Intrinsic Deflections, Local Excitation and Transmembrane Action Potentials

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The steepest rise of the action potential obtained by an ultramicroelectrode from the most superficial subepicardial muscle fiber in situ of the tortoise was compared with the intrinsic deflection of the unipolar direct electrocardiogram and the surface electrocardiogram obtained from approximately the same region. No specific point on the intrinsic deflection, such as the highest or lowest point, could be regarded as indicating the arrival of excitation. Surface electrocardiograms obtained by the capillary ultramicroelectrode could not be regarded as simple second derivatives of the action potential, they resembled more unipolar direct electrocardiograms.

WHEN direct electrocardiograms are used to study the propagation of the excitation wave in the heart, investigators are confronted with the problem of what part of the deflection should be regarded as indicating the arrival of excitation beneath the exploring electrode. Lewis termed the deflections which result from the excitation process immediately beneath the contacts as intrinsic, whereas those which are yielded by the activation of the muscle lying at a distance from the actual contact points as extrinsic. Actually Lewis and most of the investigators thereafter adopted the summit of R as the indication of the arrival time of the excitation wave in the interpretation of their data. Others, following the practice of Wilson, regard as intrinsic the deflection which ends either at the apex of the S wave or at the base line. The reason for choosing a definite point was that the time for the intrinsic deflection was regarded as almost instantaneous. According to Sodi-Pallares, however, this interval lasts from 0.01 to 0.04 second in dogs, and much longer in cold-blooded animals, as our data will show. A few attempts have also been made to determine the proper point on unipolar leads which indicates local excitation, by making comparison with adjoining bipolar leads; but different conclusions were reached. Sodi-Pallares found that the time of arrival of activation corresponded to the lowest part of the intrinsic deflection or its lower third and Toyoshima concluded that it lay somewhere during the intrinsic deflection, but that it could not be defined as a specific point. In view of these controversies and general beliefs still existent, the following research was undertaken, employing the intracellular microelectrodes of Ling and Gerard.

METHOD

A search of the literature failed to disclose previous application of the microelectrode technic to tortoise hearts. Accordingly we measured the diameter of fibers and found that they ranged from 30μ to 80μ; in other words they were similar in size to those of other animals in which the technic has been used.

Glass capillary microelectrodes with an external tip diameter of less than 0.5μ were inserted into single ventricular and atrial muscle fibers in situ of tortoises by the use of a micromanipulator. The tortoise shell was removed, the heart was exposed and pressed by the method of Woodbury and associates, modified by the authors. The microelectrodes were filled with 3M KCl solution according to Nastuk and Hodgin. The indifferent electrode was a grounded silver plate which was dipped into 3M KCl solution and 3 per cent Ringer-agar contained in a small glass cylinder. This was placed on a convenient region of the body of the tortoise.

To record the steep rise of the action potential, a cathode follower preamplifier with a tube (1620) of small grid current (10^-11 amps.), a D-C amplifier and a cathode ray oscillograph (3-channel type using an electronic switch) were employed. With the application of capillary electrodes having resistance up to 40 MΩ, no recognizable distortion could be found with the sweep speed employed in this experiment.

Comparisons were made by the following two methods:

1. Unipolar direct electrocardiograms were ob-
tained by a metal plate ring with an external diameter of 3 mm. and an internal diameter of 2 mm. This ring with its support served also for the purpose of restraining the movement of the heart. The indifferent electrode was Wilson's central terminal. The microelectrodes were inserted into the cardiac fibers within the rings placed on each region of the heart. The action potential was obtained from several regions inside the ring and also under the ring by shifting it slightly.

2. Two microelectrodes were used. One was inserted into the cell and the other was placed outside the cell as close as possible to the first, usually 0.2 mm. or 0.3 mm. apart and at most within 0.5 mm. distance, to obtain the surface electrocardiograms. By both methods an attempt was made to record the action potential from the most superficial muscle fibers.

RESULTS

Just before the microelectrode was inserted into the cardiac fiber, an electrocardiogram was obtained which was called the surface electrocardiogram by Woodbury and associates. In this case the tip of the electrode is so small that this can be regarded as being outside of a single muscle fiber, even in the heart in situ. Cole and Curtis stated that with an isolated cell, the density of current flowing through the membrane is the second derivative of the curve of the action potential, supported by their experiments on the large cells of Nitzetella and the giant axon of the squid. Churney and associates showed by experimentation that this theory holds on a linear strip of turtle atrial muscle immersed in a volume conductor. If the surface electrocardiogram were actually the second derivative of the monophasic action potential even in the heart in situ, the former would be a clearer indication of the arrival of excitation than the latter. Since our preliminary experiments showed that the shape of the action potential was not so different from RS type as its second derivative, the surface electrocardiogram obtained by the capillary microelectrode was examined as to whether it always showed an RS type or not. At the same time, the unipolar direct electrocardiogram was taken by the ring and compared with it. Our result showed that the surface electrocardiograms were different in their contours depending on the electrode position on the heart. They resembled much more the unipolar direct electrocardiograms obtained from the same regions and imitated their variety. Representative examples, shown in figures 1 and 2, revealed that the surface electrocardiogram cannot be regarded just as the second derivative of the membrane action potential, leading us, therefore, to adopt the action potential itself.

The membrane potential of the tortoise cardiac fiber is essentially the same as that of the frog cardiac fiber as reported by Woodbury and associates and by Trautwein and associates. The values were discarded when the resistance of electrodes decreased, or their tips were found, by microscopic examination upon withdrawal, to be broken, and also when the values were less than 50 mv, as Woodbury and Trautwein did, in consideration of probable significant damage of the cell membrane. The mean value of 10 selected data of the tortoise ventricular fibers was 66 mv (54 to 86 mv) in
the resting potential, 76 mv (60 to 100 mv) in the action potential and 10 mv (1 to 36 mv) in the overshoot. The duration of the action potential varied from 0.6 to 1.4 seconds. The action potential of the atrial fibers is narrow in shape and of short duration. The plateau of the repolarization, characteristic of ventricular muscle, is lost to some extent and declines more rapidly. The mean value of 10 selected data was 56 mv (50 to 63 mv) in the resting potential, 65 mv (55 to 90 mv) in the action potential, 9 mv (2 to 30 mv) in the overshoot. Its duration varied from 0.4 to 0.7 seconds. No significant difference could be found between the data of the left and right atria. The inflection point of the rising limb of the membrane action potential was regarded as indicating the arrival time of the excitation wave at the muscle fiber in which the microelectrode is inserted. Actually the time of the steepest slope of the rising limb of the membrane action potential was used.

The duration of the intrinsic deflection in electrocardiograms of the tortoise ventricle ranged from 45 msec to 120 msec., whereas that of the whole rising limb of the action potential, i.e., the duration of the depolarization, ranged from 20 msec. to 40 msec. Therefore, although we may well regard the whole duration of the latter as the indication of arrival in the usual cases, we adopted the part of the steepest rise, the duration of which ranged only from 2 to 10 msec, and which is practically the same as the inflection point.

Representative examples are shown in figure 2. Record 1 was obtained from the cardiac apex, and the steepest rise of the action potential corresponded to the point slightly lower than the summit of the R spike of the intrinsic deflection. Record 2 was derived from the left side of the ventricle, and the steepest rise corresponded to the point slightly higher than the nadir of S in the intrinsic deflection. Record 3 was obtained from the middle point on the anterior surface of the ventricle, and here the intrinsic deflection was too steep to locate the exact corresponding point, but it almost corresponded to the peak of the R. Record 4 originated from the base of the ventricle near the left atrium, the steepest rise corresponding to the point slightly higher than the nadir of S.

With the same localization of the ring action potential was taken from fibers in several regions. By shifting the ring slightly the electrode was also inserted into the muscle fibers which had been beneath the ring. Depending on the muscle fibers, their steepest rise corresponded sometimes to slightly different points of the intrinsic deflection, but the distribution of the points was limited to so small a range that usually their corresponding points could still be represented by a point, compared with the duration of the intrinsic deflection. Such a mean point with each localization is summarized in figure 3. Therefore the arrival time should be interpreted as having a small width around points in this figure for the following reasons: (1) the arrival time may have some width, which will be discussed later, and (2) these points represent the mean points of the data with several muscles in each location. The conclusion follows that the arrival time may correspond to any point on the intrinsic deflection depending on the part of the heart studied; the steepest rise sometimes corresponded to the summit of the R spike, sometimes to the nadir.
FIG. 3. The time points of arrival of the excitation wave on the intrinsic deflection of unipolar electrocardiograms of the tortoise heart. Arrows indicate points corresponding to the steepest rise of the membrane action potential.

Fig. 4. Comparison of the membrane action potentials (lower tracings) with the surface electrocardiogram (upper tracings). Both were taken by capillary microelectrodes. These records were obtained from the following regions: 1 middle part of ventricle, 2 cardiac apex.

of the S, but often to any point between the two.

Since in the preliminary experiment the surface electrocardiogram obtained by a capillary microelectrode resembled the unipolar direct electrocardiogram rather than a pure second derivative of the action potential, another question came up—since the tip of the microelectrode is so small, can any specific point of the intrinsic deflection of the surface electrocardiogram be regarded as indicating the arrival of the excitation? This was examined by a second method, by placing the intracellular and extracellular microelectrodes as close together as possible by aid of a binocular microscope. The result was about the same as by the first method. The steepest rise of the action potential corresponded to any of the points on the intrinsic deflection of the surface electrocardiogram and no specific point on the latter could be regarded as an indication of the arrival of the excitation. Moreover, on rare occasions the steepest rise corresponded to a point outside the intrinsic deflection, i.e., to some of the points on the extrinsic deflection. Our representative examples are shown in figure 4.

**DISCUSSION**

The microelectrode inserted into the cell obtains local electrical changes, being least influenced by the electrical field outside the cell, and the depolarization phase of the membrane action potential can be considered to indicate the exact time point of the arrival of the excitation wave at that cell. Although it is usual that the duration of the depolarization of the membrane action potential is quite brief compared with that of the intrinsic deflection, the former is influenced, among other things, by temperature and external sodium concentration and, occasionally, the time width cannot be ignored. Therefore, in general, we adopted the inflection point of the rise according to Cole and Curtis. Davson explained this as follows: when the approaching disturbance has reached a certain distance from the microelectrode, the electrotic effects, spreading ahead of the active region, make the recording instrument inscribe the initial deflection, i.e., the foot of the action potential. At a certain point in time, when the active region has come sufficiently close, the region beneath the electrode is excited as the result of the excitatory influence of the electrotic potential, and at this point the rate of the rise of the action potential increases to a maximum. This point is the point of inflection on the rising phase of the action potential. Schaefer and Trautwein seem to reach the same opinion with the heart muscle. Propriety of this statement in regard to the heart muscle might be still argued, partly because these experiments of Cole, Schaefer and others were performed by taking the monophasic action potentials from outside the cells. However, which point of the rise should be regarded as indicating the local activation,
or whether or not the whole duration of the rising limb of the action potential should be regarded as such, did not influence our conclusion much. In many examples the whole duration of the rise could be regarded as a point, compared with the duration of the intrinsic deflection, and these points were still distributed through various points on the latter.

**Summary**

The steepest rise of the membrane action potential of the most superficial subepicardial muscle fiber obtained by the intracellular capillary microelectrode from the tortoise cardiac fibers in situ, was compared with the intrinsic deflection of the unipolar direct electrocardiogram and the surface electrocardiogram obtained from approximately the same region.

No specific point on the intrinsic deflection, such as the highest or lowest point, was found which could be regarded as indicating the arrival of the excitation wave at the subepicardial muscle beneath the exploring electrode.

Consequently in experiments in which the time difference between the highest and the lowest points on the intrinsic deflection cannot be ignored, unipolar direct leads are not a suitable technic. Experiments already performed should be re-examined by the microelectrode technic or the adjacent bipolar leads.

The surface electrocardiogram obtained by a capillary microelectrode cannot be regarded as a simple second derivative of the membrane action potential. It resembled more the usual unipolar direct electrocardiogram and varied in shape in different regions of the heart.

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**Summario in Interlingua**

Le plus acute ascendita del potential de action membranal in le plus superficial fibra del musculo subepicardial, obtenite per medio del microelectrodo capillari intracellular ab le fibras cardiac del tortuca, esseva comparate con le deflexiones intrinsec del electrocardiogramma directe unipolar e le electrocardiogramma superficial obtenite ab approximative-mente le mesme region.

Eesseva trovate nulle puncto specific in le deflexion intrinsec—sia le plus alte, sia le plus basse, etc.—qué poteva esser reguardate como indication del arrivata del unda de excitation al musculo subepicardial infra le electrodo exploratori.

Ergo, in experimentos in que le differentia temporal inter le plus alte e le plus basse punctos in le deflexion intrinsec debe esser prendite in consideration, derivationes directe unipolar non representu un technica appropriate. Experimentos jam executate per ille technica debe esser re-examine per medio del technica microelectrodic o con derivationes bipolar adja-cente.

Le electrocardiogramma superficial obtenite per un microelectrodo capillari non pote esser considerate como un simple secunde derivato ab le potential de action membranal. Ilo resimilava plus tosto le usual electrocardiogramma directe unipolar e variava in su contornos in diferente regions del corde.

**References**

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