Transmural recording of monophasic action potentials

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Transmural recording of monophasic action potentials. Am J Physiol Heart Circ Physiol 282: H855–H861, 2002; 10.1152/ajpheart.01172.2000.—To investigate the possibility of transmural recording of repolarization through the ventricular wall, KCl monophasic action potential (MAP) electrodes positioned along plunge needles were developed and tested. The MAP electrode consists of a silver wire surrounded by agarose gel containing KCl, which slowly eluted into the adjacent tissue to depolarize it. In six dogs, a plunge needle containing three KCl MAP electrodes was inserted into the left ventricle to simultaneously record from the subepicardium, midwall, and subendocardium. In six pigs, eight plunge needles containing three KCl MAP electrodes and two plunge needles containing similar electrodes except for the absence of KCl were inserted into the ventricles. In three guinea pig papillary muscles, a KCl electrode was used to record MAPs along with two microelectrodes for recording transmembrane potentials. Transmural MAP recordings could be made for >1 h in dogs and >2 h in pigs with a significant decrease in MAP amplitude over time but without a significant change in MAP duration. With the electrodes without KCl in pigs, the injury potentials subsided in <30 min. When the pacing rate was changed to alter the action potential duration and refractory period in dogs, the MAP duration correlated with the local effective refractory period (r = 0.94). The time course of the MAP duration recorded with a KCl MAP electrode in guinea pig papillary muscles corresponded well with that of the transmembrane potential recorded with an adjacent microelectrode. It is possible to record transmural repolarization of the ventricles with KCl MAP electrodes on plunge needles. The MAP is caused by the KCl rather than being a nonspecific injury potential.

 METHODS

This study was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham Medical Center. It complies with Section 6 of the Animal Welfare Act of 1989 and adheres to the guiding principles outlined in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23). Three different experiments were performed, each in a different animal species. They are described in Parts I, II, and III.

 Part I

 Electrode preparation. On the basis of the concept that an MAP is recorded when a local injury potential is created in the tissue adjacent to the active MAP electrode but not adjacent to the reference electrode (6), we designed a KCl MAP electrode that causes a local injury potential by diffused KCl. Plunge needles (19 gauge, 1.1 mm in outside diameter).
were constructed that contained three pairs of bipolar MAP electrodes (Fig. 1A). Each pair of electrodes consisted of a reference and a KCl electrode 2 mm apart. Both reference and KCl electrodes were insulated silver wires 0.075 mm in diameter, each passing through a small hole in the plunge needle. The holes through which the reference electrodes passed were sealed with epoxy resin, whereas the holes through which the KCl electrodes passed were left open. The inside of the plunge needle was filled with agarose gel, which contained 20% KCl in the first two animals and 30% KCl in the remaining animals. Because the KCl was trapped in the agarose gel, it diffused slowly through the open holes when it was inserted into the myocardium, causing local tissue depolarization. The distance between two adjacent pairs was 5 mm so that the plunge needle containing three pairs of KCl MAP electrodes would record signals from the subepicardium, midwall, and subendocardium (Fig. 1A).

**Canine experiments.** Six mongrel dogs weighing 15–20 kg were anesthetized with thiopental sodium (13–27 mg/kg) for induction and then isoflurane (1–3%) inhalation with 0.5% oxygen for maintenance through a respirator (Harvard Apparatus; South Natick, MA). A catheter was inserted into the descending aortic artery through the right femoral artery to record arterial blood pressure and to obtain samples for measuring blood gas and electrolytes. Ringers lactate solution was continuously infused through a foreleg vein and supplemented with KCl, NaHCO3, and CaCl2 when needed to maintain a normal metabolic status. Arterial blood pressure and the surface electrocardiogram (ECG) were continuously displayed and recorded.

The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. Either four or nine conventional plunge needles were inserted 8–10 mm apart into the anterior free wall of the left ventricle. A plunge needle containing three KCl MAP electrodes was then inserted 2–3 mm from one of the conventional plunge needles. Signals from all the electrodes were digitally recorded simultaneously by a 528-channel mapping system in the bipolar mode for the KCl MAP electrodes and in the unipolar mode for the conventional plunge needle electrodes at 10 times gain with a 0.01-Hz high-pass filter, a 1,000-Hz low-pass filter, and a digitization rate of 2,000 14-bit samples/s. All data were stored on tape and optical disk for off-line analysis.

In the first four dogs, transmural MAPs were recorded for >1 h to see how MAP amplitude and duration changed with time. Continuous recording began 5 min after insertion of the KCl MAP plunge needle. Transmural MAPs were recorded simultaneously with conventional plunge needles around the MAP plunge needle to see whether the eluted KCl distorted propagation across the region around its insertion. In these four dogs, four conventional plunge needles were used, whereas in the last two dogs, nine conventional needles were used. The ERP was determined near the MAP recording site from the central conventional plunge needle by an S1-S2 pacing protocol in all six dogs. After 10 S1 stimuli at twice diastolic threshold strength, an S2 premature stimulus was given. The S1-S2 interval was initially timed so that the S2 stimulus occurred near the end of repolarization in the KCl MAP recordings. The S1-S2 interval was then shortened in 2-ms decrements until the S2 stimulus failed to induce an action potential. This S1-S2 interval was defined as the ERP. Ten S1 stimuli were also given without an S2 stimulus, and the maximum upstroke to the time of 90% repolarization back to the takeoff potential (APD90) was determined for the last S1 stimulus. The ERP and APD90 were determined at two different S1 pacing rates, 350 and 200 ms.

**Data analysis.** The amplitude of the MAP (APA) was defined as the potential difference between the diastolic baseline and the maximum amplitude of phase 2. The duration of the MAP was determined as the interval (in ms) from the time of onset of MAP depolarization identified by the APD90. Transmural dispersion of repolarization was determined by subtracting the shortest APD90 from the longest APD90 recorded from the three KCl MAP electrodes along the plunge needle. Activation time was defined as the time at which a local activation (depolarization) was recorded. Activation times were referenced to the earliest activation recorded at any of the three MAP electrodes on the needle, which was called time 0. Statistical analyses were performed using a repeated-measures ANOVA with a post hoc t-test for determination of individual group differences. A regression analysis was used to determine the relation between APD90 and refractoriness. A P value of <0.05 was considered significant.

![Fig. 1. Schematic representation of experimental recordings. A: a plunge needle containing 3 monophasic action potential (MAP) electrodes used to record from the subepicardium (Epi), midwall (Mid), and subendocardium (Endo) in part I. The KCl electrode is on the left side of the needle and the reference electrode is on the right for each of the 3 MAP electrode pairs. B: a plunge needle of the type used in part II, with the 3 KCl electrodes on the left side and the 3 reference electrodes on the right side of the needle, which contains a nylon tube within a nylon sheath. C: microelectrode experimental preparation, in which the transmembrane action potentials from guinea pig papillary muscles were simultaneously recorded by two microelectrodes along with the KCl MAP electrode recordings. One (TP1) of the transmembrane potential recordings was made near the KCl electrode while the other (TP2) was made near the reference electrode 3 mm away from the KCl electrode.](http://ajpheart.physiology.org/10.22032/s246e1/2002.06.01.03.02.04.2017)
**Electrode preparation.** New plunge MAP electrodes were designed in which the metal needle was replaced by a nonconducting tube and sleeve in case the highly conductive metal needle was shunting current and distorting the recordings (Fig. 1B). The new MAP electrode consisted of a 1.1-mm-diameter nylon tube inserted into a thin nylon sleeve. When assembled, the outer diameter of the structure was 1.6 mm. There were three reference electrodes 2.5 mm apart, each of which consisted of a silver wire 0.127 mm in diameter, which was pierced through the sleeve and sealed with cyanoacrylate adhesive. The nylon tube was then inserted into the sleeve, trapping the reference electrode wires between the sleeve and the tube. On the opposite side of the tube, three 0.8-mm-diameter holes were drilled through the sleeve and tube wall 2.5 mm apart. Identical silver wires were pulled down the shaft of the tube and out each hole. The proximal end of the tube was filled with epoxy resin. Agarose gel was injected through the tip of the assembly until it was pushed out of each hole. The tip was then sealed with cyanoacrylate adhesive, and the agarose gel was trimmed flush with the outer edge of the sleeve. Thus each plunge needle contained three MAP electrode pairs 2.5 mm apart with the two electrodes of the pair separated by 1.6 mm.

In the standard MAP electrodes, the agarose gel contained 30% KCl. For this particular experiment, other electrodes were prepared in which no KCl was in the agar to determine whether the MAP recording was specifically caused by the KCl or whether it was a nonspecific injury potential caused by the needle itself.

**Porcine experiments.** Six pigs, ranging in body weight from 32 to 40 kg and with heart weights of 186 ± 24 g, were anesthetized with 25 mg/kg intravenous thiopental sodium and maintained with isoflurane in 100% oxygen. Each pig was intubated with a cuffed endotracheal tube and ventilated with a mixture of room air and oxygen through an Ohio anesthesia ventilator (Airco). Surface ECG, femoral arterial blood pressure, and temperature were monitored. Blood gases and pH were monitored and maintained within normal physiological ranges. The chest was opened by a median sternotomy, and the pericardial sac was opened to expose the heart for placement of plunge needles.

Eight MAP plunges with KCl were placed into the ventricles, two in the anterior left ventricle, two in the lateral left ventricle, two in the anterior right ventricle, and two in the lateral right ventricle. Two plunges without KCl were also placed, one in the anterior and one in the lateral left ventricle. Recordings were made from these electrodes for >2 h with the 528-channel mapping system described in Part I. APA and APD₉₀ were determined every 30 min after needle insertion as described in Part I.

**Guinea pig experiments.** Three guinea pigs weighing ~300 g were anesthetized with pentobarbital sodium (75 mg in 1.5 ml saline) injected into the abdomen. The heart was rapidly excised through a median sternotomy and immersed in cold Tyrode solution. The Tyrode solution contained (in mM) 129 NaCl, 1.8 CaCl₂, 1.1 MgCl₂, 4.5 KCl, 1 Na₂HPO₄, 20 NaHCO₃, and 11 glucose. The left ventricular anterior papillary muscle, ~5–6 mm long and 2–3 mm wide, was removed and pinned on silicon rubber in the center of a 2 × 2-cm tissue bath. The tissue was then continuously superfused with Tyrode solution bubbled with a mixture of 95% O₂ and 5% CO₂, giving a pH of 7.35–7.40. Solution temperature was maintained in the range of 35–36°C. The papillary muscle was paced by bipolar electrodes at one end of the tissue with a stimulator controlled by a Macintosh II computer.

The MAP was recorded by an electrode pair similar to those used in parts I and II except that the KCl MAP electrode was at the tip of the needle and touched the surface of one end of the papillary muscle while the reference electrode was applied at the other end of the tissue (Fig. 1C). Glass capillary tubes were pulled to form microelectrodes that had an impedance of ~10 MΩ when filled with 3 M KCl. Two microelectrodes were used simultaneously to record transmembrane potentials from two separate sites to see whether the MAP represented the action potential near the KCl electrode, the reference electrode, or neither electrode. One microelectrode recorded at a site <1 mm from the KCl electrode, whereas the other microelectrode recorded at a site <1 mm from the MAP reference electrode (Fig. 1C). Each microelectrode was mounted on a motorized micromanipulator (WPI DC3001, World Precision Instruments; Sarasota, FL) and was connected to the input of a differential preamplifier (WPI Duo 773 Dual Microprobe System, World Precision Instruments) with an Ag-AgCl wire. The signals from the microelectrodes as well as from the KCl MAP electrode were simultaneously recorded with a four-channel data acquisition system with direct current coupling after preamplification. Signals were recorded digitally with 12-bit accuracy at a rate of 8,000 samples/s. The data were stored on optical disks for later computer analysis.

Simultaneous recordings of the microelectrode action potentials and the MAP were performed at two pacing rates, 350- and 200-ms S1-S1 intervals, to see whether the MAP recordings reproduced the repolarization of the transmembrane potential recordings in the microelectrode recordings and whether accommodation of repolarization occurred similarly for both the transmembrane potential and MAP. The transmembrane potentials were recorded before and after the MAP electrode was applied to see whether the KCl MAP electrode altered repolarization.

**RESULTS**

**Part I**

**Changes in MAP recordings with time.** There were no significant changes in the mean value of APD₉₀ at the times of 0, 30, and 60 min for all three MAP recording locations (Fig. 2A). However, the mean value of APA significantly decreased with time for all three locations (Fig. 2B). The amplitude of APA of the transmural MAP decreased by more than one-third after recording for 1 h.

The R-R interval was 466 ± 27, 444 ± 40, and 454 ± 47 ms at 0, 30, and 60 min, respectively, after the insertion of the KCl MAP electrodes. The transmural dispersion of APD₉₀ was 13 ± 7, 12 ± 2, and 11 ± 2 ms at 0, 30, and 60 min, respectively. The shortest APD₉₀ was at the epicardial electrode and the longest was at the midwall electrode for these recordings from the anterior left ventricle. The transmural dispersion of activation time was 19 ± 2, 18 ± 2, and 19 ± 1 ms at 0, 30, and 60 min, respectively. None of these variables changed significantly with time.

**Changes in APD₉₀ and ERP with change in pacing rates.** Both the APD₉₀ and ERP were significantly decreased when the pacing rate was decreased from...
350 to 200 ms (Fig. 3A). The APD\(_{90}\) and ERP were highly correlated with a slope not significantly different from 1, with APD\(_{90}\) only 11–12 ms longer than the ERP (Fig. 3B).

**Effects of KCl MAP electrodes on nearby activation.** In two animals, the effects of the KCl MAP electrodes on the three-dimensional activation sequence were studied. As shown in Fig. 4 for one of the animals, the earliest activation (time 0) was detected at the subendocardium and propagated toward the midwall and subepicardium in the mapped region both before and after the plunge needle containing the KCl MAP electrodes was inserted. The activation times across the mapped region were not changed by the insertion of the KCl MAP electrodes. As shown in Fig. 4C, the morphology of the signals at the center of the mapped region before the insertion of KCl MAP electrodes was similar to that after the insertion of KCl MAP electrodes 2–3 mm away. Activation times at the KCl MAP electrodes were nearly the same as those recorded by the nearby conventional plunge needle (Fig. 4B). Results for the other animals were similar. These results suggest that the activation sequence is not grossly affected by insertion of the KCl MAP electrodes.

**Part II**

**Changes in MAP recordings with time.** The changes in time with the insulated type of MAP electrodes in pigs was similar to those changes observed with the original electrode design in dogs (Fig. 5). APA decreased from 17 ± 4 mV at 0 min to 12.5 ± 4 mV at 60 min and to 11.4 ± 4 mV at 120 min. These values are significantly different. However, APD\(_{90}\) did not change significantly over this same time period. It was 187 ± 12 ms at 0 min, 189 ± 17 ms at 60 min, and 185 ± 14 ms at 120 min.

By 30 min, no MAP-like signal was recorded in the electrodes that lacked KCl in the agarose (Fig. 5). This finding indicates that the MAP signal recorded with the KCl electrode is specifically due to the KCl and is not simply an injury potential in response to the insertion of the electrodes.

**Part III**

**Simultaneous recording of transmembrane potentials and MAPs.** The results for the three guinea pig papillary muscle experiments were similar to each other. Application of the KCl MAP electrode to the papillary muscle had a minimal effect on the nearby transmembrane potential (Fig. 6). The superimposed tracings labeled TP1’ + TP2’ + MAP in Fig. 6H demonstrate that 1) the time course of the MAP (dashed line) was almost...
Fig. 4. Effects of KCl MAP electrodes on activation. 
A: activation times (in ms) obtained from 9 conventional plunge needles recording electrical signals from the subepicardial, midwall, and subendocardial before insertion of the KCl MAP plunge needle. 
B: activation times recorded after the insertion of the KCl MAP plunge needle 2–3 mm from the conventional plunge needle at the center of the recording array. 
The subscripted numbers near the center of each of the 3 levels of recordings represent the activation times for the transmural MAPs. 
C: electrical recordings for the activations recorded by the conventional plunge needle at the center of the recording array before (tracings a, b, and c) and after (tracings a’, b’, and c’) the insertion of the KCl MAP electrodes (tracings a”’, b”’, and c”’). The time and voltage scales are shown at the bottom right.

Fig. 5. Examples of intramural MAPs recorded by the same electrodes at times of 0, 30, 60, and 120 min. The MAP electrodes whose recordings are shown in A contained KCl within the agarose, whereas those electrodes whose recordings are shown in B did not. Time 0 was shortly after all electrodes had been inserted. The time scale is given at the bottom right.
DISCUSSION

The present study demonstrates that 1) the time course of the MAP recorded by the KCl MAP electrode pair was similar to that of the transmembrane potential recorded by the microelectrode near the KCl electrode; 2) the MAP recordings can continue for at least 2 h without a significant change in APD_{90} but with a significant decrease in amplitude; 3) electrophysiological alterations caused by a KCl MAP electrode cannot be detected in action potentials recorded with a microelectrode <1 mm away or in the activation sequence recorded with conventional plunge needles 2–3 mm away; and 4) APD_{90} in the MAP recording is highly correlated with the ERP in the same region.

MAP recording has a long history, dating back to at least 1882 when Burdon-Sauderson and Page (1) recorded MAPs in a frog heart. In 1951, Hoffman et al. (9) experimentally demonstrated that the time course of repolarization followed that of the transmembrane potential recorded by a microelectrode. Modification of the MAP electrode by replacing the suction electrode with a contact (or pressure) electrode widened its use (5, 8, 17). Because the MAP electrode is an effective, simple technique to detect local repolarization, it has been widely used in both basic and clinical studies (2, 14, 16, 19–21).

Repolarization of the action potential has been recorded by various techniques to investigate arrhythmogenic mechanisms. In isolated tissues or in situ beating hearts, repolarization has been recorded over the epicardium or endocardium by optical techniques, glass microelectrodes, and MAP electrodes with either suction or pressure (3, 5, 8, 9, 20, 21, 25, 26). Although these techniques are effective in recording surface repolarization, none currently have the ability to record repolarization intramurally. The morphological and electrical structure of the ventricular myocardium has complicated three-dimensional characteristics. Therefore, a complete understanding of the mechanisms of ventricular arrhythmias requires information about the transmural distribution of repolarization.

The underlying mechanism by which MAP electrodes record a signal resembling an action potential is still not fully known. The recording is thought to be generated by creation of an injury current (6, 11, 24). An MAP electrode consists of two electrodes, one of which creates a local depolarization beneath and around it, whereas the other electrode serves as a reference in the nearby tissue. An injury current has been hypothesized to be associated with the potential difference recorded between these two tips, with a negative potential in the depolarized region with respect to the reference electrode during diastole and a monophasic action potential in the depolarized region during excitation (6). The local depolarization and hence the injury current can be produced by pressure (5, 8, 20, 21), suction (9), or KCl (15). Weissenburger and co-workers (23) reported the use of multiple intramural MAP recordings, all using a single electrode in a region exposed to KCl recorded differentially with respect to multiple reference electrodes, one at each intramural recording site (23). In contrast, we used KCl, which was locally released at each intramural recording site from agarose gel within each plunge needle. The slowly released KCl probably caused a local depolarization, thus causing the recorded MAPs. The present study (Fig. 6) demonstrated that the time course of the repolarization followed that of the transmembrane potential recorded near the KCl electrode instead of that near the reference electrode. Although the MAP recordings in the present study continued for >2 h without a significant change in APD_{90}, the APA decreased with time. The decrease in MAP amplitude is probably due to a dilution of the local KCl concentration by the continuous blood perfusion. Therefore, the technique of MAP recording in the present study may not be suitable for long-duration experiments lasting many hours.

The present study also demonstrated that diffusion of KCl away from the MAP electrode was not sufficient to markedly alter the propagation of activation or the shape of the action potentials near the KCl MAP electrode, as shown in Figs. 4 and 6. These results suggest that the KCl MAP electrode may have only a minimal effect on the electrophysiological properties of the
nearby myocardium. This may be due to the blood circulation in the myocardium preventing a large increase in KCl in the nearby tissue. The effects of KCl MAP electrodes on ischemic tissue, in which blood flow is reduced or absent, are unknown (12, 13, 18, 22).

An important electrophysiological property of the cardiac action potential is accommodation, i.e., change in action potential duration and refractoriness with a change in heart rate. The slower the heart rate, the longer the action potential duration and refractoriness, and vice versa (8). The parallel changes in MAP APD$_{90}$ and refractoriness with a change in pacing rate and the good correlation between MAP APD$_{90}$ and refractoriness (Fig. 3) suggest that MAPs recorded by the KCl MAP electrodes reflect the underlying myocardial electrophysiological properties.

In conclusion, transmural repolarization of the ventricles during normal rhythm and ventricular arrhythmias can be observed with KCl MAP electrodes. The intramural MAP recordings in the present study continued for 2 h without a significant change in APD$_{90}$ and without obvious effects on nearby electrical activity. Future studies are required to determine the number of KCl MAP electrodes that can be used simultaneously for transmural mapping of ventricular repolarization.

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