Optical Mapping of Conduction Patterns of Normal and Tachycardia-Like Excitations in the Rat Atrium

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Abstract: Multiple-site optical recording of transmembrane voltage activity (MSORTV), using a voltage-sensitive dye and a 12×12-element photodiode array, was employed to monitor action potentials in the rat atrium. Atrial preparations including the sinus node area, caval area, and atrial septum were dissected from adult rat hearts and stained with a voltage-sensitive merocyanine-rhodanine dye (NK2761). For suppression of optical artefacts due to contractile movements, a bathing medium containing 2,3-butanedione monoxime (BDM: 10–20 mM) was used. The spread of spontaneous excitation from the pacemaker was assessed optically by timing the initiation of the action potential-related extrinsic absorption changes. The optical signals were recorded from more than 300 contiguous loci in

the atrium by sliding the photodiode array over the image of the preparation; in this way, the intra-atrial conduction pattern of spontaneous excitation was mapped. The obtained maps revealed a non-radial spread of excitatory waves originating in the pacemaking area over the atrium. Furthermore, we also mapped the conduction pattern of long-lasting tachycardia-like excitation evoked by a short train of electrical stimulation (5–10 Hz, 1–2 s) applied with a bipolar electrode. These maps suggest that excitatory waves are propagated in a circular pathway which often surrounds the ostium of the superior or inferior vena cava. Various patterns for the pathway were also demonstrated optically. [*Japanese Journal of Physiology*, 47, 179–188, 1997]

Key words: atrial excitation, conduction pattern, multiple-site optical recording, tachycardia-like excitation, re-entry.

A complete understanding of the conduction pattern of excitation in the atrium has long been sought to resolve major questions in cardiac physiology and pathophysiology. However, investigations into such issues have been hampered by the technical difficulties and limitations in the use of conventional electrophysiological methods for simultaneously recording action potentials from many sites in a preparation.

Recently developed optical methods for monitoring membrane potential activity using a multi-element photodiode array provide a powerful tool for simultaneously recording cellular electrical events from multiple areas of the nervous system (for reviews, see Salzberg [1] and Cohen and Leshner [2]) and the heart

(for reviews, see Kamino [3, 4]). Using similar methods, we have assessed the conduction pattern of excitations in early embryonic hearts (for reviews, see Kamino [3, 4]) and adult amphibian hearts [5, 6]. Nevertheless, since it is difficult to control movement artefacts due to contractions without affecting electrical excitation, investigations in mammalian hearts have been encumbered by serious difficulties. Recently, using 2,3-butanedione monoxime (BDM), we have been able to overcome the obstacle of movement artefacts.

In this work, we investigated the propagation of excitation in adult rat atrium treated with BDM, using a voltage-sensitive dye together with a 12×12-element

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photodiode array. We report here the conduction pattern of the spontaneous excitatory wave from the pacemaker in the right atrium, and the conduction pattern of the tachycardia-like excitation evoked by a short train of electrical stimulation applied with a bipolar electrode. Some of the results described here were previously presented in preliminary form [7, 8].

METHODS

Preparations. Adult rats (Wistar strain, 400–600 g) of both sexes were anesthetized with ether via inhalation. The hearts were quickly removed and bathed in an ice-cold bathing solution. The right atrial preparations including the sinus node area, caval area, and atrial septum were isolated, and the lateral wall of the right auricle was incised. The preparations were attached, positioning the endocardial side upward, to the silicone (KE106LTV, Shin-etsu Chemical Co., Tokyo, Japan) bottom of a simple chamber by pinning with tungsten wires. The preparations were kept in an oxygen-equilibrated bathing solution with the following composition (in mM): NaCl, 149; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; Tris-HCl buffer (pH 7.4), 10; and glucose, 10.

Staining. The isolated atrial preparations were stained in a bathing solution containing 0.4–0.6 mg/ml of a merocyanine-rhodanine dye (NK2761) [9, 10] for 30 min. After staining, the preparations were washed with several changes of normal bathing solution.

Optical measurement. The optical method used in this experiment was identical to that described in previous reports [3–5]. Light from a 300 W tungsten-halogen lamp (Type JC-24V/300W, Kondo Sylvania Ltd., Tokyo, Japan) was collimated, rendered quasimonochromatic using an interference filter with a transmission maximum at 703 ± 15 nm (Asahi Spectra Co. Tokyo, Japan), and focused on the preparation by means of a bright field condenser. The objective ($\times 1$) and photographic eyepiece ($\times 2.5$) projected a real image of the preparation onto a 12×12 -element silicon photodiode matrix array (MD-144-4PV; Centronic Ltd., Croydon, UK) mounted on an Olympus Vanox microscope (Type AHB-L-1, Olympus Optical Co., Tokyo, Japan). The magnification was $\times 2.5$ for the recording of spontaneous excitation, and was reduced to $\times 2$ by shortening the distance between the eyepiece and the photodiode array for the recording of tachycardia-like excitation. Each pixel (element) of the array detected light transmitted by a square region ($560 \times 560 \mu\text{m}^2$ or $700 \times 700 \mu\text{m}^2$) of the preparation. The output of each detector in the diode array was entered into an amplifier via a current-to-voltage con-

verter. The amplified outputs from 127 elements of the detector were first recorded simultaneously on a 128-channel recording system (RP-890 series, NF Electronic Instruments, Yokohama, Japan) and then entered into a computer (LSI-11/73 system, Digital Equipment Co., Tewksbury, MA, USA). The 128-channel data recording system was composed of a main processor (RP-891), eight I/O processors (RP-893), a 64K word wave-memory (RP-892), and a videotape recorder. The program for the computer was written in assembly language (Macro-11) called from FORTRAN, under the RT-11 operating system (Version 5.1). Optical recording was carried out in a still chamber without continuous perfusion of the bathing solution, at room temperature of 26–29°C. The incident light was turned off except during the measurement period.

RESULTS

Reducing the beating movement artefacts

In the optical recording of electrical activity from contractile heart tissues, optical signals generally contain light-scattering changes due to beating movement [4, 5]. Accordingly, in a strongly beating heart stained with voltage-sensitive dyes, action potential-related optical signals are often masked by larger mechanical movement artefacts. In order to minimize the beating without affecting membrane excitability, we used BDM for the suppression of muscle contraction [11].

Figure 1 shows three traces of the spontaneous optical signals recorded simultaneously from a stained right atrial preparation of rat heart. The left traces were obtained in normal bathing solution at 700 nm, and the middle and right traces were obtained in the presence of BDM (20 mM) at 700 and 620 nm, respectively. The large movement artefacts, which are seen in the left traces, were strongly suppressed in the presence of BDM, as shown in the middle traces (note the difference in the vertical scales for the control and for the BDM-containing solution). In the latter condition, the signals consisted of two components that were identified according to wavelength dependence. The 1st component (indicated by the arrowheads) was wavelength dependent, while the 2nd component, which followed the 1st, was independent of wavelength. The 1st component was completely eliminated at 620 nm, the null wavelength of the action potential signal with NK2761, whereas the 2nd component remained at 620–630 nm. These spectral 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

Fig. 1. The effects of 2,3-butanedione monoxime (BDM) on the spontaneous optical signals detected from a rat atrial preparation stained with a merocyanine-rhodanine dye (NK2761). The left traces were obtained in a normal bathing solution at 700 nm, and the middle and the right traces in a BDM-containing (20 mM) solution at 700 and 620 nm, respectively (note the difference in the vertical scales for the control and BDM-containing solutions). Optical signals detected simultaneously at three different positions (J-7, H-1, and D-5) are shown. Note that, by the application of BDM, the movement artefacts were markedly reduced and the spontaneous action potential-related optical signals (arrowheads in the middle traces) became easy to

observe. These signals were eliminated at 620 nm (right). All the recordings were made in a single sweep at room temperature (26–29°C). The directions of the arrows on the optical traces indicate decrease in transmittance and the lengths of the arrows represent the stated value of the change in light intensity divided by the dc-background transmitted light intensity. The inset at the bottom illustrates the array elements of the photodiodes on the image of the atrial preparation. Black grids indicate the positions at which the signals were obtained. In this and subsequent figures, S, I, and C on the image of the preparation indicate the ostia of the superior vena cava, inferior vena cava, and coronary sinus, respectively.

induced by the heart beat. Similar signals were also observed in adult frog hearts in which contraction was suppressed by a low Ca^{2+} solution. The empirical criteria required to distinguish an action potential-related optical signal and a beating-related optical change have been reported previously [4, 5].

The optical recordings shown in the figure indicate that there are regional differences in the directions, sizes, and shapes of the 2nd components. In the presence of BDM, the direction of the 2nd component detected at positions J-7 and H-1 was opposite to that of the 1st component, while the directions of the 1st and 2nd components were the same at position D-5. These differences in direction, size, and shape of the 2nd component may be due to regional variations in the degree and topological patterns of the reduced contraction. However, under these experimental conditions, the initiation of the 1st component of the optical signal related to the spontaneous action potentials could be measured.

In this preparation, the 1st-signals preceding the movement signals could be detected from several positions in the preparation in the normal bathing solution. There were small delays between the timings of

Fig. 2. The delay measured in the presence of BDM (20 mM) plotted against that in normal bathing solution. Each point corresponds to one position within the recording area. The origin corresponds to the pacemaking area in the sinus node region. The slope of the straight line is 1. The data were obtained from the same preparation as the data shown in Fig. 1.

the feet of the signals, and these delays corresponded to the conduction time of excitation. As shown in Fig.

2, in the graphical analysis using the delay measured in the absence of BDM plotted against that in the presence of BDM, no significant changes were observed in the delay of excitation conduction. This result indicates that the application of BDM does not affect the excitation conduction pattern in the atrial preparation. Thus, we routinely used BDM-containing solution (10–20 mM) in order to reduce movement artefacts.

Spreading of spontaneous excitation

To map the conduction pattern of spontaneous excitation over the whole area of a preparation, optical recordings were made by successive displacements of the photodiode array over the image of the atrial preparation. An example of optical recording from an atrial preparation is shown in Fig. 3. The traces are arranged so that their relative positions in the figure correspond to the relative positions within the area of the preparation imaged onto the detectors. Each element of the array detected light transmitted by a square region ($560 \times 560 \mu\text{m}^2$) of the preparation. Simultaneous 127-site optical recording was performed nine times by moving the photodiode array over nine positions on the image of the atrium. Most such measurements were carried out within about 5 min. During the measurements, although the cardiac rhythm sometimes became slightly irregular in the presence of BDM, the pattern of the spread of spontaneous excitation was stable: the variation of the conduction pattern among excitatory waves was negligibly small within each simultaneous 127-site optical recording, and the conduction pattern in the atrial preparation did

not change as a whole during the period for the serial 127-site optical recordings from nine positions. When the photodiode array was repositioned, the incident light was turned off except during the measurement period: little dye bleaching was noted. These procedures were essentially the same as those in our previous experiments using frog atrial preparations [5].

In the comparison of the timings of the feet of the action potential-related 1st-signals on the multiple-site optical recording of action potentials, we looked for the pacemaking area where the spontaneous action potential-related 1st-signal first occurred, and we measured the delays in the timings of the feet of the 1st-signals in non-pacemaking areas in comparison with the 1st-signal in the pacemaking area. For example, in the preparation shown in this figure, the delays could be measured from 395 positions. On the basis of these data, using interpolation computing, we drew isochrone curves of the spread of excitation in the atrium. In this manner, the conduction pattern of spontaneous excitation in the rat right atrium was mapped.

Examples of the maps are shown in Fig. 4. Map A is made from the data shown in the previous figure, and maps B1 and B2 from the data obtained from a different preparation at two different timings. On these maps, the pacemaking area is indicated by the pink square, and the delays in the timings of the feet of the 1st-signals in the non-pacemaking areas in comparison with the pacemaking area are classified into 16 color-coded steps with intervals of 5 ms. The boundary lines between different color-coded areas are the isochrone curves. To the right of the maps, the

Fig. 3. Optical signals accompanied by spontaneous action potentials. The 127-site simultaneous optical recordings were made nine times by sliding a 12×12 -element photodiode array on the image of the preparation in BDM-containing (20 mM) solution. The inset at the lower left corner illustrates the positions of the photodiode array on the image of the atrial preparation. For this preparation, the timings of the feet of the action potential-related optical signals were measured at 395 positions.

Fig. 4. Examples of maps of the conduction pattern of spontaneous excitation obtained from two different right atrial preparations.

The pacemaking area is indicated by the pink square, and the delays in the timings of the feet of the 1st-signals in non-pacemaking areas in comparison with that in the pacemaking area are classified into 16 color-coded steps with intervals of 5 ms, which are indicated by the color bar to the right of map A. The boundary lines between the different color-coded regions are the isochrone curves, which were drawn using interpolation computing, and the areas shown in gray are the areas where the delay could not be measured due to noise or artefacts. Map A was constructed from the data shown in Fig. 3. Map B1 was constructed from the data obtained at the beginning of the measurement, and map B2 from those obtained from the same preparation after about 10 min. In maps A and B2, the pacemaker is located in the sinus node region, whereas in map B1, it is located near the atrioventricular node (indicated by the arrowhead). Sketches of the preparations, in which the crista terminalis and some thick pectinate muscle fiber bundles are drawn, are shown on the right in the same scale. U and L indicate the upper and lower branches of crista terminalis, respectively. S, I, and C are defined in the legend for Fig. 1.

sketches of the preparations are shown in the same scale.

In maps A and B2, it can be seen that the pacemaking area is located near the ostium of the superior vena cava, suggesting that the sinus node acts as the pacemaker; that the excitation does not propagate radially over the atrium; and that there seem to be some routes of propagation of the excitatory wave. In twelve atrial preparations tested, two main pathways of excitation conduction were observed: one was the route along the lower (posterior) branch of the crista terminalis, and the other was that between the ostia of the superior and inferior vena cava. An additional route along the upper (anterior) branch of the crista terminalis was also observed in three preparations.

In map B1, the excitation spread pattern was quite different from that in maps A and B2. In this map, the action potential first appeared near the ostium of the coronary sinus (indicated by the arrowhead), suggesting that the atrioventricular node or its neighboring area (tissue) acted as the pacemaker, and that the excitation then spread over the whole atrium. In this preparation, the pattern was observed at the beginning of the measurement, and then the pacemaking area spontaneously shifted to the sinus node region after about 10 min, as shown in map B2. Such a pattern, in which the pacemaker was located near the atrioven-

tricular node, was observed in 5 of the 20 preparations tested.

Tachycardia-like excitation evoked by the electrical stimulation

We next examined the disturbance of normal spreading pattern of the spontaneous excitation using electrical stimulation. For this purpose, the magnification of the microscope was reduced to $\times 2$ in order to detect optical signals from a wider area of the atrial preparation without sliding the photodiode array. In this experimental condition, each element of the array detected light transmitted by a $700 \mu\text{m}$ square region of the preparation. An example of the map of the conduction pattern of spontaneous excitation is shown in Fig. 5. Although some peripheral parts of the preparation were omitted, the spreading pattern of spontaneous excitation from the pacemaking area could be mapped, and the observed pattern was similar to that shown in maps A and B2 in the previous figure.

Figure 6 shows the optical signals evoked by a short train of current pulses (about $\times 3$ diastolic threshold intensity, duration 1 ms, 10 Hz, 1 s) applied with a bipolar electrode placed on the preparation. In this figure, the optical signals detected simultaneously from seven different positions are represented in the seven traces (a–g), and the timing of the current pulse is in-

Fig. 5. The map of the conduction pattern of spontaneous excitation obtained from a right atrial preparation at reduced magnification. The delays are classified into 10 color-coded steps with intervals of 10 ms, which are indicated by the color bar to the right of the map. The pink area of the preparation surrounding the map is the area where the optical signals were not recorded. A sketch of the preparation is shown on the right in the same scale. The frame drawn on the sketch indicates the area of optical recording, and the red-filled circle indicated by the arrow-head is the position of the stimulating electrode used in the experiment, for which the data and map are shown in Figs. 6 and 7. Other information is the same as that in Fig. 4.

Fig. 6. Optical signals evoked by a short train of electrical stimulation. Optical signals detected simultaneously from seven different positions (a-g) are shown. The time course of the electrical pulse (about $\times 3$ diastolic threshold intensity, 1 ms, 10 Hz, 1 s) applied with a bipolar electrode placed on the preparation is shown in the bottom trace (Stim.). The asterisks indicate the pulses that evoked the action potential marked by arrows. A part of the optical signals at 2 s after the start of stimulation is shown at a higher sweep speed on the right. The feet of the action potential-related optical signals are indicated by arrowheads. A sketch of the preparation and the positions of seven detectors of the array are shown at the bottom. The filled circle indicated by the arrowhead (Stim.) indicates the position of the stimulating electrode. The data were obtained from the same preparation for which the map is shown in Fig. 5.

Fig. 7. The map of the conduction of tachycardia-like excitation evoked by electrical stimulation. Maps were constructed from the data shown in Fig. 6.

Maps 1 and 2 show the conduction of excitation during the period of 2,000–2,250 and 2,350–2,650 ms, respectively, after the start of stimulation; the isochrone curve "0" (between pink and red) indicates the wave front of the excitatory wave at 2,000 ms for Map 1 and at 2,350 ms for Map 2. The timings of the feet of the 1st-signals are classified into 13 color-coded steps with intervals of 25 ms indicated by the color bar to the right of Map 2. The thick green lines on the maps (indicated by arrowheads) denote the apparent "blocked area," and the red curved arrows are the estimated conduction pathway of the excitatory wave in the area outside of the map. A schematic diagram of the conduction pattern of the excitatory wave is shown on the right. The thick green line indicated by the arrowhead is the blocked area, and the red curved arrows are the conduction pathway of the excitatory wave. Other information is the same as that in Fig. 4. Note that the interval of the isochrone curves is 5 ms for Fig. 4 and 25 ms for this and subsequent figures.

Fig. 8. The second example of the map of the conduction of tachycardia-like excitation evoked by electrical stimulation (about $\times 3$ diastolic threshold intensity, 1 ms, 5 Hz, 1 s). Maps 1 and 2 show the conduction of excitation during the periods of 2,025–2,250 and 2,275–2,575 ms, respectively, after the start of stimulation: the isochrone curve "0" (between pink and red) indicates the wave front of the excitatory wave at 2,025 ms for Map 1 and at 2,275 ms for Map 2. The timings of the feet of the 1st-signals are classified into 14 color-coded steps with intervals of 25 ms indicated by the color bar to the right of Map 2. A sketch of the preparation at a reduced scale and a schematic diagram of the conduction pattern of the excitatory wave are shown on at bottom. The red-filled circle indicated by "Stim." on the sketch is the position of the stimulating electrode. Other indications are the same as those in Fig. 7.

Fig. 9. The third example of the map of the conduction of tachycardia-like excitation evoked by electrical stimulation (about $\times 3$ diastolic threshold intensity, 1 ms, 5 Hz, 2 s). Maps 1 and 2 show the conduction of excitation during the periods of 2,525–2,725 and 2,750–2,950 ms, respectively, after the start of stimulation: the isochrone curve "0" (between pink and red) indicates the wave front of the excitatory wave at 2,525 ms for Map 1 and at 2,750 ms for Map 2. The timings of the feet of the 1st-signals are classified into 9 color-coded steps with intervals of 25 ms indicated by the color bar to the right of Map 2. In this preparation, two blocked areas, indicated by a thick green line and a green rectangle in the maps and the scheme, were observed and the numerals (1 and 2) on the scheme indicate the two routes of excitation conduction. Other indications are the same as those in Figs. 7 and 8.

indicated in the bottom trace (Stim.). The first pulse evoked an action potential that propagated to the whole recording area, but the next two pulses evoked no action potential. Subsequently, the fourth, seventh, and tenth pulses evoked action potentials that propagated to the whole recording area. After the end of the

train of pulses, excitation appeared rhythmically with an interval of about 420 ms, which is much shorter than that of the spontaneous rhythm of the physiological pacemaker (about 1.4 s). This rhythmical excitation was sustained for about 10 s and terminated spontaneously. Similar events of tachycardia-like excita-

tion were evoked several times in this preparation.

As can be seen in the traces on the right, the timings of the feet of the action potential-related optical signals could be measured during this tachycardia-like excitation. The action potentials appeared in the order of a, b, c, d, e, f, and g, indicating that the excitatory wave propagated downward, to the right, and then upward. We measured the timings of the feet of the action potential-related signals in comparison with the start of stimulation in the whole recording area, and constructed maps of the propagation of the excitatory waves.

In Fig. 7, two examples of those maps are shown. Map 1 shows the position of the wave front during the period of 2,000–2,250 ms after the start of the train of pulses, and Map 2 for the period of 2,350–2,650 ms. In Map 1, the action potential first appeared at the left upper margin of the recording area indicated by the isochrone curve of 2,000 ms (the line between the pink and red areas). The excitatory wave propagated downward, to the right, upward, and then exited from the recording area at the center of the upper margin. No wave fronts were observed during the period of 2,275–2,325 ms. Subsequently, the action potential reappeared at the same entry site as in Map 1, which is indicated by the isochrone curve of 2,350 ms in Map 2. In Map 2, the pattern of propagation is similar to that of Map 1, and all the other excitatory waves which appeared after the termination of stimulation also propagated by this pattern (data not shown). In these maps, a “blocked area” over which the excitation could not propagate appeared between the entry and exit of excitation (the thick green line indicated by the arrowhead). Note that this “blocked area” was not observed in the map of spontaneous excitation shown in Fig. 5. From these maps, it is reasonable to deduce that the excitable wave that exited from the recording area re-entered from the entry zone, and that the tachycardia-like excitation observed here resulted from the establishment of a circular pathway of the propagation of excitatory waves.

This type of tachycardia-like excitation was evoked in 5 of the 6 preparations tested. Two other examples of the conduction map of the tachycardia-like excitation are shown in Figs. 8 and 9. In Fig. 8, the whole pathway of circular conduction of excitation could be mapped. The excitatory wave propagated around the blocked area, which was located near the center of the recording area, in the counterclockwise direction with a cycle length of about 340 ms. This tachycardia-like excitation was quite stable and lasted for over 10 min.

In Fig. 9, the conduction pattern of the excitatory wave is more complex. In this preparation, two

blocked areas were observed: one is on the left half of the recording area, the other is around the ostium of the superior vena cava. Although the excitatory wave propagated basically in the clockwise direction with a regular cycle length of about 240 ms, as can be seen on the maps, two wave fronts were observed on both sides of the ostium of the superior vena cava (isochrone curves 0–50 ms in Map 1 and 25–75 ms in Map 2). These two waves converged in the center of the recording area, propagated upward in the leftmost area, and then divided again into two waves: one of them turned direction and propagated downward between the two blocked areas (route 1), and the other exited from the recording area near the left upper corner and re-entered near the right upper corner (route 2). Although the lengths of the two routes were different, the position where they converged was stable, suggesting the difference in the conduction velocity between the two routes. This tachycardia-like excitation was sustained for about 20 s and terminated spontaneously. In this preparation, another tachycardia-like excitation, of which the conduction pattern was similar, was evoked by the next train of stimulation after 50 s.

DISCUSSION

In this work, we conducted multiple-site optical recordings of the electrical activity in the atrium of the adult rat using a voltage-sensitive dye and a multiple-element photodiode array, and we mapped the excitation spread pattern of spontaneous excitation and of that disturbed by electrical stimuli. This is the first report of the optical mapping of electrical activity in the mammalian atrium.

Multiple-site optical recording of transmembrane voltage activity (MSORTV) with voltage-sensitive dye offers two principal advantages over conventional electrical techniques: it is possible to obtain optical recordings from the very small cells which are inaccessible to microelectrode impalement, and spatially-resolved mapping of electrical activity can be conducted by simultaneously monitoring electrical activities at multiple sites in the preparation [1–4]. Thus, the optical method has been applied to map the conduction patterns of cardiac excitation [3–6, 12–16]. We previously assessed the conduction pattern of spontaneous excitations in early embryonic hearts [3, 4] and adult frog hearts in which the contraction was suppressed by a low Ca^{2+} solution [5, 6]. In these experiments, the contraction of the heart muscle cells was either absent or small enough to permit optical assessment of the conduction pattern of excitation.

However, it has generally been difficult to control muscle contraction without altering the electrical excitability of the mammalian heart.

Recently, BDM has been identified as a chemical which suppresses muscle contraction without substantially changing membrane excitability [11], although the mechanism of action seems to be complex: this chemical affects both membrane currents and the contractile system (for a review, see Sellin and McArdle [17]). It has been reported that the BDM concentration used in this study (10–20 mM) affects the maximum rate of rise of cardiac action potential [11, 18], and it is possible that this may affect the conduction pattern of the action potential. Nevertheless, as shown in Figs. 1 and 2, no systematic changes were observed in the conduction of excitation in the BDM-containing solution (20 mM) in which the movement artefacts were suppressed. This chemical has already been introduced in the optical study of cardiac excitation [15].

In the typical conduction pattern shown in maps A and B2 of Fig. 4, although there were some differences among preparations, the excitatory wave did not propagate uniformly from the pacemaker in the sinus node region over the whole atrium. The present results support the concept of “non-radial” propagation in the mammalian atrium [19, 20]. Although the basic pattern of the “preferential route” was the same as that reported for the rabbit atrium [20], the incidence of the route along the upper (anterior) branch of the crista terminalis was low in this study of rat atrial preparations, suggesting interspecies differences in the excitation conduction pattern of the atrium. In contrast, using the bullfrog atrium, we previously noted a radial conduction pattern [5].

In addition, the atypical pattern shown in map B1 of Fig. 4 is of interest, and suggests that pathophysiological phenomena such as “ectopic atrial or AV-junctional rhythm” and “pacemaker shift” could also occur in this *in vitro* experiment, and that the optical method described here is a useful tool for the analysis of these phenomena.

This study is also the first report of the optical recording of tachycardia-like excitation in mammalian atrial preparations. As shown in the maps in Figs. 7–9, since the circular pathway of excitation was demonstrated, this tachycardia-like excitation seems to be categorized as “re-entry rhythms”. It is likely that this tachycardia-like excitation has some analogy with the “atrial flutter” in humans because of the fast regular rhythm and the stable re-entrant circuit [21]. We previously reported the circus movement tachycardia in frog atrium monitored optically [6].

Although the area of “unidirectional block” was not clearly demonstrated in this study, the area where the direction of the propagation of excitation was reversed between the initiation phase and sustained phase of the tachycardia-like excitation was observed in the maps. For example, at the left upper corner of the recording area shown in Fig. 7, the excitation evoked by the stimulation pulse propagated from the site of the electrode in the upper right direction at the initiation phase (data not shown), and subsequently, after the end of stimulation, propagated in the lower left direction in the sustained phase as shown in the maps. From this result, it is suggested that unidirectional block occurred around this region.

In the confirmation of the re-entrant circuit, slow conduction velocity is one of the important factors [21]. As can be seen in the maps, the conduction velocity was generally slower during the tachycardia-like excitation than that during the normal spontaneous spread of excitation (note that the interval of the isochrone curves is 5 ms for Fig. 4 and 25 ms for Figs. 7–9). In the initial phase of tachycardia-like excitation, the conduction velocity of the second and subsequent excitatory waves evoked by stimulation decreased about by half in comparison with that of the first wave, and this slow conduction continued through the sustained phase (data not shown). This reduction in conduction velocity by repetitive stimulation seems to play an important role in the establishment of the re-entrant circuit. That the experiment was conducted at room temperature is also likely to have been advantageous to re-entry.

In this study, the blocked area which did not appear during normal spontaneous excitation was clearly observed in each preparation during tachycardia-like excitation. The blocked area usually had an irregular string-like shape with a length of several millimeters and often contained the ostium of the superior or inferior vena cava. This type of blocked area was reported by Alessie *et al.* [22]. In addition, as shown in Fig. 9, a blocked area without apparent anatomical obstacles and one with a large anatomical obstacle were both observed. The conditions for the generation of the blocked area and the establishment of re-entrant circuit are still obscure at this time.

Experiments to study the excitation conduction pattern in the atrium have been carried out using rabbit and dog hearts. This study is the first of the functional mapping of the rat atrium. The main reason we used the rat was the size of the atrial preparation: in our optical recording system, the rabbit atrium is too large to mount on the stage of the microscope. To compare the present results with the data obtained from larger

mammals, we are planning to accommodate our recording system to larger preparations.

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REFERENCES

- Salzberg BM: Optical recording of electrical activity in neurons using molecular probes. *In: Current Methods in Cellular Neurobiology*, Vol. 3, Electrophysiological Techniques, ed. Barker JL and McKeivry JF, Wiley-Interscience, New York, pp 139–187, 1983
- Cohen LB and Leshner S: Optical monitoring of membrane potential: methods of multisite optical measurement. *In: Optical Methods in Cell Physiology*, ed. DeWeer P and Salzberg BM, Wiley-Interscience, New York, pp 71–99, 1986
- Kamino K: Optical studies of early developing cardiac and neural activities using voltage-sensitive dyes. *Jpn J Physiol* 40: 443–461, 1990
- Kamino K: Optical approaches to ontogeny of electrical activity and related functional organization during early heart development. *Physiol Rev* 71: 53–91, 1991
- Komuro H, Sakai T, Hirota A, and Kamino K: Conduction pattern of excitation in the amphibian atrium assessed by multiple-site optical recording of action potentials. *Jpn J Physiol* 36: 123–137, 1986
- Sawanobori T, Hirano Y, Hirota A, and Fujii S: Circus-movement tachycardia in frog atrium monitored by voltage-sensitive dyes. *Am J Physiol* 247: H185–H194, 1984
- Sakai T, Momose-Sato Y, Hirota A, Sato K, and Kamino K: Optical mapping of conduction patterns of excitation in the rat atrium using a voltage-sensitive dye. *Jpn J Physiol* 43 (Suppl 2): S85, 1993
- Sakai T and Hirota A: Optical mapping of reentry patterns of excitation in the rat atrium using a voltage-sensitive dye. *Jpn J Physiol* 45 (Suppl 1): S63, 1995
- Kamino K, Hirota A, and Fujii S: Localization of pace-making activity in early embryonic heart monitored using a voltage-sensitive dye. *Nature* 290: 595–597, 1981
- Salzberg BM, Obaid AL, Senseman DM, and Gainer H: Optical recording of action potentials from vertebrate nerve terminals using potentiometric probes provides evidence for sodium and calcium components. *Nature* 306: 36–40, 1983
- Li T, Sperelakis N, Ten Eick RE, and Solaro RJ: Effects of diacetyl monoxime on cardiac excitation-contraction coupling. *J Pharmacol Exp Ther* 232: 688–695, 1985
- Dillon S and Morad M: A new laser scanning system for measuring action potential propagation in the heart. *Science* 214: 453–455, 1981
- Salama G, Lombardi R, and Elson J: Maps of optical action potentials and NADH fluorescence in intact working heart. *Am J Physiol* 252: H384–H394, 1987
- Müller W, Windisch H, and Tritthart HA: Fast optical monitoring of microscopic excitation patterns in cardiac muscle. *Biophys J* 56: 623–629, 1989
- Davidenko JM, Pertsov AV, Salomonsz R, Baxter W, and Jalife J: Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle. *Nature* 355: 349–351, 1992
- Rohr S and Salzberg BM: Characterization of impulse propagation at the microscopic level across geometrical defined expansion of excitable tissue: multiple-site optical recording of transmembrane voltage (MSORTV) in patterned growth heart cell culture. *J Gen Physiol* 104: 287–309, 1994
- Sellin LC and McArdle JJ: Multiple effects of 2,3-butanedione monoxime. *Pharmacol Toxicol* 74: 305–313, 1994
- Coulombe A, Lefevre IA, Deroubaix E, Thuringer D, and Coraboeuf E: Effect of 2,3-butanedione 2-monoxime on slow inward and transient outward currents in rat ventricular myocytes. *J Mol Cell Cardiol* 22: 921–932, 1990
- James TN: The connecting pathways between the sinus node and A-V node and between the right and left atrium in the human heart. *Am Heart J* 66: 498–508, 1963
- Sano T and Yamagishi S: Spread of excitation from the sinus node. *Circ Res* 16: 423–430, 1965
- Janse MJ: Reentry rhythms. *In: The Heart and Cardiovascular System*, ed. Fozzard HA, Haber E, Jennings RB, Katz AM, and Morgan HE, Raven Press, New York, pp1203–1238, 1986
- Allessie MA, Lammers WJEP, Bonke IM, and Jan Hollen EE: Intra-atrial reentry as a mechanism for atrial flutter induced by acetylcholine and rapid pacing in the dog. *Circulation* 70: 123–135, 1984