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. 195(6): 1287-1301. 1958.—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.

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 Grunbusch (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.
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Papillary Muscle

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are used in conjunction with d.c. recording it is possible
situated at the edge of the perfusion chamber, and rec-
ordered on a four-channel Sanborn 'Poly-Viso' at a
paper speed of 50 mm/sec. The sensitivity was adjusted
so that the records obtained by the microelectrode and
section electrode were of equal amplitude.

RESULTS

Records in which the monophasic action potential
obtained with a suction electrode are compared with the
transmembrane action potential recorded with an intra-
cellular microelectrode have been obtained from the
excised cat papillary muscle and from the in situ rabbit
ventricle. The records obtained from the papillary
muscle are discussed first since they permit a more rigor-
ous analysis of the similarities and differences between
records obtained by the two methods in terms of shape,
amplitude, overshoot and site of origin of the potentials.

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 elec-
trodes 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 record-
ing. 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
carcin 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 ven-
tricle in which a resting potential of 21.33 mv and a posi-
tive phase of 90.25 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 1C 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 began with an R wave. In ventricular extrasystoles the dip was present in nearly all intracellular curves, it was usually negligible and the action potential was identical with the descending branch of the synchronously registered intraventricular pressure curve. When proper application of the suction electrode resulted in an action potential proper. The afterpotential was 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. 4C). At other times both the transmembrane and the suction electrode records showed negative afterpotentials throughout; the intracellular tracing (lower curve) shows negative afterpotentials in both curves, synchronous with U waves in the electrocardiogram (ECG). D–F, the suction electrode tracing (lower curve, reversed polarity) shows negative afterpotentials throughout; the intracellular tracing (upper curve) shows no afterpotentials in D, negative afterpotentials in E and positive afterpotentials in F. The time lines in this and the following figures are 0.1 and 0.02 sec.

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. 4C). At other times both the transmembrane and the suction electrode records showed 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
Fig. 4. Simultaneous tracings registered with the intracellular electrode (T) and the suction electrode (S, reversed polarity), in the perfused rabbit heart. At the arrow the amplitude of the suction action potential suddenly decreases and negative afterpotentials become more pronounced, while the intracellular potential shows no change. B, same as A. At the arrow the intracellular electrode left the cell.

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, 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 40 mV have been recorded with suction electrodes. 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 the resting potential results from this fact. 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. 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, 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