The Two Components of the Human Atrial Action Potential

By Alexandre Fabiato and Françoise Fabiato

ABSTRACT

Action potentials studied in 36 human atrial strips at 27°C were found to be separated into two components. Simultaneous recordings with two microelectrodes demonstrated an independent conduction of the second component through selective and variable pathways. Increased separation of the two components was elicited by higher rate and lower intensity of stimulation, high K⁺, low Ca²⁺, hypertonic solutions as well as by low temperature. Opposite procedures resulted in a more homogeneous and less variable excitation. Consequently, low rate and high intensity of stimulation allowed the study of the membrane permeabilities related to the two successive depolarizations. The first was suppressed by tetrodotoxin, the second by MnCl₂, and both by low Na⁺ solutions. Hence, it was concluded that the separation was related to a nonhomogeneous excitation of the preparation and that the two components are triggered by two relatively independent depolarizations using different channels. Furthermore, the small amplitude of the first depolarization, without overshoot, and its modifications with stimulation intensity suggest that it might be due to an electrotonic spread or to a junctional mechanism.

KEY WORDS: cardiac electrophysiology, electrotonus in the heart, membrane permeabilities, functional syncytium, conduction, junctional mechanism.

Sleator and de Gubareff (1, 2) have studied the effects of lowering temperature upon electrical and mechanical activities of the human atrial muscle. They observed an action potential (AP) with two successive depolarizations, accompanied by two mechanical components, in response to a single stimulus and interpreted this phenomenon as a reflection of intrinsic properties of cell membrane specific for man and chimpanzee. Alternatively, lowering membrane potential by hypothermia may produce conduction disturbances which could help to explain this phenomenon. The aims of the present study were to assess the degree of homogeneity of excitation in the preparation and to define the membrane permeabilities underlying the two components of the AP. Preliminary results on the latter point were reported in an abstract (3).

Methods

A strip of atrial muscle was taken from each of 57 patients at operation for repair of congenital cardiac defects. Of these, 36 were selected for the present study according to the following criteria: (1) the absence of recent use of digitalis or antiarrhythmic drug prior to surgery, (2) patients less than 15 years old, to diminish the likelihood of fibrous tissue (preventing proper placement of the microelectrode), and (3) absence of automaticity of the preparation. In most cases, the sample was taken from the apex of the right atrial appendage. Immediately after excision, it was placed in an oxygenated Krebs-Ringer solution at about 27°C and taken to the laboratory. As far as possible, it was cut into strips with parallel bundles. The strips were approximately 5–10 mm long, 1–3 mm wide, and a few tenths of a millimeter thick.

The preparation, horizontal in the bath, was connected by two silk threads to a strain gauge force transducer and to a micromanipulator.
allowing adjustment of length. The mechanical recording was used as an index of the activity in the whole preparation and was correlated with localized electrical recordings (APs). Action potentials were obtained on the subendocardial surface by “floating” microelectrodes (filled with 3M KCl; resistance, 10–30 megohms) connected to a negative capacitance preamplifier. Two independent microelectrodes were used for simultaneous recordings, and zero potential was determined before and after every recording. In lengthy recordings, zero might drift, and when the difference between the two determinations was less than 10 mv, an average was used. The recordings were displayed on a 502A or 565 Tektronix oscilloscope. Stimulation was generally applied through a pair of platinum insulated wires 0.5 mm apart (localized stimulation). A “massive stimulation” was sometimes applied through two large platinum plates (15 mm long, 5 mm wide) placed on either side of the preparation, 5 mm away. Intensity of stimulation was measured by placing a known resistance in the circuit.

The 5-ml bath contained a solution with a flow rate of 4 ml/min. The components of the “normal” medium were, in mm: NaCl 131; KCl 4.5; CaCl₂ 2.16; MgCl₂ 0.25; NaHCO₃ 11; NaH₂PO₄ 0.6; dextrose 11.0. This medium was saturated by a mixture of 97% O₂ and 3% CO₂; pH was 7.4 ± 0.1. Temperature was maintained at 27°C throughout in all but five experiments, in which it was varied between 37 and 24°C. The concentrations of NaCl, KCl, and CaCl₂ were varied as indicated in the section on results. When NaCl was reduced, the osmolarity of the solution was maintained with sucrose or choline chloride. Tetrodotoxin was injected into the bath (0.8–3.2 ‰g/ml). Manganese chloride (MnCl₂) was injected (1–4 mm) in a tris-HCl buffered solution deprived of NaH₂PO₄ and NaHCO₃. In three experiments, a beta-receptor-blocking agent, similar to propranolol was used (butiridine, Simes International, 10⁻⁶M).

**Results**

**HUMAN ATRIAL ACTION POTENTIAL AT LOW TEMPERATURE**

Lowering temperature in five experiments resulted in a decrease of the resting potential, accompanied by modifications similar to those previously described (1, 2). At about 32°C a notch appeared between spike and plateau and the amplitude of the spike decreased. At about 27°C, this notch was widened into two clearly separate components with eventual disappearance of the second one. At 25°C, multiple component APs were recorded. Thus, the most typical double component APs were obtained at 27°C, and this temperature was chosen for study.

The features of the electrical activity at 27°C appeared quite variable. Some APs were reduced to a first component (Fig. 1A, first tracing), while others exhibited two components (second tracing). Frequent transitions between these two types of APs were observed (Fig. 1B). The resting potential was −60 to −80mv; the peak of the first depolarization remained below the zero potential (−10 to −30 mv). High speed recordings demonstrated very important variations in the configuration of the first depolarization (Fig. 1C), which was either (1) S-shaped, with a maximal slope over 100 mv/msec, for a stimulation frequency of 0.5 Hz or (2) asymmetrical and exhibiting two or several steps. The plateau following this initial spike was very low (−40 to −60 mv) and of

![FIGURE 1](http://circres.ahajournals.org/)

Typical aspects of electrical and mechanical activity at 27°C. A: Top trace, action potential (AP); bottom trace, contractile force. Recordings in the same cell with 6-second interruption (this resting potential was one of the largest observed). B: Superimposition of two successive APs demonstrating transition between presence and absence of a second depolarization. C: Initial spike in two cells on the same preparation. D: Wenckebachike phenomenon. Horizontal scales: solid-line scale = 1 second; broken-line scale = 50 msec. Vertical scales on the right = 100 mv (zero not designated when the upper limit of the scale was its average value; see text for technique of determination).
Atypical aspects. A, B, C: Top trace, AP; bottom trace, force; scales as in Figure 1. A: Large initial spike and small second depolarization (absent in the third response). B: Second depolarization starting from a variable potential level. C: Very low first component. D: Two APs recorded simultaneously in two bundles of the preparation; interelectrode distance, 3 mm; AP a recorded in a site proximal to stimulation site, b, in a more distal point. In a, there is only one component similar in shape and timing to the second component of b.

Figure 2

Double-component APs were characterized by a second depolarization arising from the plateau of the first component. This second depolarization demonstrated an overshoot of 5-10 mV, and its whole amplitude was generally greater than that of the initial spike, while its maximal slope was considerably slower (5-20 mV/msec for a stimulation frequency of 0.5 Hz). These two electrical components were accompanied by two mechanical events, the first of which was generally of greater amplitude. Potentiation (Fig. 1A) and summation (Fig. 1D) were observed. The interval between the two depolarizations was variable and sometimes this variation was cyclic (Fig. 1D). Thus a progressive lengthening was observed until the disappearance of the second depolarization, following which it reappeared with a short interval, resulting in a phenomenon similar to the Wenckebach phenomenon observed in clinical arrhythmias with conduction disturbances.

Many atypical aspects were observed. In rare cases, the initial spike was larger than the second depolarization and had an overshoot (Fig. 2A). The second depolarization could start from a variable potential (Fig. 2B). The first component could be reduced to a fluctuation of potential without initial spike (Fig. 2C) and, in few cells, the first component was absent (Fig. 2D). Sometimes nonhomogeneous excitation was demonstrated by asynchrony of the mechanical events recorded from the whole preparation as compared to the localized electrical ones (Fig. 2C). However, in most cases simultaneous recordings with two microelectrodes were needed to demonstrate nonhomogeneity.

Simultaneous recordings with two microelectrodes

In rare cases, simultaneous recordings with two microelectrodes exhibited two very different electrical activities, such as those illustrated in Figure 2D: coexistence of a double component AP with an AP reduced to an activity similar in shape and timing to the usual second component. In most cases, the initial spikes of the two explored cells were synchronous (distance between the cells indicated in the legends of the figures), while various degrees of desynchronization could be demonstrated between the two second components. Sometimes, the second depolarization was recorded earlier from the electrode more distal to point of stimulation than from the proximal electrode (Figs. 3B and 3D). Consequently, selective conduction pathways were demonstrated, rendering quantitative measurement of conduction velocity meaningless.

In about 30% of the cases (Fig. 3A), the second depolarizations seemed synchronous. However, even in these cases, fluctuations might appear in the respective timing of second depolarizations in the two cells (Fig. 3B) if there were variations in the interval between the two components of each cell. In about 40% of the cases, the desynchronization...
Simultaneous recordings with two microelectrodes. All recordings obtained with the two microelectrodes placed in the same bundle. In each panel, a = AP recorded at the point proximal to the localized stimulation site, b = AP recorded at the distal point. Inter-electrode distance in mm: 0.5 in A, 4 in B, 0.5 in C, 3 in D. Third tracing in each panel is force. Scales as in Figure 1.

A: Synchronous second components in the two cells. B: Variations in order of appearance of the second components in two cells concurrent with variations of the interval between the two components in each cell. C: Obvious desynchronization; enlargement of the second response in C (arrows and numbers indicate corresponding parts in the two APs: see text). D: Progressive increase of the interval between the second components in two cells with eventual disappearance of the second response in one cell (Wenckebachlike phenomenon).

was obvious. An example is given in Figure 3C; enlargement in C shows that (1) the beginning of the second depolarization in "a" corresponds to a prepotential in "b"; (2) this prepotential is followed by a large depolarization in "b", which (3) corresponds to a hump in "a." This interpretation is further suggested by the fact that the hump in "a" became larger when the interval between the two components in "b" increased (Fig. 3C). In the remaining 30%, the desynchronization was responsible for a high degree of block or for a Wenckebachlike phenomenon between the second components of the two cells (Fig. 3D). The preceding results were obtained with two microelectrodes in the same bundle and asynchrony was not significantly related to inter-electrode distance.

Hence, simultaneous recordings with two microelectrodes usually demonstrated a desynchronization between surface cells. Moreover, conduction between interior and surface of the preparation, which cannot be assessed directly, might be more impaired than surface conduction.

EFFECTS OF FREQUENCY AND INTENSITY OF STIMULATION

When conduction is slow and nonhomogeneous: (1) high rate causes fatigue phenomena, which disappear at low rate; (2) by increasing intensity of stimulus more fibers are excited and more homogeneous response occurs. Consequently, the degree of homogeneity of excitation in this preparation could be assessed indirectly by varying frequency and intensity of stimulation. In the following experiments, recordings were obtained with two microelectrodes, but they were no more

Effects of stimulation frequency. Recording of AP in A, of AP and force in B,C,D,E; scales as in Figure 1. A: Progressive shortening of the cycle with progressive lengthening of the interval between the two components. B: Sudden decrease in frequency; absence of the second component at high frequency and presence at low frequency. C: Critical frequency for the second component to appear for every alternate AT. D,E: Recordings made in the same cell; D: sudden increase in the frequency (producing alternating responses as in C); increase of the interval between the two components in the double component APs (seen in the first and third complexes) and progressive modification of the plateau in the single component APs; E: sudden decrease in frequency.
informative than those obtained with a single microelectrode, suggesting that alterations in conduction occurred between the interior of muscle strip and the surface rather than between surface cells.

Higher frequency of stimulation (above the basic frequency which was 0.3-0.7 Hz) caused an increase of the interval between the two components (Fig. 4A), while at low frequency it was reduced to a notch between spike and dome-shaped slow phase (Fig. 4B, last AP). During high frequency stimulation, the second component disappeared (Fig. 4B, first two APs), while it always occurred at low frequency. A critical frequency for the appearance of the second component for each second AP was found (Fig. 4C). Sudden variations in frequency might cause the second depolarization to be preceded by a prepotential (Fig. 4B, last AP). Furthermore, variations of frequency modified the configuration of the plateau following the initial spike, as it was observed during the staircase phenomenon (Figs. 4D and 4E).

When the intensity of stimulation was increased (above the threshold, which was 0.5-1 ma with localized stimulation), the peak tension was enhanced and the duration of contraction was shortened with merging of the two components. This effect, suggesting a "resynchronization," remained after beta-receptor blockade; it was observed with localized stimulation as well as with massive stimulation, demonstrating that this latter kind of stimulation could fail to provoke a homogeneous response when its intensity was not sufficiently high (Fig. 5A).

Increasing intensity of stimulation could change an AP with two depolarizations into an AP with only a notch between spike and slow phase (Fig. 5C). In contrast, the decrease of stimulation intensity caused important variations in shape of the initial spike (Figs. 5D and 5E). With strong stimuli, the initial spike was rapid, S-shaped, and of large amplitude, while remaining below zero potential. Lowering of intensity resulted in (1) an increasing latency, (2) the appearance of a long-lasting prepotential, (3) a striking decrease in amplitude, and (4) maximal slope of the initial spike.

As in the earlier study (1), it was possible to evoke a second component by stimulation during the first plateau with an intensity 5-10 times higher than the threshold needed to obtain the first component. Furthermore, the evoked second depolarization was preceded by a long latency which was magnified when the stimulation intensity was lowered (Fig. 5F).

**FIGURE 5**
Effects of stimulation intensity. Scales as in Figure 1, except broken-line scales = 2 msec and in A (vertical) scale = 5 ma. A: Recording of force and of intensity of massive stimulation. B: Superimposition of two successive APs evoked respectively with high (a) and low (b) intensity of localized stimulation. C: Three successive APs recorded simultaneously in two cells of two bundles; interelectrode distance, 6 mm; third trace = force; reversible transformation of APs with a notch (strong massive stimulation) into APs with two clearly separated components (low intensity). D,E: Effects of varying stimulation intensity on the shape of the initial spike; recording point separated by 8-10 mm from the localized stimulation site. E: High speed (left) and low speed (right) recordings in the same cell, corresponding artifacts of stimulation indicated by solid arrows, corresponding parts of the two recordings indicated by open arrows. F: Recording of AP and force. Evoked second components with high (first AP) and low (second AP) intensity of stimulation.
FIGURE 6
Effects of inhibitors of membrane permeabilities. Scales as in Figure 1, except broken-line scale in A = 2 msec. A: Action of tetrodotoxin (0.8 µg/ml) on the initial spike. B, C, D: Action of tetrodotoxin (2 µg/ml) on AP and force; B: decrease of the first component; C: disappearance of the response to stimulation. Between B and C, washout of tetrodotoxin and increase of stimulation intensity; D: Recovery period; initial spike practically absent in the first AP (brief surge of potential due mainly to the stimulation artifact). E, F: Action of MnCl₂ (2 mM) on AP and force; E: disappearance of the second depolarization; F: after 120 seconds under MnCl₂.

INHIBITORS OF MEMBRANE PERMEABILITIES AND IONIC MODIFICATIONS

A low stimulation rate (0.3–0.7 Hz) with a sufficiently high intensity (5 mA), allowed the second component to occur for each AP in most preparations at 27°C. These conditions were used for study of membrane permeabilities.

Tetrodotoxin caused a decrease in amplitude and in slope of the initial spike (Fig. 6A) without modification of the second depolarization. After a longer time of action of this drug, the second depolarization could be abolished while plateau and contraction remained (Figs. 6B, 6C, and 6D). In contrast, MnCl₂ (Figs. 6E and 6F) always resulted in shortening of the first plateau and a disappearance of the second depolarization, with no modification of the initial spike.

Decrease of CaCl₂ (0.2 mM) or Ca²⁺-free media produced various types of effects (several of them were observed in every preparation). A notch between spike and slow phase could be widened in two separate components, resulting in a phenomenon similar to a paired pulse stimulation (2). Consequently, a "postextrasystolic" potentiation might cause an enhancement of force (paradoxical in low Ca²⁺, emphasizing difficulty in making electro-mechanical correlations in this preparation). Double component APs could be changed into multiple component APs. Widening of the second component could be observed without lengthening of the first plateau. Lengthening of the first plateau could be accompanied by a Wenckebach-like phenomenon of the interval between the two components (Fig. 7D). In all cases, the slope of the initial spike was decreased.

Elimination of NaCl (concentration below 20% of the normal) or elimination of both NaCl and CaCl₂ resulted in disappearance of the initial spike and of the second depolarization. They reappeared consistently when NaCl was

FIGURE 7
Various effects of Ca²⁺ deprivation. Recording of AP and force; scales as in Figure 1. A: First AP, in normal medium exhibiting a slow phase separated from the initial spike by a notch; second and third APs: effect of Ca²⁺ deprivation. B: first AP, normal medium; second AP, multicomponent AP, deprivation of Ca²⁺. C, D: The AP in normal medium has been omitted (it had the same configuration as in B). C: increase in duration of the second component without lengthening of the first plateau. D: three successive APs exhibiting a Wenckebach-like phenomenon (duration of the cycle, 3 seconds).
restored, whereas they never reappeared when CaCl₂ was added (without NaCl). Identical results were obtained by use of either sucrose or choline chloride as an osmotic substitute for NaCl.

Increase of KCl (8 mM) produced a lowering of the resting potential and the occurrence of multicomponent APs. Moderate lowering (2.5–4 mM) of KCl could result in the shortening of the interval between the two components. Solutions containing 0.5 mM of KCl produced hyperpolarization, widening and, finally, disappearance of the second component (Fig. 8A). The reappearance of the second component caused by return to normal medium was often preceded by prepotentials (two first APs in Fig. 8B).

Hypertonic solutions, by addition of sucrose (50 mM), resulted in multicomponent APs.

Discussion

Double-component APs have been described in the atrioventricular node (4, 5), where conduction is slow and selective pathways are present. Moreover, splitting of the AP into two components has been elicited at the Purkinje-ventricular junction (5–8) or in ventricular muscle (9–11) by various procedures causing conduction disturbances. They include procaine (6), anoxia (5), localized injury (8), hypertonic solutions (9), stretch (10). Hence, a relationship between poor conduction and splitting of the AP seems likely in human atrial muscle at low temperature and could explain facts not readily explained by ionic conductances in each cell.

Membrane Permeabilities Underlying the Two Components

The initial spike, suppressed by tetrodotoxin, seems to be linked to the rapid Na⁺ channel (12). The second depolarization, suppressed by MnCl₂, seems to be related to the slow inward channel which might be used either by Na⁺ or Ca²⁺ (13, 14). Experiments in low Na⁺, low Ca²⁺, alone and together, show that a certain amount of Na⁺ is required for the occurrence of the second depolarization, whose slope is Ca²⁺-dependent. The first plateau is related to a slow inward current balancing a small K⁺ outward current (slow development of the delayed rectification), as evidenced by shortening of this plateau under MnCl₂ or acetylcholine (1).

Therefore, according to a hypothesis based on the ionic permeabilities, the maintenance of the membrane potential at a critical value during the long-lasting plateau would allow the reactivation of an inward current, producing the second depolarization. On the contrary, the plateau level appears to be quite variable, suggesting that the occurrence of the second component is not related to a threshold in each cell. Moreover, a secondlike component may exist without any first component (Fig. 2D). Furthermore, the various effects of low Ca²⁺, and the multicomponent APs under high K⁺, are not readily explained on the basis of the single cell activity. These observations support the hypothesis of partial independence of the two components and of conduction disturbances between them.

Nonhomogeneous Excitation of the Preparation

This partial independence of the two components is evidenced by the simultaneous recordings with two microelectrodes, which demonstrate selective and sometimes variable pathways for the second depolarization. Conduction disturbances can even cause a lack of correlation between electrical and mechanical events. This poor conduction seems to be related to the low resting potential (15) caused by hypothermia. The human atrial muscle appears to be more sensitive to this
factor than the sheep Purkinje fibers, in which definite reduction of the resting potential occurs only below 20°C (16).

A given degree of separation of the two components caused by hypothermia may be modified by well-known factors which alter conduction. In this sense, these factors can be considered to induce variable degrees of nonhomogeneous excitation, including (1) a notch between spike and dome-shaped slow phase, (2) two clearly separate components, (3) two-to-one block (Fig. 4C) or Wenckebachlike phenomenon (these terms being used only in a comparative way), (4) either total disappearance of the second component or occurrence of multicomponent APs (severe conduction disturbance can cause either complete block, or multiple wave fronts). Transformation from (1) to (4) can be induced by factors of nonhomogeneous response, which include lowering temperature, lowering intensity of stimulation, increasing frequency, high K+ media or recovery after low K+, low Ca²⁺ or Ca²⁺-free media, and hypertonic solutions. In contrast, transformation from (4) to (1) can be elicited by raising of temperature, increase of intensity of stimulation, lowering frequency, and moderate lowering of K⁺.

Hence, it may be concluded that the splitting of the AP into two (or several) components is related to a nonhomogeneous excitation of the preparation. This conclusion is complementary to the earlier study (1) in which the possibility of an independent conduction of the second component is suggested, but not used to explain the splitting of the AP.

MECHANISM FOR THE SPLITTING OF THE AP

The slow conduction of the second component could be explained by the fact that it travels during a relative refractory period through partially depolarized fibers. However, low and variable amplitude of the initial spike, in contrast with relatively large and constant amplitude of the second component, suggests the possibility that only the second component would be related to the activity of the impaled cell. The initial spike would then be attributed to electrotonic spread of another kind of AP characterized by a large spike with overshoot, such as those in Figure 2A (when the microelectrode is placed in a cell generating this latter kind of AP, the small second component would be attributed to electrotonic spread, i.e., reverse of the usual recording). The different membrane permeabilities related to these two kinds of APs might be explained by a transition between two tissues. The second component could be the activity of regular contractile atrial cells, while the first component might be electrotonic spread of an AP with rapid spike and long plateau, generated in atrial conduction tissue or in other contractile cells in better functional condition (less depolarized).

This hypothesis is further supported by the following. (1) It explains the inconsistency with the law relating slope and amplitude of depolarization (15) i.e., occurrence of small amplitude accompanied by rapid slope of the initial depolarization. (2) Amplitude and maximal slope of an electrotonus depend on the space constant and the distance between site of origin and recording point; if the explored cell is far enough from any cell generating an AP with rapid spike, the first component may become small (Fig. 2C) or nonexistent (Fig. 2D). (3) Increase of stimulation intensity may lead to a more homogeneous activation of the preparation and to an electrotonic spread over a shorter distance, resulting in a spike with larger amplitude and maximal slope, thus explaining the striking deviation from the well-established law of all-or-nothing depolarization. (4) In contrast, factors promoting a nonhomogeneous excitation such as lowering temperature or decrease of stimulation intensity result at the same time in decrease of amplitude of the first component and increase of interval between the two components, both facts being explained by longer electrotonic pathways.

If the myocardium is considered to be a functional syncytium with low-resistance intercalated disks (17), blocks between the two components can be considered as related to the geometry of this tissue (8): increase of surface area could result from transformation
by branching of one type of fiber into another or from selective conduction pathways. Impulses traveling from a small area of active membrane may fail to depolarize a large area which may be subsequently depolarized by the convergence of impulses using other pathways. Some cells, with functional rapid channels but remote from the recording site, could be involved in this second wave front, thus explaining how tetrodotoxin, after complete diffusion into the strip, could suppress the second component. If the second component does not occur spontaneously, it can be evoked by stimulation with intensity sufficiently high to depolarize the required area of membrane.

Alternatively, if cell-to-cell junctions, at the level of intercalated disks, are considered as a critical factor for impulse transmission, as suggested in one of the earliest studies of similar phenomena (9) and supported by many arguments (18), the first component could be interpreted as a junctional potential representing the excitatory interaction of an activated cell upon its contiguous neighbor. Dissociations of the intercalated disks have been reported in atrial appendages removed from patients with congenital heart diseases (19, 20). Furthermore, the fact that too low or too high K+ concentrations promote nonhomogeneous excitation, while the interval between the two components is the shortest for an optimal K+ concentration, might support the hypothesis that K+ accumulation in the intercalated disk clefts may play some role in the intercellular transmission process (21). But it must be emphasized that there is no direct evidence in the present study for the nonsyncytial functional nature of heart muscle.

Irrespective of what is ultimately decided about the fundamental nature of the two AP components, it presently appears that the demonstration of a nonhomogeneous excitation does help to explain the previously described phenomenon (1), whose reproducibility and dependence on temperature are confirmed.

Acknowledgment

We are indebted to Pr. E. Coraboeuf for advice and encouragement.

References


