Conduction Pattern of Excitation in the Amphibian Atrium Assessed by Multiple-site Optical Recording of Action Potentials

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Abstract Spontaneous action potentials were monitored from multiple sites in the bullfrog atrium using a voltage-sensitive merocyanine-rhodamine dye together with a 100-element photodiode matrix array, and we have assessed the spread of the excitation from the pacemaker. Isochrone curves of conduction were obtained by timing the initiation of the action potential-related optical signals: we constructed maps of the spread. Excitatory waves appeared to conduct radially from the pacemaking area over the atrium, and the conduction velocity in the left atrium exceeded that in the right atrium.

Key words: conduction pattern, atrial excitation, multiple-site optical recording.

A complete understanding of the conduction pattern of excitation in the atrium is necessary to answer major questions in cardiac physiology. However, investigations into such a problem have been hampered, because of a technical limitation in the use of microelectrodes for simultaneous recording of action potentials from many sites in a preparation.

Recently, introducing a square photodiode matrix array, optical methods for monitoring membrane potential activity have been developed and they provide a powerful tool for simultaneous recording of electrical activity from multiple areas of the central nervous system (Salzberg et al., 1977, 1983; Grinvald et al., 1981, 1982, 1984; Orbach and Cohen, 1983; also for reviews, see Grinvald, 1985; Cohen and Lesher, 1986), the salivary gland (Senseman et al., 1983) and the heart (Dillion and Morad, 1981; Salama et al., 1981). Using a similar method, we also have assessed the conduction pattern of spontaneous excitations in early embryonic hearts (Sakai et al., 1983; Komuro et al., 1985; Hirota et al., 1985a, b). These results suggest that the multiple-site optical recording system might also be used to monitor conduction patterns in the adult heart.
In the present work, we have investigated electrical propagation in the bullfrog atrium, using a voltage-sensitive dye together with a multiple-site optical recording system to monitor action potentials simultaneously from 100 different areas. We report here the conduction pattern of the spontaneous excitation wave from the pacemaker in the atrium. Some of the results described here were presented in a preliminary form (HIROTA et al., 1984).

MATERIALS AND METHODS

Preparations. Bullfrogs (Rana catesbeiana) of both sexes 400–500 g were anesthetized with a spinal injection of urethane (20%, 1.0–2.0 ml). The heart was quickly removed and bathed in a Ringer solution at room temperature (25–28°C). The whole atrium was isolated, spread out, and then the atrial septum was excised.

Bathing solutions. The preparation was attached, positioning the epithelium or endothelium side upward, to the silicone (KE 106LTV; Shinetsu Chemical Co., Tokyo, Japan) bottom of a simple chamber by pinning it with tungsten wires, as shown in Fig. 1, and bathed in a solution of the following composition (in mM): NaCl 118, KCl 3.3, CaCl2 2.7, Tris-Cl buffer (pH 7.3) 10.0.

Staining. The isolated atrium was incubated in a Ca2+-free bathing solution containing 0.15 mg/ml of a merocyanine-rhodamine dye (NK 2761; KAMINO et al., 1984).

Fig. 1. Photomicrographs of the atrial preparations isolated from two bullfrogs attached to the silicone bottom of a chamber by pinning it with tungsten wires, for optical measurements. A, epicardial surface; B, endocardial surface. The scale given at the bottom applied to both preparations.

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1981) for 30 min. After that, the preparation was washed with several changes of a 
Ca$^{2+}$-free bathing solution. The dye was purchased from Nippon Kanko Shikiso 
Kenkyusho Co. Ltd. (Okayama, Japan).

Optical measurement. The optical method for monitoring of action potential 
was the same as that described previously (HIROTA et al., 1985a, b). The preparation 
chamber was mounted on the stage of an Olympus Vanox microscope (Type, ABH-
L-1, Olympus Optical Co., Ltd., Tokyo Japan) placed on a vibration isolated 
table. Bright field illumination was provided by a JC24V-300 W halogen-tungsten 
lamp (Kondo Sylvania Ltd., Tokyo Japan) driven by a stable DC power supply 
Incident light was collimated, passed through a heat filter (32.5B-76, Olympus 
Optical Co.), rendered quasi-monochromatic with a 700 ± 11 nm (for measuring of 
action potential-related change) or 610 ± 9 nm (for contraction-related change) 
interference filter (Type 1F-S, Vacuum Optics Co. of Japan, Tokyo Japan), and 
focused onto the epi- or endo-myocardial surface of the atrium by an aplanatic/ 
achromatic condenser. A long working distance objective (S plan or S plan Apo) 
and a photographic eyepiece formed a magnified real image of the heart at the 
image plane. Magnifications were ×2.5, ×10, or ×12. The transmitted light 
intensity at the image plane was detected using 100 elements of a 10 × 10 square 
array of silicon photodiodes (MD-100-4PV, Centronic, Ltd., Croydon, U.K.). The 
image of the preparation was positioned on the array, and a drawing was prepared 
of the heart superimposed on the photodiode matrix. The outputs of the detectors in 
the diode array were fed to amplifiers via current-to-voltage converters. The 
amplified outputs from 100-elements of the diode array were first recorded 
simultaneously on a 112-channel data recording system (RP-890 series with seven 
I/O processors RP-893, NF Electronic Instruments, Yokohama, Japan), and then 
were fed into a computer (LSI-11/73 system, Digital Equipment Co., Maynard, 
MA, U.S.A.).

RESULTS

Optical signals accompanying action potentials

When we optically monitor action potentials from contractile tissues using 
voltage-sensitive dyes, optical changes generally include light-scattering changes 
due to mechanical movement (BAYLOR and OETLIKER, 1977; VERGARA and 
BEZANILLA, 1976; MORAD and SALAMA, 1979; SAWANOBORI et al., 1981; KASS, 1981; 
HIROTA et al., 1985a). Accordingly, in a strongly beating heart stained with voltage-
sensitive dyes, action potential-related optical signals are often covered by a larger 
mechanical movement artifact. Therefore, in such a case, it is a serious problem to 
minimize the contribution of beating artifacts.

In order to minimize the beating, we have thus used as a bathing solution a 
Ringer solution from which Ca$^{2+}$ was removed. Figure 2 shows simultaneous 
measurements of optical changes and electrical action potentials in an atrium
isolated from a frog, in a Ca$^{2+}$-free solution. The preparation was stained with a voltage-sensitive merocyanine-rhodanine dye (NK 2761). Optical recordings were obtained simultaneously from five different areas, using a 700 ± 11 or 610 ± 9 nm interference filter, and the intracellular recording was made with a microelectrode having a resistance of 30 MΩ. The inset on the right of the optical traces illustrates the array elements of the photodiodes which were positioned on the image of the atrial preparation. The preparation was bathed in a Ca$^{2+}$-free Ringer solution. The direction of the arrows on the optical traces indicates a decrease in transmittance and the length of the arrows represents the stated value of the change in intensity divided by the DC-background transmitted light intensity.

In the Ca$^{2+}$-free solution, the contraction movements were gradually reduced and the action potential-related optical signal and the artifact (light-scattering change) due to the contraction could be easily identified. The signals consisted of two components that were identified according to the time course. The time course of component 1 (1st-signal) was related to the action potential, and component 2 (2nd-signal) followed component 1 somewhat later. The 1st component is wavelength dependent, while the 2nd component is independent of wavelength. As shown in Fig. 2, the 1st component was completely eliminated when a 610 ± 9 nm
interference filter was used, whereas the 2nd component remained at 620–610 nm. These characteristics indicate that the 1st component is indeed the absorption change due to the action potential while the 2nd component corresponds to a light-scattering change induced by heartbeat. Similar signals are also observed in small beating embryonic hearts and the empirical criteria required to distinguish between an action potential-related signal and a beating-related optical change were previously established (Fujii et al., 1980; Hirota et al., 1985a, b).

In the optical recordings shown in Fig. 2, the sizes of the 2nd signals varied among the elements. The 2nd-signals detected by elements 29 and 30 were very small. The direction of the 2nd-signal by elements 30, 39, and 59 were opposite to that of the 1st-signal, while in trace 4, the direction of the 1st- and 2nd-signals were the same. Thus, it is suggested that the differences in the size and the shape of the 2nd-signals were due to regional variations in the degree and topological pattern of the reduced contractions: in areas 29 and 30, the contractions were almost reduced, and in areas 4, 30, and 59, small contractions remained. Nevertheless, in such a condition, we could measure the initiation of the 1st-signals related to the spontaneous action potentials.

The size of the action potential-related 1st signal was approximately the same among different areas. Only in area 59, the 1st-signal was somewhat smaller than others. This is probably due to the superposition of a large 2nd-signal with the direction opposite to that of the 1st. In voltage-clamp experiments using giant axons of squid (Loligo pealei), absorption changes in voltage-sensitive merocyanine-rhodanine dyes are directly proportional to the membrane potential change (Ross et al., 1977; Gupta et al., 1981). Therefore, in the preparation used in the experiment shown in Fig. 2, there were probably no differences in the degree of action potential activity among the areas of the optical recordings. It also shows that the tissue was uniformly stained.

**Real-time optical recording of action potentials**

Figure 3A illustrates an example of simultaneous optical recording of spontaneous action potentials from 95 adjacent areas in the stained atrial endocardium, using a 10 × 10 element photodiode array.

The arrangement of the optical signals corresponds to that of the photodiode array elements illustrated in Fig. 2. Although most of the signals included the 2nd-signals, the action potential-related 1st signals could be easily identified. We also verified that the 1st component disappeared at 610 nm. In addition, an electrode measurement was made simultaneously in area 38. The recording is shown in the lower right-hand corner. Although the spontaneous rhythmic action signals were synchronized among the 95 different areas, there were short delays between timing of the upstrokes of the signals at a higher sweep speed (Fig. 3B). These delays correspond to the conduction time of excitation.
Similar measurements were made several times by sliding the photodiode array on the image of the atrium. For example, as shown in Fig. 4, the 10 x 10-element Japanese Journal of Physiology

Fig. 3. Optical changes accompanied by spontaneous action potentials simultaneously measured by 95 photodiodes from a stained atrial endocardium of the bullfrog. The optical measurement was made with a 700 ± 11 nm interference filter. The microelectrode recording was obtained from area 38, and is shown on the lower right portion of the array. Recordings in B were obtained at a higher sweep speed, in order to see the difference in the time of the upstrokes of the action potential-related 1st signals.

**Spreading of excitation**

Similar measurements were made several times by sliding the photodiode array on the image of the atrium. For example, as shown in Fig. 4, the 10 x 10-element
The quadruplet of real-time optical monitorings of optical changes from a stained atrial endocardium of the bullfrog. The 100-site simultaneous optical recordings were made at four times by sliding a 100-element photodiode array, in the order of I→II→III→IV, on the image of the preparation. The paired series of signals indicated by double arrows was obtained twice from the same position.
Fig. 5. A. Numerical representation of the spread of excitation in an atrial endocardium. The numerical values in the grid display the conduction times (in ms) of the excitations from the pacemaking area. The pacemaking area corresponds to the grid filled by "O." OSV is the ostium of the sinus venosus. The grid superimposed on the drawing indicate the size and positions of the photodiode array elements. In areas indicated by triangles, the 1st-signals were not identified.
Fig. 5. B. Isochrone curves constructed by the data shown in A. Spacing of isochrone lines displays the conduction velocity, one isochrone interval represents the approximate values of the propagation distance per 10 ms. Dash-lines correspond to the position of the atrial septum and PA corresponds to the pacemaking area.
other areas. The results are shown in Fig. 5. In this figure, the grid superimposed on the drawing indicates the size and positions of the photodiode array element; the numerals on the grid display, on corresponding elements, the delays in ms. From this numerical table, we could assess the location of the pacemaking area, and express quantitatively that excitation propagated progressively over the atrium from the pacemaking area.

**Mapping.** On the bases of these data, computing by means of interpolation, we drew isochrone curves of the spread of excitation in the atrium. In the map shown in Fig. 5B, we noted: (i) the pacemaking area was located near the ostium of the sinus venosus, and the area was roughly estimated to be about 2.8 mm²; (ii) although the isochrone curves were frizzy, the excitation propagated radially over the atrium; and (iii) the excitation propagated in the left atrium faster than in the right atrium.

To assess the conduction velocity of the excitation, along the five directions indicated by lines a, b, c, d, and e in Fig. 5B, we plotted the delay of the initiation of the 1st-signal against the straight distance from the pacemaking area (Fig. 6): the area marked by an asterisk in Fig. 5A was regarded as the zero-point reference for the distance.

Figure 6 shows that, in the left atrium, the delay of initiation of the action
Fig. 7. Additional six samples of endocardial (preparations F-43, F-50, and F-52) and epicardial (preparations F-45, F-47, and F-51) activation maps for six different bullfrog atrial flaps, representing the spread of excitation from the pacemaking area. Note that the location of the pacemaking area varies somewhat with every preparation. Dash-lines indicate the position of the atrial septum.
potentials is approximately proportional to the distance from the pacemaking area, in the directions indicated by \( a, b, \) and \( c \), and that the conduction of the excitation along direction \( e \) in the right atrium is slower than that in the left atrium, and the velocity decreases with the distance. The conduction velocity of the excitation was roughly estimated to be \( 0.14 \text{--} 0.3 \text{ m/s} \) in the left atrium and to be \( 0.02 \text{--} 0.2 \text{ m/s} \) in the right. As a control experiment in a normal Ringer solution, we also measured the conduction velocity in the atrium preparation by impaling each site with two microelectrodes, and obtained a value of \( 0.22 \pm 0.6 \text{ m/s} \) (\( n = 4 \)) in the right atrium. This value is in good agreement with the conduction velocity obtained by optical measurements, and shows that the conduction was not altered in the \( \text{Ca}^{2+} \)-free solution.

Furthermore, we made similar measurements in both the atrial endocardium and epicardium using more than 30 preparations, and constructed maps of the spread of the spontaneous excitation in the atrium. The six examples are shown in Fig. 7. These maps indicate that the pattern of spread of the excitation is basically similar in all preparations and that there are no differences in the patterns between the atrial epicardium and endocardium.

**DISCUSSION**

The present study demonstrates the pattern of spread of spontaneous action potentials in the bullfrog atrium, using a voltage-sensitive merocyanine-rhodamine dye together with a 100-element photodiode matrix array. The multiple-site simultaneous optical recordings of action potentials provide critical advantage over conventional microelectrodes measurements when investigating the spreading of excitation in the heart.

With reference to the spread of excitation in the atrium, the question “Is the spread of excitation waves radial or nonradial?” has arisen, mainly in reference to mammalian hearts. Based on finding in electrical experiments, it has been suggested that activity in the atrium commences in the S-A node and spreads outward like the wave produced when a stone is dropped into still water (e.g. Lewis, 1915; Paes de Calvalho et al., 1959; Sano and Yamagishi, 1965; Opthof et al., 1985).

Our results in the present optical experiment provide interesting data on the spread of spontaneous excitatory activity in the frog atrium. From the maps illustrated in Figs. 5 and 7, it is concluded (i) that basically, electrical excitation is generated in a narrow pacemaking area which lies close to the sinus venosus, and conducts radially over the atrium, and (ii) that the conduction velocity in the left atrium is higher than that in the right atrium. Using a rapid laser scanning method, Dillon and Morad (1981) measured the propagation of action potentials evoked by stimulation of the frog heart. The pattern they obtained is essentially similar to our results.

The isochrone curves shown in Figs. 5 and 7 show a zigzag-like shape. It seems likely that such a characteristic feature of the curves is related to the complexity in
the network of the cardiac fibers. However, we have never obtained unequivocal evidence of a specialized conduction system or of a preferential conduction pathway in the frog atrium.

In the experiment described in this paper, methodologically, there are limitations and difficulties. Movement artifacts are the most serious factor to be considered in a beating heart. In addition to the use of a Ca$^{2+}$-free solution, as Dillon and Morad (1981) have pointed out, choice of fluorescent dyes might be useful to minimize the movement artifact. The photodiode array having 100 elements of $1.4 \times 1.4$ mm square was positioned over the image plane formed after $\times 2.5$, $\times 10$, or $\times 12$ magnification. Each element would thus, monitor signals from many cells. To analyze the conduction pattern of excitation in more detail, an apparatus with a photodiode array constructed of many more smaller elements and a higher magnification would be required.

We are now attempting to increase the number of simultaneously monitored portions. The ability to record electrical activity simultaneously in more areas should enhance understanding of the basis of the cardiac function under normal or pathological conditions.

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