Ventricular Action Potentials, Ventricular Extracellular Potentials, and the ECG of Guinea Pig

Toshifumi Watanabe, Pentti M. Rautaharju, and Terence F. McDonald

From the Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada

SUMMARY. Action potentials were recorded from different regions of the guinea pig ventricle to characterize regional differences in waveform configuration, and to acquire insight into the generation of the T-wave of the electrocardiogram. Isolated tissue preparations were driven at 1 Hz, and microelectrodes were used to map accessible surface regions of the epicardium, endocardium, and septum. There were minimal differences in regional resting potentials (mean —87 mV) and amplitudes (mean 122 mV), but $V_{\text{mam}}$ in the epicardium (mean 110 V/sec) was much smaller than elsewhere (mean 247 V/sec). The action potential duration at the —80 mV repolarization level was longest in the papillary muscles (mean 154 msec), shortest in the septum (mean 126 msec), and generally 10–15 msec longer at the base than at the apex. The characteristics of intramural action potentials were inferred from measurements on enzymatically isolated myocytes, the rationale being that most dissociated myocytes originated from intramural cell layers. The action potentials in about 40% of the myocytes had durations similar to those recorded from the tissue surface (110–170 msec), and the remainder ranged from 170–290 msec long. The existence of longer-than-surface action potentials in the ventricle was also inferred from the body surface electrocardiogram and from bipolar electrograms of isolated left ventricles. In both cases, the Q-T intervals could be accounted for only by action potentials longer than those recorded from the ventricular surface. (Circ Res 57: 362-373, 1985)

THE body surface electrocardiogram(s) (ECG) of many mammals, including human (e.g., Waller, 1887; Wilson et al., 1931), exhibit a concordance in the polarity of the QRS complex and the T-wave. The explanation for the presence of a concordant T-wave is that some regions with longer action potentials must be excited earlier and repolarize later than regions with shorter action potentials (e.g., Wilson et al., 1934; Burger, 1957; Harumi et al., 1966; Toyama et al., 1967).

This theoretical consideration spurred the search for ventricular regions with appropriate long and short action potential durations. Even so, the sum total of reliable information on the nature of this heterogeneity is not large. A major reason is that it is difficult to map the ventricular action potential configuration with intracellular microelectrodes in the in vivo setting. The solutions have been to estimate regional differences in waveform configuration, and to acquire insight into the generation of the T-wave of the electrocardiogram. Isolated tissue preparations were driven at 1 Hz, and microelectrodes were used to map accessible surface regions of the epicardium, endocardium, and septum. There were minimal differences in regional resting potentials (mean —87 mV) and amplitudes (mean 122 mV), but $V_{\text{mam}}$ in the epicardium (mean 110 V/sec) was much smaller than elsewhere (mean 247 V/sec). The action potential duration at the —80 mV repolarization level was longest in the papillary muscles (mean 154 msec), shortest in the septum (mean 126 msec), and generally 10–15 msec longer at the base than at the apex. The characteristics of intramural action potentials were inferred from measurements on enzymatically isolated myocytes, the rationale being that most dissociated myocytes originated from intramural cell layers. The action potentials in about 40% of the myocytes had durations similar to those recorded from the tissue surface (110–170 msec), and the remainder ranged from 170–290 msec long. The existence of longer-than-surface action potentials in the ventricle was also inferred from the body surface electrocardiogram and from bipolar electrograms of isolated left ventricles. In both cases, the Q-T intervals could be accounted for only by action potentials longer than those recorded from the ventricular surface. (Circ Res 57: 362-373, 1985)

Methods

Ventricular Tissue Preparations

Hearts were taken from male guinea pigs (250–300 g), and placed in oxygenated (95% O2-5% CO2) normal Krebs' solution (mm): NaCl, 113.1; KCl, 4.6; CaCl2, 2.45; MgCl2, 1.2; NaH2PO4, 3.5; NaHCO3, 21.9; and glucose, 50. After the atria were removed, incisions were made from base to apex along the septum to obtain a left or right ventricular wall preparation, or septum. The preparation was pinned to the waxed floor of a Perspex bath (5 ml volume) perfused with normal Krebs' solution (10 ml/min). The solutions were maintained at 36 ± 0.5°C, pH 7.4. Stimulation with 50% suprathreshold pulses, 1 msec long, was applied to the basal part of the preparation through a bipolar Ag-AgCl electrode. Stimulus frequency was 1 Hz.
during the initial equilibration period (10–15 minutes) and during all subsequent periods, unless otherwise specified.

Action potentials were recorded with 3 M KCl-filled microelectrodes (8–10 mf), which were connected to a high input impedance amplifier (M-750, WPI) via an Ag-AgCl pellet. The reference electrode was a 3 M KCl/Ag-AgCl unit which was connected to the reference terminal of the amplifier. The reference electrode was placed in a downstream position close to the tissue; its position had no detectable effect on action potential parameters. The first derivatives of the action potentials (dV/dt) were derived electronically to obtain maximum rates of rise (V_{max}). The action potentials and dV/dt were displayed on a storage oscilloscope (5113, Tektronix).

**Isolated Myocytes**

Ventricular myocytes were isolated from male guinea pigs (250–300 g) by a method similar to that described previously (Watanabe et al., 1983).

The hearts were excised and attached to the bottom of the Langendorff column (60-cm height). The solutions added to the Langendorff column were equilibrated with 95% O₂-5% CO₂ and were maintained at 36 ± 0.5°C, pH 7.4. Cell isolation procedures consisted of the following three stages of perfusion. The first perfusate was Ca-free Krebs’ solution (normal Krebs’ solution without CaCl₂) to wash out the blood remaining in the heart cavities and coronary arteries. After 3–4 minutes of the initial perfusion, 50 ml of calcium-free Krebs’ solution containing enzymes (300 U/ml collagenase type V, and 200 U/ml trypsin, type XII, Sigma) were added to the column, and recirculated for 5 minutes. Finally, calcium-free Krebs’ solution was introduced to wash out the residual enzyme solution in the heart. The ventricles were cut into small fragments with iris scissors, and myocytes were dispersed by gentle stirring in oxygenated calcium-free Krebs’ solution. The suspension then was filtered by gravity through a nylon gauze (250-μm mesh). Finally, the calcium concentration in the cell suspension was raised to 2.45 DIM by the addition of CaCl₂. Cell suspensions were stored in plastic vials (room temperature) preparatory to experimental use.

A Perspex bath (volume 1 ml) with a bottom formed by a microscope coverslip was attached to the stage of an inverted microscope (type D, Zeiss). A few drops of freshly isolated myocytes (within 1 hour of isolation) were placed in the bath, and the myocytes were allowed to settle to the bottom (about 5 minutes). The bath then was perfused with normal Krebs’ solution gassed with 95% O₂-5% CO₂ at a rate of 2 ml/min. The temperature and pH of solutions in the cell suspension were maintained at 36 ± 0.5°C and 7.4, respectively. A TV system (Sony) was employed for visual observation of the cells and their contractile activity.

Cells were impaled with 3 M KCl-filled microelectrodes (20–50 MΩ) connected to a high input impedance amplifier (M707, WPI) via an Ag-AgCl pellet. The reference electrode was a 3 M KCl/Ag-AgCl unit, and this was connected to the reference terminal of the amplifier. Action potentials were elicited by passing short current pulses (5–20 nA, 0.5 msec, 1 Hz) through the recording microelectrode. Action potentials and dV/dt were displayed and recorded in the same manner as described for the ventricular wall preparations.

**Electrocardiograms and Extracellular Potentials**

Standard bipolar ECG (leads I, II, and III) were recorded from the body surface of supine guinea pigs anesthetized by continuous application of ether (Baker Chemical Co.). Needle electrodes (Propper Co.) were placed on four limbs, and the ECG were recorded via an isolation amplifier (8811A, Hewlett-Packard) and a low-pass filter (0.05–1000 Hz). The signals were displayed on an oscilloscope (5113, Tektronix) as well as on a paper recorder (7702B, Hewlett Packard).

Extracellular potentials were recorded from excised left ventricles of guinea pigs. Two Ag-AgCl electrodes were placed in the bathing solution near the ventricles to form a lead I-like system. The extracellular potentials from the bipolar electrodes were recorded and displayed in the same manner as those from the body surface potentials.

**Data Analysis**

Action potentials from the tissue and myocytes were characterized in terms of the amplitude, resting potential, V_{max}, and durations at the +10- and -80-mV repolarization levels (APD_{10}, APD_{-80}). (Durations were also measured at the +20-, -20-, and -50-mV levels of repolarization, but these provided no useful additional information.) After impalement of the tissue or myocytes, the microelectrode was allowed to seal-in for 5–10 minutes to obtain a stable action potential configuration. Photographs (35-mm camera; Pentax, Asahi) were taken from tracings of the action potentials and dV/dt on the face of the oscilloscope. All measurements of the parameters were made from enlarged (15X) projections of 35-mm film (projector: 500 Reader Printer, 3M Co.). Values were expressed as

**FIGURE 1. Heterogeneity of the action potentials obtained from different regions on the surface of guinea pig ventricles. Representative action potentials recorded from the base of the left epicardium (upper left), left septum (upper right), and right endocardium (lower left) were superimposed on those recorded from the respective apical surfaces. The lower right panel shows a representative action potential from a right papillary muscle. Stimulation rate 1 Hz.**
mean ± SD, with the exception of those in Figures 2 and 3 (mean ± se for clarity of presentation). The parameters were compared statistically by Student's t-test.

**Results**

**Mapping of the Action Potentials on the Ventricular Tissue Surfaces**

Action potentials were recorded from the epicardial, endocardial, and septal surfaces of the right and left ventricles in 48 hearts. Each surface was visually divided into base, middle, and apex. With the addition of right and left papillary muscles, the total number of designated regions was 20. Action potential characteristics of each region were determined by analyzing records obtained from three separate sites within a given region in each of six hearts (i.e., \( n = 18 \) action potentials per region). Typical examples of regional action potentials are illustrated in Figure 1.

**Action Potential Amplitude, Resting Potential, and \( V_{\text{max}} \)**

The highest mean value of the action potential amplitude among the regions was 126 mV, and the lowest was 118 mV (Fig. 2). The highest values were recorded from the endocardium and the papillary muscles (average 125 mV); the lowest values were recorded from the epicardium and septum (average 120 mV). In general, there were no significant differences \((P > 0.05)\) within the endocardial, epicardial, or septal surfaces, but the amplitudes recorded from the papillary muscles and the endocardial regions were significantly greater \((P < 0.01-0.001)\) than those recorded from the epicardial or the septal regions. The mean resting potentials in the regions were between −85 and −90 mV (Fig. 2). The highest values (mean ± sd) were recorded in the papillary muscles (−90 ± 4 mV) and the lowest in the apical regions of the septum and the basal region of left epicardium (−85 ± 4 mV).

There was no significant difference \((P > 0.05)\) in \( V_{\text{max}} \) within the major surfaces, between corresponding surfaces of the right and left ventricles, or between the endocardium (average 248 V/sec), the papillary muscle (average 256 V/sec), and the septum (average 243 V/sec). However, there was a marked difference \((P < 0.001)\) between the latter surfaces (endocardium, septum, and papillary muscles) and the epicardium (average 110 V/sec). A similar difference in \( V_{\text{max}} \) values (endocardium > epicardium) has also been observed in dog (e.g., Kimura et al., 1982) and rat (Watanabe, 1983) ventricles. The explanation for the low epicardial values in the guinea pig is not immediately obvious, since the average resting potential in the epicardium (−86 mV) was within 1-2 mV of that in the endocardium or septum. One possibility is that a structural difference between the epicardium and the other regions is responsible, since the direction or pattern of propagation in the ventricle can affect \( V_{\text{max}} \) (e.g., Spach et al., 1981; Joyner, 1982). However, stimulus patterns designed to vary the direction of propagation in the guinea pig preparations did not produce large changes (more than 10%) in \( V_{\text{max}} \).

**Action Potential Duration**

Action potential duration was measured at the +10 mV and −80 mV levels of repolarization (APD+10, and APD−80). A summary of the results is shown in Figure 3. The longest durations were observed in the papillary muscles (149-159 msec, APD−80) and the shortest in the septal regions (118-134 msec, APD−80).

In view of the literature in this area (see reviews by Noble and Cohen, 1978; Burgess, 1979; Scher and Spach, 1979), and possible implications for T-wave generation, it was especially interesting to find out whether the action potentials in the endocardium, left ventricle, and basal regions were longer than those in the epicardium, right ventricle, and apical regions, respectively.

There was no systematic difference between the
The regional means from the three hearts were almost identical to those in Figure 3, and individual regional durations were easily within 2 SD of the data in the larger series.

Action Potentials in the Intramural Cell Layers: Experiments on Sliced Tissue and Isolated Myocytes

Ventricular wall segments were isolated from guinea pig hearts and cut transversely with fine scissors or a razor blade to expose intramural surfaces. Microelectrode impalements were attempted after a 130-minute stabilization period during which the preparation was stimulated at 1 Hz. Unfortunately, 20 experiments of this type did not produce consistent results. In most of the preparations, impalements of the exposed intramural surfaces suggested that the sites were electrically inactive, or generated action potentials with amplitudes lower than 100 mV and durations shorter than 100 msec. In four preparations, action potentials approaching 110 mV in amplitude were recorded, and these had durations that were longer (ca. 180 msec, APD$_{-80}$) than those recorded from the uncut ventricular surfaces.

Given these inconsistent results, we decided to take a different approach to the measurement of electrical activity in the intramural layers. Action potentials were recorded from a large number of single myocytes isolated from guinea pig ventricles, and the measurements from these were compared with the data obtained from the tissue surface impalements. The rationale was that most myocytes, perhaps 85–90%, would originate from the intramural layers rather than from the surface layers. Therefore, if the intramural action potentials were appreciably different from the surface action potentials, this should be observed in the distribution of the myocyte data. The calculation above is based on estimates of the surface area and total volume of
guinea pig ventricular tissue, and on the assumption that the microelectrode can reach cells up to 150 \( \mu \)m deep from the surface (see Tranum-Jensen and Janse, 1982).]

Disaggregation of guinea pig ventricles produced both rod- and round-shaped myocytes. About 70% of the isolated myocytes had a damaged, rounded-up appearance, and most of these incorporated the vital stain, trypan blue (0.1%, 10-minute exposure). The rod-shaped myocytes accounted for about 30% of the total population and were 119 ± 34 \( \mu \)m long and 18 ± 14 \( \mu \)m in diameter (\( n = 300 \), mean ± SD). These myocytes excluded trypan blue (0.1%, 10-minute exposure), and did not beat spontaneously. Cross-striations were prominent, and the length of individual sarcomeres ranged from 1.6–2.1 \( \mu \)m (mean ± SD: 1.8 ± 0.2 \( \mu \)m, \( n = 100 \) myocytes). As observed under the electron microscope (Bustamante et al., 1981), the myofilaments and Z-bands of these cells were well-ordered, and the mitochondria and other intracellular organelles had a normal appearance.

Short current pulses (100 nA, 0.5 msec, 1 Hz), applied through a microelectrode positioned just outside the cell, triggered twitch-like contractions. Action potentials were recorded from the myocytes by passing current pulses (5–20 nA, 0.5 msec, 1 Hz) through intracellularly positioned microelectrodes. On average, it required about three cells to obtain one stable impalement. The threshold potential was —61 ± 5 mV (\( n = 20 \), mean ± SD), a value similar to that reported for guinea pig ventricular tissue [—64.7 mV (Kishida et al., 1979)].

Although the action potential in a given myocyte remained stable (<3% change in amplitude or resting potential, and <10% change in duration) for periods exceeding 12 hours, the action potential duration varied considerably from myocyte to myocyte. This is illustrated by the records presented in Figure 4. Despite the fact that all three of the myocytes in this experiment were isolated from the same heart, the action potential duration in one cell (panel C, APD\(_{90} \) 268 msec) was nearly twice as long as in another (panel A, APD\(_{90} \) 126 msec). In experiments on 230 myocytes from more than 100 hearts, the APD\(_{90} \) ranged from 40–200 msec and the APD\(_{90} \) from 110–290 msec. The values (mean ± SD) of the resting potential (—89 ± 5.5 mV), amplitude (128 ± 5.4 mV), and \( V_{\text{max}} \) (245 ± 58 V/sec) were consistent with those obtained from impalments of the tissue surface layers (Fig. 2). It is also worth noting that in five of the myocytes sampled, the \( V_{\text{max}} \) values of 100–120 V/sec were far outside the normal range. However, since the other action potential parameters were not unusual (amplitude 120–130 mV and APD\(_{90} \) 130–150 msec), it is possible that these myocytes originated from the low \( V_{\text{max}} \) epicardial surface layers (Fig. 2).

The action potential durations in the myocytes (\( n = 230 \)) were compared with those in the surface layers of the tissue (\( n = 480 \); data from the previous section) by constructing histograms of probability versus duration range (Fig. 5). The data from the tissue surface were distributed over a relatively narrow range with one well-defined peak at each potential level (APD\(_{90} \) 70–90 msec, APD\(_{90} \) 130–150 msec). Although the myocyte data overlap the tissue values, they are more spread out, with most durations exceeding those in the tissue.

It is unlikely that the isolated cells exhibiting long action potential durations were Purkinje fiber cells, because action potentials recorded from Purkinje fiber bundles during 1-Hz stimulation of ventricular tissue preparations had the following characteristics: maximum diastolic potential approximately —90 mV, amplitude ca. 130 mV, \( V_{\text{max}} \) ca. 400 V/sec, an early rapid repolarization (phase 1) to about +15 mV, and an APD\(_{90} \) of 180–200 msec. Since action potentials possessing these characteristics were not found in any of the isolated cells, it is improbable that the longer durations recorded in myocytes were due to the presence of Purkinje fiber cells.

Ventricular Action Potentials and the ECG

Three sets of experiments were designed to assess the linkage between ventricular action potential configuration and the guinea pig ECG. First, the body surface ECG was recorded to establish standard features of the guinea pig ECG and to reconstruct ventricular gradient vectors (Wilson et al., 1931). Second, extracellular potentials generated by excised guinea pig ventricles driven at 1 and 5 Hz were...
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FIGURE 5. Frequency distribution of the duration of action potentials (stimulation rate 1 Hz) recorded from guinea pig ventricular tissue (open dotted blocks, total n = 480) and isolated myocytes (solid blocks, total n = 230). The durations in some myocytes were similar to those in the tissue, but most were considerably longer.

FIGURE 6. Panel A: bipolar lead electrocardiograms (leads I, II, and III) recorded from the body surface of a guinea pig. The heart rate, QRS duration, and Q-T interval in 10 animals averaged (mean ± SD) 5.2 ± 0.4 Hz, 19 ± 4 msec, and 120 ± 10 msec, respectively. Panel B: simultaneous recordings of extracellular potentials and action potentials (papillary muscle) in an excised left ventricle of guinea pig driven at 1 Hz (left) and 5 Hz (right). The APD values at 1 and 5 Hz were 153 msec and 95 msec, respectively. The Q-T intervals of 200 msec (1 Hz) and 138 msec (5 Hz) in the extracellular potentials are too long to be explained by the action potential durations of the papillary muscles, even though these action potentials were longer than any others recorded on the surfaces of the preparation (range 128–151 msec, 1 Hz, n = 24). The vertical calibration bar indicates 0.5 mV for ECG, 2 mV for the extracellular potentials, and 40 mV for the action potentials. The horizontal bar indicates 100 msec for all records.

extracellular potentials and action potentials were recorded from 10 preparations of isolated left ventricles. Figure 6B presents the records obtained from one of these preparations during stimulation at 1 Hz (left) and 5 Hz (right). The shapes of the ST-T segment and T-wave in the extracellular potential recording (5 Hz) were similar to those in the body surface ECG (lead I). The following values were extracted from these experiments (mean ± SD): QRS duration 34 ± 7 msec, Q-T interval 204 ± 11 msec with 1 Hz stimulation, and QRS duration 30 ± 6 msec, Q-T interval 135 ± 10 msec with 5-Hz stimulation. The respective QRS and T-area amplitudes...
were consistent and are well-described by the slopes of the diagonal lines bounding the tissue and the myocyte data; i.e., increasing the frequency from 1-5 Hz reduced the APD_{so} by 40-50%. In no instance was the reduction smaller than 35% or larger than 55%. The extent of this shortening with frequency is in good agreement with that shown in Figure 6B, and with earlier studies on isolated guinea pig papillary muscles (e.g., Reiter and Stickle, 1968). From the results shown in Figures 6 and 7, it is difficult to see how action potentials in the surface layers can account for the Q-T intervals.

One possible reason why the action potentials in the myocytes are longer than those in the tissue is that activity-related accumulation of [K+]o around cells in the tissue shortens the action potential by increasing K^+ permeability and stimulating outward electrogenic pump current. A method of testing this hypothesis is to (1) measure the tissue depolarization associated with a sudden increase in activity, (2) estimate the extent of the [K+]o accumulation from the depolarization, (3) repeat (1) and (2) in isolated myocytes, and then (4) measure the action potential shortening when myocytes are exposed to estimates of tissue activity [K+]o. The rationale is that sudden high frequency stimulation of quiescent or slowly beating cardiac tissue induces a rapid diastolic depolarization ([K+]o accumulation) prior to a gradual repolarization due to electrogenic pump stimulation (Vassalle, 1970; Nosek, 1980; Aomine et al., 1983).

In the present case, the maximum depolarization can be translated to an effective [K+]o, because the behavior of the resting membrane in both guinea pig ventricular tissue and myocytes closely resembles that of a K^+ electrode when [K+]o is between 4.6 and 15 mM (Baumgarten et al., 1981; Bustamante et al., 1981). The maximum depolarization in middle endocardium (n = 4), middle epicardium (n = 4), and papillary muscle (n = 4) preparations from left ventricles was 5.1 ± 1.9 mV (SD, n = 12) when the driving rate was increased to 5 Hz from a basal 0-0.3 Hz. This suggests an increase in effective [K+]o from a basal level near 4.6 mM to about 5.6 mM at 5 Hz.

Myocytes are also expected to exhibit activity-related [K+]o accumulation, since several likely areas of restricted diffusion (e.g., T-tubules, caveolae) are still present after isolation. This was borne out by the depolarization of 3.1 ± 1.7 mV in eight myocytes when a 1-minute rest was followed by 5 Hz stimulation (Fig. 8, A and B). The extent of the depolarization suggests that accumulation raises [K+]o around myocytes from 4.6 to about 5.2 mM. Therefore, accumulation is larger around cells in situ than those in isolation, and the question is whether the higher [K+]o in situ affects the action potential duration. The tests were conducted on six myocytes stimulated at 1 Hz (APD_{so} = 153 msec at 1 Hz, 95 msec at 5 Hz), not long enough to explain the Q-T intervals.

The dependence of the action potential duration on stimulation rate was examined in 20 myocytes with a representative range of action potential durations at 1 Hz (APD_{so} between 120 and 260 msec), and in 32 regional tissue sites (two basal and two apical sites in the left and right endocardium, epicardium, and septum, plus four left and four right papillary muscles). The effects on the APD_{so} of increasing the driving rate from 1-5 Hz for 1 minute are shown in Figure 7. Although individual impalements are not identified in the figure, the results were consistent and are well-described by the slopes of the diagonal lines bounding the tissue and the myocyte data; i.e., increasing the frequency from 1-5 Hz.
Figure 8. Effects of post-rest stimulation and elevated external $K^+$ on electrical activity in isolated myocytes. Panel A: depolarization of the resting potential with 5-Hz stimulation after a 60-second rest. Panel B: action potential configuration at the time of maximum depolarization during 1- and 5-Hz stimulation after 60-second rests. Panels C and D: depolarization and action potential shortening in response to an increase in $[K^+]_e$ from 4.6 to 6 mM (panel C) or 7.4 mM (panel D) for 10 minutes (1-Hz stimulation). Time calibration for panel D also applies for panel C. The records (four different myocytes) indicate that post-rest stimulation produces a maximum depolarization of roughly 3 mV (panels A and B), and that exposure of myocytes to liberal estimates of accumulated $[Ca^{2+}]_{situ}$ (see text) shortens the action potential by 10% or less.

Discussion

The configuration of the action potential in guinea pig ventricle has been determined by recording action potentials from the surface of tissue preparations and from dissociated myocytes. Comparisons between tissue regions and between tissue and myocytes indicated that there were only small differences in mean action potential amplitudes (range 118 to 126 mV) and resting potential (range −85 to −90 mV) during 1 Hz stimulation. Mean $V_{max}$ values were around 240 V/sec except in the epicardial tissue regions and in a small number of myocytes where values around 110 V/sec were observed. Large differences were observed in action potential durations. APD₉₀ values in tissue surface regions ranged from 110–170 msec, whereas the range in myocytes was from 110–290 msec.

The discussion that follows concentrates on the heterogeneous nature of the ventricular action potential duration. First, the results of the present study are compared with those obtained from other species. This is followed by an examination of the myocyte data and their validity as an indicator of intramural cell electrical activity. Finally, the ECG and the extracellular potentials will be discussed with reference to the action potential configurations observed in the isolated tissue and myocytes. The hypothesis under consideration is that long action potential durations in the intramural regions can explain the Q-T intervals.

Regional Differences in the Ventricular Action Potential Duration

Methodological Considerations

Most studies dealing with regional differences in the action potential duration have utilized methods other than the intracellular microelectrode (see Burgess, 1979). The indirect techniques have included measurements of the ventricular gradient, refractory period mapping, and epicardial or intramural potential distribution mapping, and the suction electrode. Each of these methods has at least one serious drawback:

1. The ventricular gradient method based on the body surface ECG provides little information on the action potential duration in designated regions.
2. Refractory period measurements are based on the assumption that the refractory period assessed from electrograms is strictly related to the action potential duration. In view of the complicated time- and voltage-dependent ionic currents governing repolarization and premature excitation, it is difficult to accept the premise that a strict correlation exists between the refractory period (electrogram) and the action potential duration everywhere in the ventricle. Furthermore, it is difficult to determine the exact moment of reexcitation under the sensing electrode.
3. A difficulty with epicardial or intramural potential mapping is that action potential durations must be derived from extracellular potential gradients occurring over the course of repolarization.
4. Suction electrodes provide monophasic action potentials from surface sites and raise questions concerning damage to the suction site, and distortion of signals due to mechanical movement.

Microelectrode studies are also not free of objec-
tion. One major concern is that action potential durations in vivo may be different from those in vitro. For example, the temperature gradients, the extracellular milieu and the mechanical stress impinging on ventricular tissue in vivo are certainly different from those in vitro. Since all of these factors have some effects on action potentials, the action potential configuration at a given site in vivo could be quite different from that in vitro. Nevertheless, a comparison of the ST-T segments in the body surface ECG with the bipolar electrograms from excised ventricles (Fig. 6) suggests that the action potential durations in the excised ventricles are not greatly different from those in vivo.

Comparisons between Regions

Since, for the most part, the results in the literature on the heterogeneity of the action potential duration are described in terms of three regional pairs, endocardium vs. epicardium, base vs. apex, and left vs. right ventricle, focusing on these pairings provides a convenient way of comparing the present results with those in the literature.

Endocardium vs. Epicardium. In the guinea pig heart, the action potential duration was almost 10 msec longer at the left endocardial base than at the left epicardial base. Otherwise, there was either no difference between the surfaces, or the epicardial events were 10–15 msec longer (right ventricle: middle and apex) (Fig. 3).

In the dog heart, it is a common view that the action potential duration in the endocardium is 10–20 msec longer than in the epicardium. This conclusion comes from functional refractory period measurements (e.g., Burgess et al., 1972; Abildskov, 1975), epicardial and intramural mapping studies (e.g., Spach and Barr, 1975, 1976), and intracellular microelectrode recordings (e.g., Solberg et al., 1974; Gilmour and Zipes, 1980; Kimura et al., 1982). Lab (1971, 1978) reached a similar conclusion in suction electrode studies on frog ventricle. In addition, he presented evidence suggesting that differences in action potential duration were related to mechanical stress [also see review by Lepeschkin (1976)].

Base vs. Apex. The action potential durations in the basal regions of guinea pig ventricles were similar to those at the corresponding apical regions in some surfaces (right and left epicardium, right septum) but were 10–15 msec longer in others (right and left endocardium, left septum) (Fig. 3). In rat endocardium, action potential durations at the basal regions were 30–60% longer than those at the apical regions in the left ventricle, and to a lesser extent, this was also the case in the right ventricle (Watanabe et al., 1983).

The application of indirect methods in other species has not led to a consensus of the base-vs.-apex question. Longer durations at the base have been hypothesized from studies on human [Wilson et al. (1931), ventricular gradient], frog [Schaefer et al. (1943), suction electrode], and dog hearts [van Dam and Durrer (1964), ventricular gradient]. More recently, the opposite relationship, a duration 10–30 msec longer in the apex than in the base, has been demonstrated for the dog heart [Burgess et al. (1972), functional refractory period; Abildskov (1975), epicardial mapping; Autenrieth et al. (1975) and Toyoshima et al. (1981), suction electrodes]. In microelectrode studies on ventricular tissue from sheep ventricles, Cohen et al. (1976) reported that action potentials in the base had longer durations than in the apex. However, the conclusions of Cohen et al. (1976) regarding sheep heart need qualification, because they compared septal base with epicardial apex.

Left Ventricle vs. Right Ventricle. The action potential durations in the left endocardium and septal base of guinea pig heart were about 10 msec longer than in the corresponding regions of the right ventricle. In contrast, the durations were about 10 msec shorter in left than in right papillary muscles (Fig. 3).

These small, unsystematic differences are in agreement with results from the canine heart. On the basis of refractory period measurements, Burgess et al. (1972) concluded that there was no difference between left and right free walls, but that there was a small difference (1–11 msec) between left and right septum (left > right). Solberg et al. (1974), in their microelectrode study, reported that left and right papillary muscles had similar durations. In contrast to the foregoing, Watanabe et al. (1983) found that the action potential in the endocardium and papillary muscle of rat heart in the left ventricle is nearly twice as long as in the right.

The results of the study on rat heart (Watanabe et al., 1983) are all the more interesting because they were obtained in the same way as the guinea pig data. Thus, whereas methodology is undoubtedly one of the factors responsible for the lack of accord noted throughout this discussion, the results obtained with rat hearts indicate that species differences must also be important. Otherwise, it is difficult to explain the large difference in action potential duration between right and left surfaces in the rat heart vs. the very small difference in guinea pig heart observed in the present study.

Action Potential Duration in the Intramural Cell Layers

There is no agreement in the literature on the action potential duration in the intramural cell layers relative to those in the epicardial and endocardial surface layers. As discussed below, experimental results on the dog have led to suggestions that the action potential duration in the intramural layers is shorter than, longer than, or in between the durations in the endocardium and epicardium.

Van Dam and Durrer (1961) reported that intramural cell layers had the shortest action potential duration and that endocardium had the longest, based on refractory period measurements in the
epicardium, endocardium, and intramural layers of dog heart. However, studies utilizing refractory period measurements (e.g., Abildskov, 1975) and intramural mapping results (e.g., Spach and Barr, 1975) led to the conclusion that there is a progressive increase in duration from the epicardial surface to the endocardial surface.

The results of a microelectrode study by Solberg et al. (1974) are not supportive of either view (although caution is warranted when comparing the in vivo situation with their in vitro approach). Solberg et al. recorded electrical activity from the epicardial and endocardial surfaces of ventricular wall strips, as well as from intramural cell surfaces created by slicing papillary muscles. Their finding was that the action potential duration in the intramural layers of papillary muscle was about 20 and 40 msec longer than in the epicardium and endocardium of ventricular free wall, respectively. Unfortunately, they had little success with impalements of intramural surfaces in slices of specimens from ventricular free wall and septal regions.

Since we were also unable to obtain direct reliable recordings from cells in the intramural layers of sliced tissue walls (traumatized tissue?), we isolated myocytes from entire ventricles and sampled the configuration of the action potential in a large number of them (n = 230). The rationale was that if each cell in the ventricle stands an even chance of becoming a viable myocyte after tissue disaggregation, and if myocytes are selected for impalement in a random manner, most of those impaled will have originated from the intramural cell layers, i.e., the region of largest mass in the ventricle. In fact, about 60% of the myocyte action potentials had a duration exceeding any recorded in tissue surface cells (Fig. 5).

Myocyte Isolation and the Action Potential Configuration

The interpretation of the myocyte data depends on the premise that, in a given myocyte, enzyme treatment, loss of contact with neighboring cells, and removal from the tissue extracellular environment, do not result in action potential characteristics that are very different from those it had in vivo. It may be some time before this premise can be completely accepted or rejected. However, there is some evidence in favor of electrophysiological similarity: guinea pig ventricular myocytes and tissue respond in like manner to the application of channel blockers, ionic substitution, changes in extracellular Na^+, K^+, or Ca^{++} concentration, and the influence on the action potential configuration between guinea pig, cat, rabbit and rat ventricular tissues are readily observed in respective myocytes [unpublished observations; see Watanabe et al. (1983) for rat action potentials]. A relevant finding from the latter study was the absence of a group of rat myocytes with action potentials distinct from those observed in the tissue surface layers. This illustrates that enzyme treatment per se does not result in an indiscriminate lengthening of the action potential. In addition, it suggests that the action potential duration observed in tissue preparations is not simply the average outcome of a tight coupling of randomly dispersed cells with widely different action potential durations. If that were the case in guinea pig ventricle, a large fraction of myocytes, with durations far shorter than those measured in the tissue, would be required to offset the long-duration myocytes.

Electrotonic interactions between cells that have different sets of membrane properties can exert an influence on the action potential duration (McDonald and Sachs, 1975; Joyner et al., 1983). The nature and degree of the influence will depend on the distribution of the durations. Since higher coupling resistances sharply reduce the interaction (Joyner et al., 1983), and since the cell-to-cell resistance is much larger across the ventricular wall than along it (Myerburg et al., 1978; Spach et al., 1981), interaction in the transverse direction will be less than in the longitudinal one. Based on the tissue surface and myocyte data, one could assign the myocytes with short durations to the surface and near surface layers, and build toward the center of the wall layers of myocytes that had progressively longer durations. In fact, there are strong indications that myocardial tissue is comprised of discrete poorly coupled bundles (Spach et al., 1981). With this arrangement, one might expect only a slight lengthening of the myocyte action potential in the surface layers, and a slight shortening in the central layers.

Cells in the tissue may experience [K^+]o, accumulation in restricted extracellular spaces and resultant modulation of the action potential duration due to electrugic Na^+–K^+ pumping. The modulation may be of a lengthening or shortening nature. For example, Cohen et al., (1976) hypothesized that the longer action potential duration in sheep septum (base) than in epicardium (apex) arose from stronger pumping in the septal tissue, rather than from differences in ionic channel currents. The lengthening was explained as follows: activity-induced accumulation of [K^+]o, leads quickly to increased pumping, depletion of effective [K^+]o below bulk concentrations, and a lengthening effect (reduced [K^+]o) that offsets a shortening one (outward pump current). A different view is that electrogenic pumping would reduce the accumulated [K^+]o, down toward, rather than below, bulk fluid concentration (e.g., Kunze, 1977), and that the higher P_K and pump current would have an additive shortening influence.

These two propositions predict opposite responses to any modulation of activity-related [K^+]o, accumulation that might occur upon cell isolation. Cohen et al. predict that cells will have shorter action poten-
tentials in isolation. This does not disturb our contention that, as observed in myocytes, there are longer-than-surface action potentials in the ventricle. The alternative argument predicts that myocytes will have longer action potentials in isolation, and if the lengthening due to the removal of $[K^+]_o$ accumulation were severe enough, say 50–100 msec at 1 Hz, our postulate would be greatly weakened.

We estimated effective $[K^+]_o$ accumulation with activity by measuring the maximum depolarization induced by a sudden increase in the driving rate (see Vassalle, 1970). In guinea pig tissues, an increase in rate from 0–0.3 Hz to 5 Hz produced an average maximum depolarization of 5.1 mV. This depolarization compares with those recorded in monkey epicardium (4.4 mV) and endocardium (5.8 mV) (Aomine et al., 1983), and in guinea pig endocardium (3.3 mV) (Nosek, 1980), during post-rest stimulation at 3–3.8 Hz. Based on a $K^+$ electrode response and $K_0$ of 140 mM (Baumgarten et al., 1981; Bustamante et al., 1981), the results suggest that effective $[K^+]_o$ increased from 4.6 to about 5.6 mM under these conditions. In myocytes, the depolarization and effective $[K^+]_o$ estimate were somewhat smaller (3.1 mV and 5.2 mM). The key question, whether a tissue-like $[K^+]_o$ accumulation around myocytes would significantly shorten the myocyte action potential, was tested by increasing $[K^+]_o$ from 4.6 to 6 then 7.4 mM in myocytes stimulated at 1 Hz. These liberal levels were chosen to simulate the possible case of longer than average accumulation in deep-lying cells. The shortening observed, 2–12% is in line with the minimal changes in pump activity (Daut and Rudel, 1982) action potential duration (Reiter and Stickel, 1968), and outward plateau current (Trautwein and McDonald, 1978) observed when similar or greater $[K^+]_o$ changes were imposed on guinea pig ventricular muscle.

In summary, action potentials in the upper range of the durations recorded from isolated myocytes may have been 10–15% longer than they were in situ due to the combined influence of cell-to-cell coupling and extracellular environment. The most likely reason for differences in action potential duration, whether the cells are in isolation or in situ, is that there are differences in net current flow through ionic channels. In contrast to the findings of Cohen et al. (1976) on sheep ventricular tissue, differences within and between guinea pig myocytes and ventricular tissue at 1 or 5 Hz were not dissipated by low stimulation rates or rests of 30–60 sec.

Ventricular Action Potentials and the ECG

The polarity of the T-wave is the same as that of the QRS complex in ECG from human subjects (Waller, 1887). This concordance of the QRS complex and the T-wave is also observed in the guinea pig, and leads to the expectation (e.g., Harumi et al., 1966; Toyama et al., 1967) that the action potential duration in the ventricle is heterogeneous, and that there are some regions with long action potentials that are triggered earlier and repolarized later than other regions with shorter action potentials.

The activity recorded from the tissue surface layers indicates that the condition of heterogeneity is fulfilled (Fig. 3). On the assumption that the activation sequence in guinea pig ventricle is similar to that in other mammalian hearts (e.g., Durrer et al., 1970; Scher and Spach, 1979), the second condition, repolarization proceeding in a direction opposite to that of activation, may be fulfilled in certain areas (e.g., endocardial and epicardial base of left ventricle) but not in others (e.g., endocardial and epicardial apex of right ventricle). However, even if that is the case, the measurements taken from the bipolar electrograms suggest that the explanation of T-wave generation lies elsewhere. The Q-T interval in excised left ventricle preparations was 204 ± 11 msec (QRS duration 34 ± 7 msec) at 1 Hz, and 135 ± 10 msec (QRS duration 30 ± 6 msec) at 5 Hz. Thus, even the longest action potential durations recorded in the tissue surface (papillary muscle: 149 ± 8 msec at 1 Hz and 90 ± 6 msec at 5 Hz, $n = 4$) were too short to account for the Q-T intervals in the electrograms. Our suggestion is that the required durations are generated by cells in the intramural layers.

If we take into account that the myocyte action potential duration may be about 10–15% shorter in situ than in isolation ([K$^+$]o accumulation, coupling), very few of the myocyte action potentials would exceed the Q-T intervals (Fig. 5). The population of myocytes having longer action potentials may be too small to contribute to the T-wave, especially if there is a high degree of cancellation with each other (Burgess et al., 1969). However, if cancellation is less than complete, they could contribute to the U-wave (a small amplitude, post-T event that often escapes detection).

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Dr. Watanabe's present address is: The Research Institute of Environmental Medicine, Nagoya University, Chikusa-ku, Nagoya, Japan.

Address for reprints: Terence F. McDonald, Ph.D., Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

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INDEX TERMS: Isolated ventricular tissue • Adult ventricular myocytes • Regional action potentials • Guinea pig ECG • Q-T interval
Ventricular action potentials, ventricular extracellular potentials, and the ECG of guinea pig.

T Watanabe, P M Rautaharju and T F McDonald

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