Ventricular Fibrillation Studied by the Microelectrode Method

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Capillary ultramicroelectrodes were inserted into ventricular muscle fibers of dogs in ventricular fibrillation and action potentials obtained were compared with deflections of unipolar direct lead, adjoining bipolar direct lead or other electrocardiograms taken simultaneously. In most stages of ventricular fibrillation neither synchronism nor any other regular time relationship could be found between them. Microelectrodes inserted at two points variously distant on various regions of the ventricular surface also failed to show any regular time relationship. Total incoordination of ventricular muscle fibers was thus supported.

There are still divergent views as to the nature of ventricular fibrillation. The theory of incoordinate continuous activity has been deduced heretofore from experiments by electrocardiographic, cinematographic or mechanical methods. The electrocardiogram is a manifestation of the electric field, therefore even the direct leads cannot clarify the electric activity of individual ventricular muscle fibers, especially since each of them acts differently in fibrillation. Cinematographic study enables us to follow the progression of gross contraction waves, but cannot define the movements of individual muscle units. It also has the disadvantage of creating visual illusions of apparent mechanical contraction waves which might be different from real excitation waves. Simple mechanical devices are affected by adjacent and underlying muscle fibers and cannot be relied upon. The use of capillary ultramicroelectrodes enables one to clarify the electric activity of single cells in situ without the need of their isolation. The purpose of this research was to re-examine the nature of ventricular fibrillation, applying this method, and to re-evaluate the correctness of the conclusions of authors using older methods.

Methods

Adult dogs were anesthetized by intravenous injection of thiopental sodium. Respiration was maintained by intermittent positive pressure. The heart was exposed by removing the sternum and opening the pericardium. Ventricular fibrillation was evoked by electric stimulation of the ventricle by means of an inductorium or by subepicardial injection of 0.5 ml. of a 0.05 per cent solution of acenitine crystals. Sometimes fibrillation occurred spontaneously after other experiments.

Glass capillary microelectrodes with an external tip diameter of about 0.5 to 1.0 μ were inserted into fibrillating single ventricular muscle fibers in situ by the use of a micromanipulator. No fixation or compression of the heart was used, but microelectrodes were held by a spiral spring made of insulated wire so that inserted microelectrodes floated freely as the heart moved. Insertion and retraction of the electrodes were made possible by this spiral spring. Occasionally two microelectrodes were inserted almost simultaneously into two ventricular muscle fibers at different locations. Penetration of the microelectrode tips into the cells was judged by the shift of the baseline. Although the configuration of the membrane action potentials were not so characteristically different from their counterparts outside the cell in ventricular fibrillation as they were in the normal state, it was still helpful in determining their position. Whether their tips were broken by insertion or not was checked by measuring their electric resistance and by examining with a microscope before and after insertion. Only data obtained by unbroken microelectrodes were used. Other details concerning the method of microelectrode experiments are described elsewhere.

Unipolar direct leads were obtained by thin cotton-wool strips, saturated with isotonic saline solution and attached to the tip of the lead wire. The Wilson central terminal was used for the indifferent electrode of this lead. The method of adjoining bipolar direct lead was similar to that described by Sodi-Pallares, which employs the principle of the differential electrogram by Garten.
and Clement, but is closer to the method of Harris. The separation of tips of its two electrodes was 1 to 3 mm.

To record the action potential obtained by the capillary electrodes a cathode follower preamplifier with a tube (1620) of small grid current ($10^{-6}$ a.), a DC amplifier and a cathode ray oscillograph (3-channel type using an electronic switch) were employed. To record electrocardiograms a C-R preamplifier was connected with the above mentioned DC amplifier and the cathode ray oscillograph. The time constant in this case was 2.3 sec.

**RESULTS**

Due to the technical reason that capillary electrodes broke easily even with spring suspensions when movement of the heart was vigorous, the results were limited to ventricular fibrillation in the stages of tremulous incoordination and to atonic fibrillation according to Wiggers' classification, and could not extend to ventricular flutter except in a few instances.

*Transmembrane Potentials of Ventricular Muscle Fibers in Ventricular Fibrillation.* The magnitude of action potentials, resting potentials, and overshoot of ventricular muscle fibers decreased in ventricular fibrillation as was reported by other authors. The range of their magnitude was quite wide, since ventricular fibrillation includes various stages between ventricular flutter and complete death of the heart. The maximum value of the resting potential in our experiments was 75 mv., which was smaller than the normal value of about 90 mv. Fifteen millivolts was the minimum value considered to be obtained intracellularly. Smaller values existed, but could not be differentiated from those taken extracellularly because of relative similarity of configuration of action potential recorded inside the cell to its counterpart outside the cell and because of occasional inconstancy of the base line in ventricular fibrillation, although in normal condition the characteristic shape of action potential and the position of the base line are criteria by which to ascertain that the tip of the microelectrode is inside the cell. In the typical case, at the beginning of fibrillation the magnitude of action potential, resting potential and overshoot was large and became smaller toward the terminal phase, but this was not always found. It was occasionally experienced that at the terminal stage a large resting potential with large typically shaped action potential was recorded from one cell when scarcely any action potential was obtained from the other cell (fig. 1). Action potentials varied widely in sizes and frequencies. Average frequencies ranged from 83 to 500/min. The smaller values were due to the fact that occasional action potentials appeared much less frequently than deflections of the electrocardiogram (fig. 3C). The appearance of the action potential was completely irregular in time, and its size and shape varied from beat to beat. Sometimes action potentials of similar sizes and shapes appeared almost regularly in time, but they were often supplanted by one or a few deflections of small, incomplete and irregular shape like premature beats (fig. 1); therefore, its magnitude ranged widely from 5 to 90 mv. Its rising phase lost its
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steepness more or less and its downstroke was quicker, losing the spike and plateau shape to some extent. The duration of action potentials became irregularly shortened. Frequently no overshoot could be found, and often action potentials were smaller than resting potentials. Even in fibrillation the isoelectric period at the level of resting potential was present in the majority of cases.

Action Potentials and Electrocardiograms. During the very early phase of ventricular

Fig. 2. Action potentials of ventricular muscle fibers in situ in the early stages of ventricular fibrillation (upper tracings) and the lead II electrocardiograms (lower tracings). Microelectrode inserted at middle point of anterior right ventricular surface. Note: synchronism exists in the very early stage of fibrillation (A and initial part of B), but not later (the rest of B and C).

Fig. 3. The time relation between action potentials and deflections of direct electrograms in ventricular fibrillation. A. Action potentials (upper tracings) and unipolar direct lead electrograms (middle tracings) obtained from the apex on the anterior surface of the right ventricle. B. Action potentials (upper tracings) and adjoining bipolar direct lead electrograms (lower tracings) obtained from a point close to the anterior descendens artery. C. Action potentials (upper tracings), lead II (middle tracings) and the bipolar direct leads between both atria (lower tracings). Action potentials were obtained from a point close to the anterior descendens artery. Note: neither synchronism nor any other regular time relation exists between action potentials and deflections of electrocardiograms. In C, even after exclusion of deflections of the middle tracings corresponding to atrial complexes of the lower tracings, if any, such relation cannot be found.
fibrillation when differentiation from ventricular flutter is difficult, action potentials of ventricular cells showed synchronism with deflections of limb lead electrocardiograms and unipolar direct lead electrograms taken from points as close as possible to the inserted points of the microelectrodes. The action potentials during this phase were large, and of almost normal configuration. Sometimes action potentials with slower depolarization upstrokes alternated with or intervened among those with more normal configurations (fig. 2). After the initial phase, throughout almost all records of ventricular fibrillation, neither synchronism nor any other regular time relationship could be found between cellular action potentials and deflections of electrocardiograms from limb leads nor unipolar direct leads obtained as close as possible to the microelectrodes, at most 1 mm. distant from them (fig. 3A). This was true even with adjoining bipolar direct leads taken similarly as close as possible to the microelectrodes (fig. 3B). Temporarily, with some of these leads, action potentials seemed to show synchronism, which lasted only for a brief duration and disappeared after a few seconds. Such occurrences undoubtedly were fortuitous. In order to exclude the possibility that atrial complexes intermingled with fibrillated ventricular complexes in electrocardiograms might obscure some regular time relationship, bipolar direct leads between the atria were recorded simultaneously with limb lead electrocardiograms and action potentials of ventricular cells (fig. 3C). The fact that no time correspondence could be found between deflections of intracellular records and those from adjoining bipolar direct leads seem sufficient to conclude that ventricular electric units must be divided into very small fractions in ventricular fibrillation. To seek further evidence for the conclusion, experiments were performed in which multiple pairs of cellular potentials were recorded.

Comparison of Two Action Potentials Obtained from Different Ventricular Cells in Ventricular Fibrillation. Two microelectrodes were inserted into two different ventricular cells at distances varying from less than 1 cm. to points on different ventricles. Figure 4 shows typical recordings from electrodes 10 and 20 mm. apart. No synchronism nor any other regular time relationship could be found between these two action potentials or between any of them and the deflections of electrocardiograms regardless of whether ventricular fibrillation was evoked by electric stimulation, by aconitine injection or by any other method. On a few occasions the two action potentials seemed to show sporadic synchronism for brief periods but even in this period the electrocardiographic deflections were asynchronous with them.

DISCUSSION
It has been usual practice that, when the deflections of two or several unipolar direct lead electrograms taken at various points on the surface of the heart showed no synchronism in fibrillation, complete dissociation of
cardiac muscle fibers was concluded. But this is not necessarily so. For instance, if the majority of cardiac muscle fibers showed weak but regular, coordinated electric activity and numerous small islands of strong but complete irregular activity were scattered throughout the heart, the same chaotic electrograms would be obtained. Such a state would also fail to be differentiated from complete dissociation by high speed cinematography. To establish cellular relationships affirmatively, it is necessary to examine the simultaneous activities of as many pairs or combinations of as many cells as possible, as was done in these experiments.

The results obtained show that each ventricular fraction excites independently and incoordinately in most stages of ventricular fibrillation and that these fractions must be very small. This incoordination, however, does not necessarily mean that an excitation wave does not proceed in any direction, as Wiggers properly pointed out. Of course our examination could not cover all the ventricular cells, although as many as possible were examined. Our examination was also limited to the superficial cells of the anterior surface of the ventricle. But it is most likely that this kind of state exists throughout the ventricle.

This study does not provide crucial evidence regarding the innate mechanism of the ventricular fibrillation. However, the synchronism of action potentials at its initial phase and occasionally in the other phases for brief periods suggest that multiple re-entries constantly changing their routes are most likely responsible at least for the maintenance of this condition. However, these findings are not incompatible with the multiple ectopic focal theory or a single circus movement theory. For example, if we consider that if many daughter waves emanate from a mother wave, each cardiac muscle fiber would be excited differently. Prinzmetal could not find any evidence of an isoelectric period in electrocardiograms of atrial fibrillation which is all-essential for the existence of continued movement. It is true that isoelectric periods were difficult to find in electrocardiograms in ventricular fibrillation also, but in intracellular recording such isoelectric periods at the level of the resting potential were mostly present. Therefore, circus movement theory cannot be excluded on this basis alone. However, theories that a single mother ring or focus is the cause of this condition were seriously challenged, for instance by Brans and Katz, who showed that flutter or fibrillation still continued after complete functional or actual separation of one atrium or ventricle from the other.

**Summary**

The capillary ultramicroelectrode method was applied to the ventricular muscle fibers of dogs in situ in ventricular fibrillation.

The resting potential, action potential and overshoot decreased to various extents in ventricular fibrillation. The magnitude and configuration of action potentials varied from beat to beat, and their frequencies were completely irregular. The rising limb of the action potential lost its steepness more or less and the descending limb often lost its spike and plateau.

Except in the initial phase and during a very brief period in the other phases of ventricular fibrillation, neither synchronism nor any other regular time relation could be found between action potentials and deflections of limb leads, direct unipolar leads or from adjoining direct bipolar leads taken as close as possible to the inserted points of microelectrodes. This held even after exclusion of atrial complexes from electrocardiograms. These findings show that the electrocardiogram cannot clarify the electric activity of single cardiac muscle fibers in fibrillation.

Two microelectrodes were inserted at points variously distant on various regions of ventricular surface. Simultaneous recording of the two action potentials with electrocardiograms revealed that neither synchronism nor any other regular time relation existed regardless of how ventricular fibrillation was evoked. These findings give sounder basis than previous works for the theory of total incoordination in ventricular fibrillation.
**SUMMARIO IN INTERLINGUA**

Le methodo a ultramieroelectrodos capillar esseva applicate al fibras ventriculo-muscular in sito in canes in stato de fibrillation ventricular.

Le potential de reposo, le potential de action, e le supercompensation se monstrava variemente reducite in fibrillation ventricular. Le magnitude e le configuration del potentialles de action variava ab un pulso al altere, e le frequentias de illos esseva completamente irregular. Le branca ascendente del potential de action perdeva su tendentia vertical plus o minus pronunciatemente, e in multe casos le branca descendente perdeva su spica e su plateau.

Exepte in le phase initial e excepte brevisimemente durante le phases subsequent de del fibrillation ventricular, nulle synchronismo e nulle altere regularitate in le interrelation temporal esseva constatabile inter le potentialles de action e le deflexiones in electrocardiogrammas ab derivationes de extremitate, ab derivationes unipolar directe, e ab adjacente derivationes bipolar directe que esseva facite in sitos le plus proxime possible al punctes inserite del microelectrodos. Isto valeva mesmo post le exclusion de complexos atrial ab le electrocardiogrammas. Iste constatationes demonstra que le electrocardiogramma es incapace a clarificar le activitate electric de unic fibras del musculo cardiac in fibrillation.

Due microelectrodos esseva inserite con variye distantias inter le un e le altere in varie regiones del superficie ventricular. Le simultane registration electrocardiographic del duo potentialles de action revelava nulle synchronismo e nulle altere correlation temporal inter illos, sin regardo a como le fibrillation habeva essite inducite.

Iste constatationes provide un plus solidc base que previe investigaciones pro le theorica del incoordination total in fibrillation ventricular.

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