center at the current injection electrode. The finite volume conductor and position of the common return current electrode affected the shape of circular concentric equipotential lines.

In Vivo Recordings

Studies were done on eight healthy 1-yr-old rats of either sex weighing 300–400 g, anesthetized intraperitoneally with 5 µg/kg body weight of fentanyl citrate and 250 µg/kg body weight of droperidol (Leptofen, Farmitalia-Carlo Erba, Milan, Italy). Additional amounts of anesthetic were administered during the experiment as needed. Under artificial respiration (rodent ventilator 7025, Ugo Basile, Comerio, Italy), the heart was exposed through a longitudinal sternotomy. Body temperature was maintained constant at 37°C with infrared lamp radiation. The sternum was covered with a plastic sheet to maintain the heart in a moist and constant-temperature environment. All procedures performed on the animals conformed to the guiding principles of the Veterinary Animal Care and Use Committee of the University of Parma (Parma, Italy). In each experiment, unipolar epicardial electrograms were recorded with sock or tulle electrode arrays containing 64 or 121 electrodes. A 10-mm silver spiral electrode was sutured to the aortic root as a reference for all unipolar recordings. The arrays were usually positioned on the anterior surface of the heart and were kept moist by periodic addition of small amounts of warm saline (37°C). Unipolar stimuli were delivered between a single electrode on the array and a common return current electrode, a heavily chlorided silver spiral, inserted into the chest wall. Stimulus duration was 5 ms during subthreshold current injection and 1 ms or less during pacing, with the stimulus strength just above threshold. During the subthreshold current injection, one of the unipolar electrograms was used to trigger a stimulator, with a programmable delay of output current pulse of variable phase, duration (100 µs to 10 ms) and intensity (10 µA to 10 mA) to be delivered just after the end of the T wave (4-channel biomedical stimulator model 425 and biphasic stimulator model 220, Crescent Electronics, Sandy, UT). To ensure stable positioning of the recording array and to minimize pressure over the epicardial surface, the wire bundles were suspended by means of a horizontal rod that reduced mechanical stress over the epicardium. At the end of the experiment, the location of the array was marked by inserting pins into the tissue at each corner of the array.

Morphological Study

After epicardial potential recording with the tulle electrode array was completed, in five rats, the hearts were directly fixed in 10% buffered Formalin for histological examination of the explored epicardial region, and fiber direction was determined. In the remaining three rats, in addition to fiber direction, the thickness of the pericardial layer was measured. To this end, the abdomen was opened, the abdominal aorta was cannulated with a PE-200 catheter filled with phosphate buffer (0.2 M, pH 7.4) and heparin (100 U/I ml), and the coronary vasculature was perfused for 10 min with phosphate buffer and fixed with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde for an additional 10 min. Small fragments of the epicardial surface of the left and right ventricular myocardium close to the area covered by the electrode array were postfixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in araldite. Thin sections were cut and stained with uranyl acetate and lead citrate, and pictures were taken at ×3,500 in a Zeiss 9M electron microscope and printed at a final calibrated magnification of ×10,000. Measurements of the visceral pericardal thickness were obtained from eight pictures of each heart at 10-µm regular intervals.

RESULTS

In Vitro Tests

To simulate the in vivo conditions, the tulle electrode array was positioned over the free surface of a fine circular sponge immersed in saline solution. The lower side of the array was in contact with the saline solution, and all electrodes exhibited uniform contact area. Biphasic unipolar current pulses through one electrode generated good-quality potential waveforms (Fig. 2A) at all other electrodes. Potential distributions (Fig. 2B) generated by current injections at selected electrodes at the center of the 8 × 8 array demonstrated that the tulle electrode array has uniform geometry. Equipotential lines were circular, with the center at the current injection electrode, and exhibited only minor irregularities. Specifically, the potential distributions displayed symmetry about the midlines and main diagonals of the 4 × 4 map array (Fig. 2B). Moreover, the distribu-
tions from the 16 maps were similar when spatially aligned to the stimulus site. Potential distribution due to a current injection through the center electrode of the higher resolution 11 × 11 tulle array is displayed in Fig. 3A. The slightly defective symmetry of the circular equipotential lines recorded in vitro by the different electrode arrays was due to the influence of the saline-bounded volume conductor and finite distance of the common return current electrode from the electrode array.

**In Vivo Recordings**

A total of eight rats were used in this study. Four rats were used to test and develop the sock electrode array, which proved unsatisfactory, as explained in METHODS. These results were discarded. The other four rats were used to test the tulle array. Two rats enabled us to demonstrate the suitability of the 8 × 8 tulle electrode array both in vitro and in vivo. Similarly, two rats were

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**Fig. 3.** In vitro (A) and in vivo (B) potential distributions recorded with higher-resolution 11 × 11 tulle electrode array during anodal current injection through center electrode (electrode 61; ⋄). Positive potential values at array sites. Equipotentials were not drawn within the 6 triangles having current injection electrode as vertex. In each map, nos. at bottom (left to right), absolute potential maximum, increment between positive equipotentials, and absolute potential minimum (all in µV). Reference electrogram refers to electrode 60, just above stimulated point. Vertical bar, time instant during anodal pulse.

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**Fig. 2.** A: potential waveforms recorded in response to biphasic unipolar current injection in 0.9% NaCl solution by 8 × 8 tulle electrode array with 1.08-mm interelectrode distance, covering 0.53-cm² surface area. Top waveform, 1-s recording from 1 of the array electrodes during current injection through electrode 29; rectangle, 45-ms time interval for display of the array waveforms below. Positive and negative current pulses had 5-ms duration, 100-mA intensity, and 20-Hz frequency. Missing waveform at electrode 29 identifies injection electrode. Highest recorded potential value was 13.7 mV. B: potential distributions recorded in the same solution as in A during positive phase (potential waveform at bottom) of unipolar current injection through a series of 16 electrodes in central region of 8 × 8 tulle array. Potential distribution due to current injection through electrode 29 (3rd row, 2nd column) refers to waveforms in A. In each map, spacing between equipotentials is 1.6 mV, value of highest equipotential is 6.4 mV, and potential value at current injection point is missing. Bottom right square below maps shows 8 electrodes surrounding current injection electrode (●) and oblique triangulation of rectangular mesh. Equipotentials were not drawn within the 6 triangles having current injection electrode as a vertex.
used to test the $11 \times 11$ tulle electrode array. This number of experiments is sufficient to demonstrate the feasibility of the method but is too small to justify a statistical analysis.

As soon as the tulle electrode array was positioned on the anterior ventricular epicardium (Fig. 4A), unipolar electrograms exhibited injury potentials (ST-T elevation; Fig. 4B), with a pattern similar to monophasic action potentials. After a few minutes, ST-T elevation diminished (Fig. 4C) to give rise to stable, good quality epicardial electrograms. The ST-T elevation was most likely due to injury caused by pressure exerted by the electrode array on the delicate and thin visceral pericardium. In the rat heart, the visceral pericardium is composed of thin layers of collagen and a mesothelial layer (Fig. 5). These layers may contain vessels, nerves, and lymphatics. In our animals, the thickness measured from the pericardial myocytes to the epicardial surface varied from 1.0 to 10.0 µm, with an average value $3.5 \pm 1.2$ µm.

Epicardial current injection. Potential distributions recorded over the anterior ventricular surface in response to unipolar anodal current injections with the $8 \times 8$ (Fig. 6) and $11 \times 11$ (Fig. 3B) electrode arrays displayed elliptic equipotential lines, with the center at the stimulation point and the common major axis parallel to fiber direction at the injection site (19, 21). In vivo maps in Fig. 6 correspond to in vitro maps in Fig. 2B because of current injection through the same 16 electrodes in the central part of the $8 \times 8$ array. Anisotropic epicardial potential distributions were obtained even by injecting low-density currents.

Sinus rhythm. Epicardial potentials recorded during sinus rhythm (as well as during ectopic activity; see Ectopic activity) demonstrated the ability of high-density electrode arrays to capture significant features of the epicardial activation observed in hearts of larger dimensions. The general time course and spatial distribution of electrical events on the epicardium for the different activations were similar in all experiments, although the details of the activation pattern varied. Sinus rhythm electrograms in Fig. 4, sinus rhythm isopotential maps in Fig. 7, and ectopic beat isopotential maps in Fig. 8 were recorded by the $11 \times 11$ electrode array over the anterior ventricular surface of the same rat.

The potential distributions of the sinus rhythm activation sequence of normal epicardial tissue are illustrated in Fig. 7. Potential distributions were entirely positive during the early stages of activation on the surface explored as displayed in Fig. 7A (4 ms after QRS onset). At 5 ms (Fig. 7B), one or more potential depressions appeared in the lower portion of the right ventricle. The potential values in these depressions reached $-10$ mV at 6 ms (Fig. 7C), and the region of densely packed equipotential lines was considered to be the electrical manifestation of an underlying activation wave front moving toward the left ventricle and basal region of the right ventricle. At the same time, one or more potential depressions appeared in the ventral aspect of the right ventricle (Fig. 7C, arrows). These events are the expression of activation wave fronts emerging at two sites of the right ventricular surface (breakthrough points). Meanwhile, another activation wave front moved from the free wall of the left ventricle toward the right ventricle (Fig. 7, D and E, top right...
corners). The activation of the ventral aspect of the right ventricular surface was completed through merging, over the interventricular septum, of two wave fronts, one coming from the right ventricle and the other coming from the left ventricle (Fig. 7F). The maximum potential jump of epicardial potentials across the wave front during sinus rhythm activation was 25 mV (Fig. 7C). The sequence of events described was stable for several hours.

Ectopic activity. Ectopic beats were elicited by delivering unipolar cathodal pulses at various sites of the anterior ventricular surface. Pacing rate was slightly higher than sinus rhythm, current density was just above threshold, and pulse duration was 1 ms to avoid overlapping of the stimulus and early activation potentials. Maps A–H in Fig. 8 were recorded during paced activation from the center of the electrode array. Two milliseconds after pacing (Fig. 8A), potential patterns appeared, with negative potentials surrounding the pacing site and two positive maxima located on opposite sides of the central region. A straight line joining the two potential maxima was parallel to the fiber direction near the stimulated point as verified by histological examination. Equipotential lines in the negative region were elongated, with the major axis perpendicular to fiber direction near the pacing site at this early stage of propagation, and the ratio between the absolute values of the potential minimum and maximum was 1.5. Open circles in Fig. 8A identify a subset of electrodes of the 11 × 11 array with a 2-mm interelectrode distance sampling the same area explored by the high-resolution array. A lower-resolution electrode array fails to record clear-cut potential patterns during the early stages of activation after epicardial pacing as in dog hearts where well-defined potential patterns appeared only at 5–8 ms after the stimulus when the ratio between the potential minimum and maximum was ~6 (33). During the subsequent 4 ms (Fig. 8, B–E), the eccentricity of early negative equipotentials gradually shifted in a direction parallel to the fibers. Eight milliseconds after pacing (Fig. 8F), the negative equipotentials became clearly elliptical, with the major axis parallel to the fiber direction and the ratio between the potential minimum and maximum increased to ~6. The subsequent pattern of ectopic activation (Fig. 8, G and H) was one of expanding negative potential ellipses, with the major axis approximately parallel to the fiber orientation. At this time, the two positive potential regions that initially appeared on opposite sides of the central negative region underwent changes, pointing to a progressive expansion and rotation in a counterclockwise (CCW) direction (Fig. 8, G and H), whereas the two maxima maintained their initial position, moving along a straight line. At 12 ms after pacing, the lower positive region moved completely outside the array boundary (Fig. 8H), followed 6 ms later by the upper positive region (data not shown). The expansion and rotation of the positive potential regions after epicardial pacing are in agreement with previous findings in dog hearts (33).

Fig. 6. Epicardial potential distributions generated by anodal current injections through the same 16 electrodes of 8 × 8 tulle array positioned over anterior ventricular surface as in Fig. 2B. Spacing between equipotentials in all maps is 700 µV, and value of highest equipotential is 4.9 mV. Positive pulse in electrogram at bottom refers to anodal current injection of 5-ms duration and 100-µA intensity delivered during sinus rhythm just after end of T wave. Major axis of elliptical equipotential lines identifies average fiber direction in region surrounding injection point. Bottom right square below maps is defined in Fig. 2B.
Mathematical Modeling

To attempt interpreting epicardial potential patterns 2 ms after pacing (Fig. 8A), we computed the potentials generated by a linear quadrupole (Fig. 9), represented by two opposite dipoles separated by a small distance \(d\) (18), immersed in an infinite homogeneous anisotropic monodomain (see Appendix B). The linear quadrupole is assumed to be an equivalent generator of the activation wave front a few milliseconds after pacing. The quadrupolar potential distribution in Fig. 9A was generated by two collinear, opposing dipoles separated by 1 mm on a uniform grid, with points spaced at a distance between grid points \(d\) of 0.5 mm at a distance of 0.125 mm from the plane of the quadrupole. Simulated potentials were displayed in a plane at a short distance from the sources because the potential distribution in the source plane is characterized by the presence of equipotential lines that cluster all around the sources due to the steep potential gradient surrounding these points. On the contrary, in a plane at a short distance from the sources, the potential gradient decreases and is similar to the gradient of measured potentials. Another reason for displaying potentials at a finite distance from the sources is that the linear quadrupole is an equivalent source that reconstructs the potential distribution at a distance from the wave front. Potential distributions were also computed at the same short distance from the quadrupole when \(d\) was decreased by a factor of two \((d = 0.25 \text{ mm}; \text{Fig. 9B})\) and four \((d = 0.125 \text{ mm}; \text{Fig. 9C})\), and the explored area was reduced to the inner squares B and C, respectively, in Fig. 9A. Simulation results indicate that equipotential lines in the interdipolar region are always elliptical, with the major axis parallel to the quadrupole axis (Fig. 9, B and C) and that only inadequate spatial sampling (Fig. 9A) fails to reveal this pattern. Lower-value negative equipotential lines outside the interdipolar region were always elongated, with the major axis perpendicular to the dipole axis (Fig. 9, A–C). Potential patterns similar to the ones displayed in Fig. 9C were generated by two opposing dipoles oriented along a diameter and evenly spaced from the center of a circular conducting medium (7).

The similarity between recorded (Fig. 8A) and simulated (Fig. 9A) potential patterns indicates that 0.5-mm spatial sampling fails to explore, with fine details, the area surrounding the stimulated epicardial point at an early stage of activation and only shows negative elliptic equipotentials, with the major axis perpendicular to local fiber direction. Because simulated potentials were computed in a plane at a short distance from the plane of the quadrupolar source, it follows from the similarity between recorded and computed potentials that the epicardial array of electrodes is also at a short distance from myocardial cells. We cannot quantify how much the visceral pericardium and the epicardial liquid layer separately affect extracellular potential, which is a fraction of transmembrane potential as a function of extracellular and intracellular resistance. Because the electrodes are separated by only a few micrometers...
from the myocytes through the visceral pericardium of rat heart, it is most likely that the smoothing action of the epicardial liquid layer plays a significant role in decreasing the amplitude of the extracellular potentials and spatial potential gradients.

DISCUSSION

The results of the present investigation demonstrate for the first time that, with a tulle net of 0.39 cm² with 121 electrodes, the potential field created by current injection and propagating wave fronts can be measured on an area covering ~20,000 epicardial myocytes, the average dimensions of which in the adult rat heart are 120 µm in length and 16 µm in diameter (17). Thus the average number of epicardial myocytes, depending on fiber orientation and neglecting the interstitial space, is 10 beneath the electrode contact area, approximately equal to 20,000 µm² (see APPENDIX A), and is 200 in each 540 × 720-µm quadrant of the array. Such a high resolution makes it possible to describe the effects of myocardial structure on cardiac electrical events close to the microscopic scale in normal or pathological conditions. Recorded epicardial potential patterns in rat hearts were similar to those obtained in dog hearts during spontaneous and paced ventricular activation (1, 33). As previously found in dogs (18, 33), potential patterns observed early in activation after paced beat can be interpreted in terms of an equivalent quadru-pole model.

Construction of the High Spatial Resolution Electrode Array

The high-density electrode array made from a patch of nylon sock that we used in preliminary experiments was unsatisfactory because of irregular geometry arising from overcrowding of the silver wire knots in a small area and an excessive electrode contact surface area. The tulle electrode array, although less elastic, was sufficiently flexible to follow the curvature of the epicardial surface of the heart and established close contact with the epicardium without constraining the ventricular mechanics. In addition, the stiffness of the tulle allowed for the construction of electrodes with a limited surface area. Although stripping only a limited surface area of the insulated silver wire required the visual inspection of each electrode under the stereom-
of conduction, however, requires higher-resolution recording. Spach and colleagues (26, 27) have suggested that a complete understanding of microscopic propagation requires mapping at a resolution approaching the dimension of the individual myocytes. Electrode arrays used in dog hearts usually sample epicardial potentials with a 2-mm or larger interelectrode distance. One previous study (6) described the activation sequence over dog epicardium with a high-density electrode array, with the electrodes evenly spaced 350 µm on a rigid mesh. High-resolution mapping of dog epicardium was also reported for the interpretation of activation times (22) and for in vivo estimation of cardiac transmembrane current (36). The array used in our study has the highest reported spatial resolution for a flexible epicardial electrode array. The high level of resolution enabled us to display early activation patterns 2 ms after the onset of the epicardial pacing stimulus (Fig. 8A). Despite this level of resolution, the early potential distributions failed to detect the central equipotential lines that are elongated along the fiber direction as revealed by the theoretical model that simulates an equivalent potential distribution generated by a linear quadrupole (Fig. 9).

The use of a miniature flexible electrode system for epicardial mapping may be extended to mouse hearts, in consideration of the growing interest in genetically modified murine models (14). The anterior aspect of a mouse heart measures ~3.5 × 4 mm. The shortest interelectrode distance that can be obtained with the tulle used is 360 µm, and a 7 × 10 electrode array with a 540 × 360-µm interelectrode distance can cover an epicardial surface area of 3.24 × 3.24 mm². However, the flexibility of such an array has to be tested in new experiments.

Epicardial Potential Response to Current Injection

Current injection has been used to assess passive electrical properties of the myocardium such as interstitial and “gross tissue” anisotropic resistivity (13, 23). Early work by Woodbury (38) demonstrated anisotropic influence of myocytes on passive current flow from one injection electrode. Clerc (5) measured longitudinal and transverse intracellular and interstitial resistivities of an in vitro calf trabecula preparation. Roberts and colleagues (23, 24) obtained values for the tissue resistivities in vivo with a method similar to the “four-electrode technique” (29). However, resistivity values measured by various investigators are inconsistent (25). Kleber and Riegger (15), who measured the electrical properties of arterially perfused rabbit papillary muscle, suggested that the relatively large differences in measured parameters may reflect the shunting effect of the current through the thin superficial liquid layer. Our results in rat hearts in vivo confirmed that the surface of the current injection electrode in contact with the epicardial liquid layer greatly affects measured potential distributions in response to current injection, and the shunting effect of the fluid is minimized by reducing the thickness of the liquid layer and the electrode area in contact with the underlying

Array Dimensions

Many groups (e.g., Refs. 6, 33) have studied epicardial potentials with arrays characterized by different interelectrode distances and number of electrodes. These techniques have been useful in understanding large-scale spatial distribution of wave fronts and potentials as a function of time. The description of the finer details

Fig. 9. A: potential distribution generated by a linear current quadrupole, superposition of 2 dipoles, separated by 1 mm, in an infinite homogeneous anisotropic monodomain. Each dipole is obtained as superposition of a point source-sink pair separated by 0.125 mm and centered at a grid point. Quadrupole is parallel to fibers that are oriented horizontally (x-axis), with axial symmetry (τ_y = τ_z) and anisotropy ratio (τ_x/τ_y = τ_x/τ_z = 2). Dotted inner squares B and C represent areas displayed in B and C, respectively, with higher resolution. Potentials were computed on a plane at a distance (d) = 0.125 mm from plane of quadrupole. B and C: potential distributions generated by the same linear quadrupole in A in a plane at d = 0.125 mm from quadrupole when distance between grid points was reduced 2 times in B (d = 0.25 mm) and 4 times in C (d = 0.125 mm). D: 21 × 21 grid points uniformly spaced (d mm), where potentials were computed. 0, □, and △ pairs, dipole positions separated by 1 mm when grid points are spaced at d = 0.5, 0.25, and 0.125 mm, respectively (A–C, respectively). In each map, nos. at bottom (left to right), potential maximum, step between positive equipotentials, potential minimum, and step between negative equipotentials (all in arbitrary units). Note that ratio between absolute values of potential minimum and maximum decreases to 1 by increasing grid resolution due to prevailing influence of single dipole potentials.
myocytes. On the other hand, by recording epicardial potential distributions in response to a current injection over small epicardial areas, the passive electrical properties of cardiac muscle may be estimated with greater detail. Specifically, the eccentricity of elliptical equipotential lines obtained by high-density epicardial mapping may provide information regarding the anisotropic electrical properties of the tissue on the basis of the bidomain model (13, 21). In addition to the measurements of tissue resistivity, the technique identifies myofiber orientation at points of unipolar (21) or bipolar (19) current injection.

High-density epicardial mapping may also provide useful information in pathological conditions. In a previous study (34), myocardial resistivity was shown to change dramatically when local ischemia is induced. Using a method based on the four-electrode technique, Steendijk et al. (30) demonstrated that, within 2 min of coronary occlusion, myocardial anisotropic electrical resistivity increased and returned to the control value after reperfusion. Fallert et al. (9) showed, with the same method, that impedance mapping revealed significantly different values for normal, ischemic, and infarcted tissues. Thus it is tempting to anticipate that high-quality, high-resolution epicardial potential recordings will make it possible to define the passive electrical properties of cardiac muscle in normal and pathological conditions. Epicardial potential distributions, which we have shown to be measurable with our electrode array, are known to be altered in a number of cardiac diseases, such as myocardial infarction, ischemia, and conduction disturbances. In addition, the distribution of injected currents and related potentials, which we have shown to be measurable with our array, is known to be altered in hearts with myocardial ischemia and infarctions (9, 30, 34). Moreover, disparity of repolarization, an arrhythmogenic condition, can be inferred from the distribution of the QRST area on the epicardium. This variable, too, can be measured with our electrode array. For instance, the recently described relationship between beat-to-beat variability of ventricular repolarization and the amount of ventricular fibrosis in rat hearts (31) may be examined more closely by means of high-resolution epicardial mapping. Finally, very little is known about the changes in myocyte volume and shape after different loads are imposed on the myocardium and the potential distributions are recorded on the epicardial surface. Similarly, variations in the amount and composition of the interstitium may greatly affect the distribution of electrotonic currents during action potential propagation along different directions, altering excitation potential patterns measured at the epicardium. In particular, during ventricular reentrant tachycardia, high-resolution epicardial maps may help in studying, with more details, the configuration of reentry pathways as recently revealed in canine hearts (37).

**Epicardial Ventricular Activation**

Epicardial potentials recorded during sinus rhythm and stimulated activity in the rat heart were consistent with the electrical activity measured in the dog heart under similar conditions. Particularly, during sinus rhythm, multiple breakthrough points were present on the anterior ventricular surface and initiated wave fronts, which moved along preferential directions probably related to the myocardial fiber direction (1). These wave fronts collided, thus terminating the activation, in the basal region of the ventricles. Early after epicardial pacing, the position of the epicardial minimum and two maxima revealed the orientation of myocardial fibers near the pacing site, whereas at later stages of activation, the CCW rotation and expansion of the positive areas correlated with the helical spread of excitation through CCW-rotating intramural fibers as previously demonstrated in dog hearts (33, 35).

In summary, the major advantage of high-density epicardial mapping with tulle electrode arrays is that stable recordings can be obtained for several hours from the same epicardial area, and the electrode array can be easily made and is durable. Moreover, because the epicardial potential estimate reflects the spatial average over an electrode area of $\sim 200 \times 10^{-6}$ cm$^2$, our technique is particularly useful to also explore small areas of myocardium in the hearts of larger animals. The high sensitivity of this methodology should provide information on the electrical correlates of the anatomic changes occurring in several heart conditions such as hypertrophy, myocardial ischemia, and infarction.

**APPENDIX A**

**Optimal Electrode Chloriding**

Geddes et al. (10) have studied the properties of silver-silver chloride electrodes as a function of the thickness of the chloride that was plated on the silver. For a piece of silver metal with a given surface, thin layers of chloride reduced the
impedance of the electrode. When the layer of chloride was too great, the electrode impedance started to increase again. The lowest electrode impedance was produced when the plating current density was limited to \(-5 \text{ mA/cm}^2\) and the thickness of chloride layer was limited in the range of 500–2,000 (\text{mA} \cdot \text{s})/\text{cm}^2.\) Contact area of our array electrodes was approximately equal to one-half of the lateral surface area of one-fourth of the loop of the knot (Fig. 1B). Thus the electrode contact area was estimated \(\frac{1}{2} \pi D_w \cdot \frac{1}{2} \pi D_t \approx 20,000 \text{ \mu m}^2 = 200 \times 10^{-6} \text{ cm}^2,\) where \(D_w\) is the 60-\mu m silver wire diameter and \(D_t\) is the 250-\mu m loop diameter, so that the 200-nA current intensity through the electrode surface area corresponds to 1 \text{ mA/cm}^2\) chloriding current density. Optimal chloriding current density and time for tulle array electrodes was estimated by evaluating electrode impedance in the 10-Hz to 1-kHz range for different chloriding current densities and time intervals. It was found that a 100-nA current intensity decreased electrode impedance to an average minimum value of 30 \text{k} \Omega in the 10-Hz to 1-kHz range for a chloriding time interval of \(>1,200\) s (Fig. 10). However, a current intensity of 200 nA or greater attained slightly lower impedance values that started to increase after 600 s. Thus a current intensity of 200 nA or greater attained slightly lower 

If we introduce new variables \(x = x' \sqrt{|\sigma^{(k)}|}, y = y' \sqrt{|\sigma^{(p)}|},\) and \(z = z' \sqrt{|\sigma^{(l)}|},\) the equation changes into the following form

\[
\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} + \frac{\partial^2 \phi}{\partial z^2} = -\frac{4\pi l}{\sqrt{|\sigma^{(k)}| |\sigma^{(p)}| |\sigma^{(l)}|}} \delta(x')\delta(y')\delta(z')
\]  

(2B)

which is similar to Poisson’s equation in an infinite homogeneous isotropic medium if we substitute \(l' = l/\sqrt{|\sigma^{(k)}| |\sigma^{(p)}| |\sigma^{(l)}|}.\)

Thus its solution is

\[
\varphi = \frac{l'}{r} = \frac{1}{r} \frac{1}{\sqrt{|\sigma^{(k)}| |\sigma^{(p)}| |\sigma^{(l)}|}} \left( \frac{x^2}{\sigma^{(k)}} + \frac{y^2}{\sigma^{(p)}} + \frac{z^2}{\sigma^{(l)}} \right)^{-1/2}
\]

(B3)

The infinite homogeneous anisotropic volume conductor is characterized by fibers parallel to the \(x\)-axis, axial symmetry about the \(y\)-axis, and the anisotropic conductivity ratio \(|\sigma^{(k)}|/|\sigma^{(p)}| = |\sigma^{(p)}|/|\sigma^{(l)}| \gg 1.\)

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REFERENCES


