Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes

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HOFFMAN, B. F., P. F. CRANEFIELD, E. LEPESCHKIN, B. SURAWICZ AND H. C. HERRLICH. Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. Am. J. Physiol. 196(6): 1297~1301. 1959.—The action potentials recorded from heart muscle with a suction electrode have been compared to those recorded with an intracellular microelectrode. It has been found that if the suction electrode is properly used the monophasic potentials recorded with it may be taken as a reliable index of the time of arrival of excitation at the electrode and as a reliable index of the shape of the action potential during the entire phase of repolarization. The suction electrode potentials differ from the microelectrode potentials in showing a lower rise velocity, a smaller amplitude, a quantitatively different reversal or overshoot and, in the beating heart, 'afterpotentials' caused by mechanical effects. When the shape of the action potential, as observed with the microelectrode, is changed by ions such as K⁺ or Ca⁺⁺ a similar change is observed in the potential recorded with the suction electrodes.

Prior to the introduction of intracellular microelectrodes all attempts to register the potential variations across the membrane of the myocardial cell used leads in which one of the electrodes was in contact with a part of the heart where the cell membranes were injured. This can be accomplished most easily and reproducibly by means of the suction electrodes (1–6). It is desirable to compare potentials obtained with intracellular electrodes to those recorded simultaneously with suction electrodes and to determine to what extent the latter actually represent variations of potential across the cell membrane. To our knowledge such a comparison has not been made until the present.

Methods

Isolated Papillary Muscles

Long thin papillary muscles isolated from the right ventricle of the cat heart were perfused with Tyrode’s solution equilibrated with 95 % O₂ and 5 % CO₂ and maintained at 37°C. Driving stimuli were applied to one end of the muscle. Microelectrodes were pulled to a tip diameter of less than 1 micron and filled with 3 M KCl by boiling. Suction electrodes were similar to those described below. Both transmembrane potentials and monophasic action potentials from the suction electrodes were recorded against ground with identical cathode followers and d.c. amplifiers set at the same sensitivity. Records were photographed on paper from a switched-beam Tektronix oscilloscope. Voltage calibration was accomplished by injecting a known voltage between the tissue bath and ground.

Perfused Rabbit Hearts

Isolated rabbit hearts were perfused with oxygenated Krebs-Henseleit solution at 37°C according to the method of Grumbach (7). The suction electrode consisted of a long spiral polyethylene tube with an internal diameter of 0.5 mm containing a silver wire extending within 1 mm of its tip. The tube was filled with the perfusion solution, placed adjacent to the ventricular surface and connected to a pump which fixed it to the myocardium by the suction developed. The flexibly mounted intracellular electrodes were of the type described by Woodbury and Brady (8) and followed the movements of the heart without causing undue injury to the cell membrane. The heart was partly immobilized at the apex by means of a subepicardial suture. Simultaneously with the two monophasic curves, an electrogram was registered, using a wick electrode placed near the A-V border of the ventricle. Unipolar leads from the three electrodes were paired with a distant electrode.
Amplitude. It has always been recognized that any form of extracellular recording detects only a part of the transmembrane potential because of shunting. The monophasic injury potential recorded with suction electrodes has been reported to be about 50 mv in turtle ventricle and about 75 mv in dog ventricle (6). The zero reference potential. B and C, slow development of a monophasic potential after application of suction (arrow in B). D, a small monophasic action potential persists for a few beats after release of suction (arrow).

**Fig. 2.** Records of monophasic action potentials and microelectrode action potentials superimposed to show their similarity. In A and B the gain has been increased on the record of the suction electrode potential to make the recorded amplitudes equal. In A the early part of the first record (arrow) shows superimposed suction and microelectrode potentials. The second record is from the suction electrode alone and the third trace is from the microelectrode alone. In B, the entire record shows two superimposed traces. C and D show a comparison of time of arrival of activity at the suction electrode (smaller amplitude) and the microelectrode. In C the microelectrode is adjacent to the drive site and the suction electrode is 1 mm distant. In D the suction electrode is in the same place as in A and the microelectrode is 8 mm away from the drive site.

**Papillary Muscle**

Amplitude. It has always been recognized that any form of extracellular recording detects only a part of the transmembrane potential because of shunting. The monophasic injury potential recorded with suction electrodes has been reported to be about 50 mv in turtle ventricle and about 75 mv in dog ventricle (6). The corresponding values obtained with transmembrane recording are 110 mv both for turtle ventricle and for dog ventricle (4). Figure 1 shows the result of measuring the amplitude of the two potentials at closely adjacent sites on a cat papillary muscle. It will be seen that the suction electrode potential is about 23.5 mv and the transmembrane potential is about 112 mv.

Reversal. Great theoretical significance is attached to the extent to which the membrane potential reverses during the action potential. When suction electrodes are used in conjunction with d.c. recording it is possible to observe that the action potential becomes positive with respect to the potential of resting uninjured muscle. It is of interest to know whether this positivity has the same significance as that seen in transmembrane recording. Figure 1A shows a series of action potentials recorded with an intracellular microelectrode.

It is of interest to know whether this positivity has the same significance as that seen in transmembrane recording. Figure 1A shows a series of action potentials recorded with an intracellular microelectrode. It will be noticed that the amplitude of the resting potential is 95 mv, the action potential 112 mv and the reversal 17 mv. In the suction electrode records, however, the ratio of reversal to total amplitude is very much larger (fig. 1C, D). It is interesting to note that the resting potential appears as soon as the suction is applied and remains fairly constant thereafter. The action potential, however, grows during the course of many beats. In our records the resting potential is about 8.5 mv and the reversed phase is about 15 mv. This agrees with earlier reports for dog ventricle in which a resting potential of 26.1 mv and a positive phase of 31.1 mv were reported (9) and for turtle ventricle in which a resting potential of 21.35 mv and a positive phase of 30.25 mv were reported (6). It is apparent that neither the absolute magnitude nor the relative magnitudes of the resting potentials and reversals recorded with suction electrodes are the same as those recorded with an intracellular microelectrode.

**Shape.** A general similarity in the shape of the action potentials recorded by the two methods is obvious. This similarity is illustrated in figure 1A and B in which the gain has been adjusted so that the action potentials are of equal apparent amplitude. It will be seen that there is a rather close agreement in the course of the entire recovery process and that the major difference is found in the steepness of the upstroke. The upstrokes are shown on a faster sweep in figure 2C and D. It is quite obvious that the rising phase of the suction electrode record is much slower than that obtained with the microelectrode.

**Site of the origin of the potential.** When allowance is made for the difference in rate of depolarization it can...
readily be seen that the appearance of depolarization in the suction electrode records corresponds in time with that seen in the microelectrode records. In figure 2C a record is shown in which the microelectrode is very close to the drive electrode and the suction electrode is about 1 mm away from it. Only a slight difference in conduction latency is apparent. When, in figure 2D, the microelectrode is moved about 8 mm away from the drive electrode and the suction electrode is unchanged in position the upstrokes are much more widely separated in time. It appears therefore that the rising phase of the monophasic action potential recorded by the suction electrode is associated with the onset of activity in the near vicinity of that electrode.

Rabbit Heart

The capillary microelectrode was assumed to register true transmembrane potentials when the resting potential was at least 80 mV, the base line was steady and the configuration of the action potential remained identical for at least 10 consecutive beats. Under these conditions the amplitude of the action potential of the rabbit ventricle was approximately 110 mV. With a properly applied suction electrode the amplitude of the action potential ranged from 20 to 50 mV, the base line was stable and the configuration of the action potential remained identical for many minutes. After approximately 3-5 minutes the amplitude of the action potential recorded by the suction electrode began to fall but its configuration remained unchanged. The amplitude usually decreased by about 50% during the following 20-30 minutes (fig. 3A).

The rapid ascent of the intracellular action potential was usually preceded by a slower component, 1 mV or less in amplitude, appearing as a step or as a dip. When the intracellular microelectrode left the cell the step was transformed into the ascending branch of an R wave (fig. 4B). The ascent of the action potential registered with the suction electrode also showed a slow initial step or dip which was synchronous with that appearing in the intracellular curve. A dip appeared usually when an electrocardiogram registered before suction was applied showed a Q wave, while a step appeared when this curve began with an R wave. In ventricular extrasystoles the dip may disappear in both the intracellular potential and the curve registered with the suction electrode (fig. 3B).

The ascent of the action potential was usually followed by a brief rapid descent ('spike') of 1-5 mV, coinciding with the descending branch of the R wave in the synchronously registered electrocardiogram. While a spike was present in nearly all intracellular curves, it was not constant in the suction curve, and whenever it was present, it was usually smaller than in the intracellular curve (figs. 3 and 4). In ventricular extrasystoles the spike may disappear (fig. 3B).

The duration and configuration of the descending branch of the action potential was nearly identical in the suction and the intracellular record except that in the suction record a 'negative afterpotential,' i.e. persisting depolarization, was usually seen at the end of the action potential. The amplitude of this afterpotential ranged from one-eighth to one-fifth of the total amplitude of the action potential; it was greater after long diastolic pauses (figs. 3D, 4B). The duration of this afterpotential was usually approximately equal to that of the action potential proper. The afterpotential usually coincided with the descending branch of the synchronously registered intraventricular pressure curve. When proper application of the suction electrode resulted in an action potential of high amplitude, the afterpotential was usually negligible and the action potential was identical in shape with the transmembrane action potential (fig. 4A). At other times both the transmembrane and the suction electrode curve had negative afterpotentials (fig. 3F) or the afterpotentials had opposite polarity in the two curves (fig. 3E). On many occasions afterpotentials appeared in the transmembrane curve shortly before the capillary electrode worked itself out of the cell, as indicated by decrease in voltage and appearance of an unsteady base line.

In the experiment illustrated in figure 4A, both the intracellular and the suction electrode potentials remained practically identical for about 2 minutes. Suddenly an afterpotential appeared in the suction electrode curve; this was accompanied by a sudden decrease in the amplitude of the action potential. This
The principle of the suction electrode is that myocardial cells are drawn forcibly into the tip of a tube; through the combined effect of pressure and the resulting ischemia the membrane of those cells are probably partially depolarized and made nonexcitable. The electrode in contact with these cells registers the potential across the membranes of the normal portion of the myocardium immediately adjacent to the opening of the electrode, reduced by the short-circuiting effect of the extracellular fluid. Because of this effect, the action potential registered even immediately after application of suction can be at best only a fraction of the true transmembrane potential difference (1, 9-11). In our experiments it was about 40% of this difference, while in skeletal muscle, which has a considerably larger cell diameter in relation to the intracellular spaces, this fraction was found to be about 60% (12). In addition to this short-circuiting effect, another factor tends to reduce the voltage of the action potential as time goes on; this is the gradual depolarization of the normal cell membranes in the vicinity of the suction electrode perhaps due to flow of injury current.

The above results suggest that monophasic action potentials recorded from cardiac muscle with suction electrodes may be used to indicate the course of repolarization with reasonable accuracy. Since little information about this phase of the electrical activity is obtained by conventional external electrodes there is an obvious advantage in the use of the suction electrode.

However, it is well to establish by a preliminary control experiment that the potential under study is similar to that recorded with the intracellular microelectrode. The major source of artifact with either method results from electrode movement. This is especially great in records from the isolated, perfused heart, since in this case the heart is suspended from a fixed aortic cannula and any movement of the heart during contraction is communicated in its entirety to the suction or intracellular electrode. It is highly probable that the negative afterpotential registered under these conditions is caused by relaxation of the ventricle during early diastole, which leads to partial withdrawal of the microelectrode from the cell or of the suction electrode from depolarized tissue (the afterpotentials are not caused by electrode polarization, since they were not changed when nonpolarizable electrodes were used (fig. 3C)).

Mechanical distortion of the glass capillary tip and grid current are other possible sources of the artifact. The afterpotential corresponds in its duration to the descending branch of the intraventricular pressure curve, and becomes higher after long postextrasystolic pauses when the force of contraction is potentiated and disappears when contraction becomes very weak under the influence of calcium-free solutions (13). It is possible that the rapid initial portion of the negative afterpotentials observed in monophasic leads from the isolated, perfused frog ventricle (14) was also caused by mechanical effects.

The records obtained with a suction electrode are not a reliable indication of the rate of depolarization of a single cardiac cell. This results from the fact that the electrodes are large and must record from many cells the activity of which is somewhat dispersed in time. If one estimates conduction velocity from figure 2, it may be calculated that the wave of depolarization requires 2 msec. to pass an electrode of 0.5 mm diameter. This may be compared with the rise time of 5 msec. observed with the suction electrode of the diameter stated.

The amplitude of the monophasic potential recorded with the suction electrode is less than that of the transmembrane potential. While there is presumably some relationship between amplitudes of the two records which depends upon the diameter and passive electrical properties of the fiber, there seems to be little point in using the suction electrode to estimate the absolute value of transmembrane potentials. It is, however, interesting to note that the use of a complicated and difficult method of analyzing the potentials obtained with a suction electrode yielded a value of 97 mv for the transmembrane potential of turtle ventricle (15) which is comparable to the value of 110 mv obtained with microelectrodes.

The reversal or overshoot of potentials during ac-
tivity is greater with suction electrodes than with transmembrane recording. Not only is the reversal greater as a percentage of the total potential but also reversed potentials of 48 mv have been recorded with suction electrodes (9). It is possible that the fibers under the suction electrode remain partially depolarized throughout the entire cardiac cycle and that the disproportion between the reversal and resting potential results from this fact (16); alternatively the presence of a group of fibers which fail to become activated during systole may distort the relationship between the reversal and the resting potential (1, 15). The value of the reversal recorded by suction electrodes is greater than any recorded with transmembrane electrodes from ventricular muscle. It is apparent that an evaluation of the amount of reversal must not be based upon the suction electrode technique.

It is interesting to note that the beginning of the upstroke in the electrical record obtained with a suction electrode corresponds closely with the arrival of activity in the vicinity of that electrode as indicated by an adjacent intracellular microelectrode. This appears to be true both when the suction electrode is used on a strip and also when it is used on the whole heart. In the latter case, however, the contribution of activity at more remote sites may be greater unless recording is differential. It will be noted that many of the monophasic records, particularly those obtained with the suction electrode, show deflections resembling QRS waves superimposed upon the monophasic tracings. This, of course, results from the fact that any pair of electrodes placed on or near the heart will detect the potential difference associated with the activation of the entire heart. Obviously this difference of potential will be recorded even if one electrode is intracellular or in contact with injured tissue. If the two electrodes are very close together such deflections will be minimized. Records obtained with the so-called ‘micro’ suction electrode (17) in which a small suction electrode is used very near to an indifferent electrode provide a monophasic record which undoubtedly is an accurate index of the arrival of activity at the electrode pair.

REFERENCES