Low-frequency extracellular potentials recorded from the sinoatrial node

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Abstract

Objective: To study the morphology of small extracellular potentials localized to the sinoatrial (SA) node and to elucidate its potential usefulness in evaluating SA node dysfunction. Methods: Extracellular potentials were recorded from the endocardial surface of the SA node in isolated right atrial preparations of rabbits through custom-made modified bipolar electrodes with high-gain amplification and a low-frequency (0.5–30 Hz) filter setting. Results: The potentials in and around the SA node under control conditions showed a variety of morphologies. In a small area near the leading pacemaker site, slow primary negative deflections were preceded by a gradual increase of the negativity (73.5±5.6 μV in amplitude, n=12). In the periphery of the SA node cranial and caudal to the leading pacemaker site, slow positive/negative deflections were recorded. In the septal side of the SA node showing very slow conduction, the electrograms showed slow primary positive deflections. Transient pacemaker shifts induced by atrial stimulation or vagal nerve stimulation were reflected well in morphologies of the extracellular potentials. In the presence of 20 μM TTX, wide and slow negative deflections were observed in the center and periphery of the SA node in association with extremely slow conduction restricted to a corridor-like area along the crista terminalis, whereas the atrial muscle surrounding the area was made inexcitable. In the presence of 1 μM nifedipine, the leading pacemaker site was shifted to the periphery of the SA node close to the crista terminalis. The negative deflection in the center and septal side of the SA node disappeared reflecting no excitation of the area. Conclusion: The endocardial extracellular electrograms recorded in and around the SA node under appropriate conditions reflect two dimensional activation sequences. They would provide useful information in recognizing the leading pacemaker site and alterations of the conductivity and excitability.

Keywords: Sinoatrial node; Extracellular potentials; Transmembrane potentials; Tetrodotoxin; Nifedipine

1. Introduction

Direct recording of low-frequency extracellular electrograms from the sinoatrial (SA) node through catheter electrodes was first introduced into clinical electrophysiology in the beginning of 1980s [1–4]. This technique was expected to have an advantage over indirect atrial pacing methods in assessment of the conduction disturbance from the SA node to the surrounding atrial muscle [5–7]. Identification of the SA node potential would also be important for preservation of the normal pacemaker activity in catheter ablation procedures to treat reentrant atrial tachyarrhythmias. The potential usefulness of this technique is, however, limited by large far-field potentials of atrial muscle close to the SA node [8,9].

Extracellular voltage changes in the vicinity of cardiac tissue are due to current crossing the cell membrane and associated current in the volume conductor surrounding the tissue. Any difference in transmembrane potential between electrically coupled cells would, therefore, cause a current through the cell interior, across the cell membrane and along the extracellular conductors, giving rise to a voltage gradient in the extracellular environment. Accordingly, if we can record extracellular potentials strictly localized to the SA node by efficient elimination of far-field potentials, their morphologies should reflect two dimensional activation sequences in and around the node. The present study was designed to verify this assumption. We recorded extracellular potentials from the endocardial surface of isolated rabbit right atria including the whole SA node using custom-made modified bipolar electrodes [10,11].

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since the proximity of the indifferent and active poles of the electrodes is expected to provide a high level of common-mode rejection, giving rise to a better isolation of localized potentials [12]. Transmembrane potentials were recorded simultaneously with the extracellular electrograms. The influence of transient pacemaker shift induced by atrial stimulation or vagal nerve stimulation was tested. The effects of tetrodotoxin (TTX) to block inward sodium current ($I_{Na}$) and nifedipine to block inward L-type calcium current ($I_{Ca}$) were also examined for better understanding of morphologies of the extracellular potentials localized to the SA node.

2. Methods

This investigation conforms with the Guide for the care and use of laboratory animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

2.1. Preparation

Rabbits weighing 1.5–2.0 kg were anesthetized with intravenous pentobarbital sodium (30–40 mg/kg). The chest was opened and the heart was rapidly excised and placed in Krebs-Ringer solution at 34°C. The right atrium was separated from the rest of the heart and opened by a longitudinal incision in the free wall to expose the endocardial surface. The atrium was then trimmed to leave a preparation approximately 15x15 mm, which included the SA node and some of the surrounding atrial muscle. The preparation was fixed in a tissue bath with the endocardial surface up and superfused with modified Krebs Ringer solution at 34°C. Solution flowed with the action of gravity at a rate of 20–25 ml/min through a heat exchanger. The bath temperature was monitored using a miniature thermistor. Experiments were carried out at 34°C because our experience is that all electrophysiological properties (including spontaneous cycle length, action potential configuration and activation sequence) are stable for much longer periods (>10 h) at 32–34°C than at 37°C. The composition of the solution was as follows (in mM): NaCl 120.3; KCl 4.0; CaCl$_2$ 1.2; MgSO$_4$ 1.3; NaH$_2$PO$_4$ 1.2; NaHCO$_3$ 25.2 and glucose 11.0 (pH 7.4). The solution was saturated with 95% O$_2$+5% CO$_2$, and bovine serum albumin (40 mg/l) was added to reduce the tissue edema during the long-time superfusion.

2.2. Recording

Extracellular potentials were recorded from 90 to 100 sites with a pair of modified bipolar electrodes (MBEs). Another pair of MBEs were used to record extracellular potentials from atrial muscle near the crista terminalis; this provided a reference signal for measuring the activation time. Each pair of MBEs was positioned using a calibrated X,Y,Z-micromanipulator with 0.1 mm precision. One pair of MBEs was also used to measure the dimensions of the preparation (e.g. Fig. 1A) at the start of each experiment by recording the coordinates of various anatomical landmarks; in this way the drawing and the recording sites shared common coordinates. Each pair of MBEs consisted of two 100 μm stainless-steel wires insulated to the tip and taped together. The indifferent pole of the electrodes was located 1.0 mm directly above the point at which the active pole contacted the endocardium. The potential difference between the two poles was recorded, and the signal was amplified with high gain (50–80 dB) and at a low frequency (0.5–30 Hz) filter setting. This filter setting is comparable to those employed in clinical electrophysiology to record extracellular SA node potentials [1–7]. Activation time at the recording site was identified by the initial negative deflection in each electrogram. The time interval between the exploring and reference electrodes was measured. The site showing earliest activation was taken to be the leading pacemaker site. Maps of the excitation spread from the leading pacemaker site were constructed by manually drawing isochrones at 5 or 10 ms intervals. The total time necessary to draw a complete map using this procedure was 45–50 min.

Transmembrane potentials were recorded from a surface cell using conventional glass microelectrodes filled with 3 M KCl (resistance, 40–50 meg ohm). For simultaneous recording of the transmembrane potential with the extracellular potential, a tip of the microelectrode for intracellular impalement was advanced in an oblique direction under the tip of the exploring MBEs. Another glass microelectrode was set close to the tip of the MBEs as a reference. These two microelectrodes were each connected by Ag–AgCl wires to a unity gain, high-input impedance differential amplifier. The upstream of the action potential was electrically differentiated to measure the maximum upstroke velocity ($V_{max}$).

Extracellular potentials and transmembrane action potentials were recorded using a chart recorder (thermal array recorder, RTA-1200; Nihon Kohden), magnetic tape (digital magnetic tape recorder, SONY, PC-108M; sampling rate 10 kHz) and Axotape software (Axon Instruments Inc., Burlingame, CA, USA) for later analysis.

2.3. Electrical stimulation

In some experiments a part of the atrial muscle or vagal nerve terminals were stimulated electrically to see the changes in the extracellular potentials recorded from the leading pacemaker site, which had been identified by mapping the potentials over the entire endocardial surface of the preparation. For atrial muscle stimulation, a pair of contiguous bipolar electrodes made of platinum wires (interpol of distance of 0.2 mm) was placed on a pectinate muscle lateral to the crista terminalis near the superior
Fig. 1. Spread of spontaneous excitation and the morphology of extracellular potentials. (A) Activation pattern of entire preparation. CT = crista terminalis; SVC = superior vena cava; IVC = inferior vena cava; SEP = interatrial septum; RA = right atrial appendage; RSARB = right branch of the sinoatrial ring bundle; LSARB = left branch of the sinoatrial ring bundle; ★ = position of a pair of modified bipolar electrodes (MBEs) to provide a reference point to measure the activation time at other sites. The extracellular potentials in a total of 95 other sites were recorded consecutively in 0.5–1.0 mm steps by another pair of MBEs. Isochrones of activation time were drawn every 5–10 ms. (B) The recording sites of extracellular potentials (open and solid circles) in the central square in panel A surrounded by dashed lines. (C) Extracellular electrograms recorded from 24 sites (solid circles in B).

edge of the preparation. Pulses used for stimulation were 1 ms in duration and twice the diastolic threshold in intensity.

The method for the stimulation of the vagal nerve terminals was essentially the same as employed in our previous studies [13,14]. In brief, a pair of 1 mm diameter silver wire electrodes (insulated to the tip) with a distance of 4 mm between the electrodes was placed on the endocardial surface of the preparation. One electrode was sited on the atrial muscle and the other was sited close to the leading pacemaker site. A train of 50 pulses (monophasic pulses of 100 μs in duration at 200 Hz) were applied during the spontaneous action potential at the leading pacemaker site (the train was triggered by the signal from a pair of MBEs situated on the atrial muscle opposite the leading pacemaker site). The intensity of the stimuli was subthreshold for excitation of atrial or nodal cells, but was sufficient to stimulate postganglionic nerve terminals. The stimulus voltage was adjusted (over the range of 10–30 V) until a typical negative chronotropic response was observed. Stimulation of β-receptors by noradrenaline released from sympathetic nerves was pre-
vented by the presence of 1 μM propranolol in the superfusate. All the effects of vagal stimulation reported here could be blocked by 2 μM atropine.

2.4. Drugs and data analysis

Tetrodotoxin (TTX, Sankyo, Tokyo, Japan) and nifedipine (Sigma Co., Ltd.) were added to the solution when required. Nifedipine was added from a stock solution (10 mM nifedipine in 50% methanol; final methanol concentration in Krebs-Ringer solution was 0.01%). During an experiment with nifedipine, the bottle containing the test solution was wrapped in metal foil to protect the nifedipine from light. Although TTX contains citrate buffer and this will buffer Ca²⁺, the decrease in Ca²⁺ concentration in Krebs-Ringer solution was small and will be ignored.

Data are presented as means or means ± SE unless otherwise specified. Students’ t-test (paired or unpaired as appropriate) or one way analysis of variance was used to test differences. A difference was considered significant at P<0.05.

3. Results

3.1. Spread of spontaneous excitation and extracellular potentials

In 12 preparations studied, the spread of spontaneous excitation under the control condition showed a similar pattern, which was in accordance with the reports by previous investigators [15–18]. Representative results are shown in Fig. 1A. Cycle length of spontaneous excitation of this preparation was constant (556-574 ms) throughout the experiments. The site of earliest activation (leading pacemaker) was located in the intercaval region 1.1 mm away from the medial border of the crista terminalis. From there, the excitation wave propagated preferentially in an oblique cranial direction toward the crista terminalis. The spread of excitation toward the interatrial septum was very slow and often almost blocked. Extracellular potentials recorded from the endocardial surface in and around the SA node (Fig. 1B) are shown in Fig. 1C. These potentials showed a variety of morphologies. In a small central area near the leading pacemaker site (l, m, p, q), a slow primary negative deflection (~25–30 ms in duration) was preceded by a gradual increase of the negativity. In the medial and cranial margin of the central area (g, k), a slow primary negative deflection of comparable duration appeared abruptly without any prepotentials. In the periphery of the SA node cranial and caudal to the leading pacemaker site (b, e, t, w), a slow positive deflection was followed by a slow negative deflection. In the septal side of the SA node showing very slow conduction (c, f, i, n), a primary positive deflection of wide duration (>30 ms) was observed. In the atrial muscle surrounding the SA node (j, o, u, x), the potentials showed a sharp positive deflection followed by a negative deflection with a duration ≥10 ms. The potentials recorded from the intercaval region close to the crista terminalis (a, d, s) had morphologies intermediate between the atrium and the SA node periphery.

Fig. 2 shows the extracellular potentials recorded from the leading pacemaker sites in four preparations. The dominant slow negative deflection (arrow head 2) with a peak amplitude of 48–91 μV (slope −1.5−−2.0 V.s⁻¹) was often interrupted by a small positive deflection reflecting a far-field potential of atrial excitation. The negative deflec-

![Fig. 2. Extracellular potentials recorded from the leading pacemaker sites in four preparations. (A) The extracellular potential recorded from three different preparations. Arrow heads indicate the negative diastolic slope (1), the negative upstroke slope (2) and the subsequent negative deflection at the time of repolarisation (3). (B) simultaneous recording of transmembrane potentials (top trace) and extracellular potentials (bottom trace) in another preparation.](https://academic.oup.com/cardiovascres/article-abstract/39/2/360/286855)
tion was preceded by a gradual increase of negativity (slope $-136$ to $-490 \mu V/s$) (arrow head 1), and normally followed by a second slow negative deflection (arrow head 3). When transmembrane action potentials were recorded simultaneously with the extracellular potentials (Fig. 2B), the initial slow negative deflection coincided with the upstroke phase of action potentials, whereas the second slow negative deflection with the repolarization phase of action potentials. The gradual increase of negativity in the extracellular potentials at the end of the electrical diastole corresponded with the terminal phase of pacemaker depolarization in the transmembrane action potentials.

Fig. 3 compares the configuration of four extracellular and transmembrane action potentials recorded simultaneously perpendicular to the crista terminalis and through the leading pacemaker site (the activation pattern of this preparation was shown in Fig. 6A). The action potential recorded from the leading pacemaker site (g) showed the steepest diastolic depolarization and the earliest upstroke phase with $V_{\text{max}}$ of $6.3 \mu V/s$. The extracellular potential had a typical morphology in the center of the SA node with smooth transition from a gradual increase of negativity to a slow negative deflection. Latent pacemaker cells in the periphery of the SA node close to the crista terminalis (site e) were then depolarized; the action potential in the periphery showed a more distinct transition from phase 4 to phase 0 depolarization, and extracellular potentials recorded from these sites, the initial slow negative deflection appeared abruptly or followed a small positive deflection. The action potential recorded from atrial muscle in the crista terminalis (site d) had the highest $V_{\text{max}}$ (95.0 $\mu V/s$) with no pacemaker potential. The extracellular potential had sharp positive/negative deflections. The septal border of the SA node (site i) was excited from the opposite side by a circulating wave front (Fig. 6A). The action potential duration was longest at the leading pacemaker site and shortest at the atrial muscle in the crista terminalis. The SA node cell at the leading pacemaker site was, therefore, depolarized first but repolarized last. This means that the leading pacemaker site would be a current source during both depolarization and repolarization, since the cells in the SA node and surrounding atrial muscle are coupled with each other through gap junctions [15,19–21]. The dual slow negative deflection of extracellular potentials at the leading pacemaker site (g: arrow heads 2 and 3) could be interpreted by such sequences of depolarization and repolarization (see Section 4).

### 3.2. Pacemaker shift

Influence of pacemaker shift on the extracellular potentials was examined by electrical stimulation of atrial muscle or vagal nerve terminals. Fig. 4 shows an experiment with atrial muscle stimulation. The transmembrane action potential recorded from the leading pacemaker site had a smooth transition from phase 4 to phase 0 depolarization, and extracellular potentials recorded from the same site had the typical morphology for the center of the SA node (slow negative deflections preceded by a gradual increase of the negativity). The action potential elicited by a premature atrial stimulation during late diastole had a sharp transition from phase 4 to phase 0 depolarization. The extracellular potential of the stimulated beat showed a slow primary positive deflection without any change in the negativity before the excitation. The normal leading pacemaker-type morphology of extracellular potentials was resumed for the subsequent spontaneous beats.

Fig. 5 shows an experiment with vagal stimulation.

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**Fig. 3.** Comparison of transmembrane potential (TMPs) and extracellular potentials (ECPs) from four sites (g, e, d, i) along a line perpendicular to the crista terminalis and through the leading pacemaker site in the preparation illustrated in Fig. 6a (control condition). The potentials are aligned in a sequence of spontaneous activation. Vertical dotted lines indicate the initiation (0 ms) of spontaneous activation. Arrow heads on the extracellular potentials at site g indicate the negative diastolic slope (1), the negative upstroke slope (2) and the subsequent negative deflection at the time of repolarisation (3).
Fig. 4. Pacemaker shift elicited by premature atrial stimulation. Top and middle traces are transmembrane potentials (TMPs) and extracellular potentials (ECPs), respectively, recorded from the leading pacemaker site of the sinoatrial node (SAN). Bottom trace shows ECPs recorded from atrial muscle (AM) lateral to the crista terminalis.

Transmembrane and extracellular potentials were recorded from the leading pacemaker site under control conditions. The atrial electrogram was recorded as a reference; there was an activation delay of 24 ms from the leading pacemaker site. Brief vagal stimulation resulted in a hyperpolarization of the transmembrane potential and suppression of pacemaker activity; the spontaneous cycle length (678 ms in control) was prolonged to 1.345 ms. The first action potential after vagal stimulation showed an abrupt transition from phase 4 to phase 0. The time difference between the action potential upstroke and the atrial electrogram was also reduced (9 ms). The morphology of extracellular potential changed from the leading pacemaker-type to the periphery-type after vagal stimulation. The normal leading pacemaker-type morphology of the extracellular potentials was resumed for the subsequent beats.

3.3. Effect of $I_{Na}$ block by TTX

The effect of $I_{Na}$ block by TTX was examined in five preparations. Application of 20 μM TTX for 40 min resulted in a significant increase in the cycle length of spontaneous excitation (from 597±19 ms in control to 640±13 ms after TTX, n=5), but regular spontaneous beating was well preserved in the presence of TTX. Characteristic changes were observed in the activation pattern of the preparation and in morphology of extracellular potentials. Representative data are shown in Fig. 6. In this preparation under control conditions, the earliest

Fig. 5. Pacemaker shift induced by vagal nerve stimulation. Top and middle traces are transmembrane potentials (TMPs) and extracellular potentials (ECPs), respectively, recorded from the leading pacemaker site of the sinoatrial node (SAN). Bottom trace shows ECPs recorded from atrial muscle (AM) lateral to the crista terminalis. To stimulate postganglionic vagal nerve terminals, a train of 50 pulses (100 μs in duration at 200 Hz) of 16 V were applied during the spontaneous action potential at the leading pacemaker site. The experiment was carried out in the presence of 1 μM propanolol.
excitation (leading pacemaker) was located in the middle of the intercaval region 1.0 mm away from the crista terminalis (Fig. 6A). From there, the excitation propagated preferentially towards the crista terminalis. The propagation toward the septal side was very slow and partially blocked. After the treatment with TTX, the site of earliest excitation was shifted a little (~0.5 mm) toward the inferior side. The distance from the leading pacemaker to the medial border of the crista terminalis (1.1 mm) was similar to control. From the new leading pacemaker site, the excitation spread very slowly in both cranial and caudal directions along the crista terminalis. Atrial muscles in the lateral side of the crista terminalis and in the septum were not excited (shaded area in Fig. 6B). The most prominent change of extracellular potentials in the center and periphery of the SA node was the widening of the dominant slow negative deflection. At the new leading pacemaker site (j), the slow and wide negative deflection (~70–80 ms in duration) was preceded by a gradual increase of the negativity. In addition, the second slow negative deflection, which had been observed in control, disappeared after TTX. There were no sharp positive/negative deflections in atrial muscles in the lateral side of the crista terminalis and in the septum; extracellular potentials in these regions showed slow miniature deflections or fluctuations (sites d, i and m).

Fig. 7A summarizes the amplitude of the main negative deflection of extracellular potentials in three different regions of the five preparations before and after application of TTX. The data were obtained from the potentials showing the leading pacemaker-type morphology under control conditions (within 0.5 mm from the leading pacemaker site), periphery-type morphology (cranial and caudal sides of the leading pacemaker site) and atrial electrograms (lateral to the crista terminalis). The potential amplitude in the atrial muscle was markedly decreased (by 97% on average). The potential amplitude in the periphery (cranial and caudal sides) of the SA node was decreased a little (by 32% on average), whereas the potential amplitude in the center was unaffected.

Fig. 8 compares the configuration of four extracellular and transmembrane potentials recorded simultaneously in the presence of 20 μM TTX along a line perpendicular to the crista terminalis (the same preparation as shown in Figs. 3 and 6). The action potential at the new leading pacemaker site (j) was similar to the leading pacemaker...
Fig. 7. Amplitude of the main negative deflection of extracellular potentials. The data were obtained from (i) potentials showing the leading pacemaker-type morphology under control condition in the center of the sinoatrial node (within 0.5 mm from the leading pacemaker site), (ii) potentials showing peripheral-type morphology (cranial and caudal sides of the leading pacemaker site) and (iii) atrial electrograms (lateral to the crista terminalis). (A) Absolute values (top) before (control) and 40–60 min after application of 20 μM TTX and percentage changes after TTX (bottom) in five preparations (n=8 for center, n=15 for periphery, n=40 for atrial muscle). (B) Absolute values (top) before (control) and 40–60 min after application of 1 μM nifedipine and percentage changes after nifedipine (bottom) in three preparations (n=5 for center, n=9 for periphery, and n=20 for atrial muscle). Value are presented in means±sem. *Significantly different from control at P<0.05.

Fig. 8. Comparison of transmembrane potentials (TMPs) and extracellular potentials (ECPs) in the presence of 20 μM TTX. The recordings were obtained from five sites (j, a, h, d, i) in the preparation illustrated in Fig. 6b. The potentials are aligned in a sequence of spontaneous activation. Vertical dotted lines indicate the initiation (0 ms) of spontaneous activation. Arrow heads on the extracellular potential at site j indicate the negative diastolic slope (1), the negative upstroke slope (2), and the subsequent positive deflection at the time of repolarisation (3).
lateral side of the crista terminalis (site d) and in the septum (site i) showed a resting potential of \(-69\sim-71\) mV with no excitation. There was no negative deflection in the extracellular potential recorded from these sites. Because of a substantial activation delay in the periphery of the SA node in the presence of TTX, the cell at the new leading pacemaker site repolarized first despite its long action potential duration. This may explain the slow positive deflection of the extracellular potential (arrow head 3) following the slow negative deflection at the new leading pacemaker site (j) (see Section 4).

We estimated conduction velocities of spontaneous excitation in the five preparations before and after application of 20 \(\mu\)M TTX by plotting distances traveled by isochrones in a direction along the crista terminalis towards the superior vena cava, and in a direction across the crista terminalis toward the right atrial appendage. The data plots under control conditions revealed two distinct conduction velocities; a slow velocity (\(\Theta_2\)) near the center of the SA node and a fast velocity (\(\Theta_1\)) from the periphery of the SA node to the surrounding atrial muscle. The average value of \(\Theta_2\) is 11–12 times larger than that of \(\Theta_1\) (Table 1). The data plots after TTX showed almost uniform conduction in either direction with velocities similar to the slow conduction before TTX (Table 1).

### 3.4. Effect of I\(_{Ca}\) block by nifedipine

The effect of I\(_{Ca}\) block by nifedipine was examined in three preparations. After application of 1 \(\mu\)M nifedipine for 40 min, cycle length of spontaneous excitation of these preparations was shortened significantly (from 577±6 ms in control to 457±55 ms after nifedipine, \(n=3\)), and their regular spontaneous activity was well preserved. In the presence of nifedipine, the leading pacemaker site was shifted from the center to the periphery of the SA node.

Representative results are shown in Fig. 9. Control data of this preparation was presented before (Fig. 1). The new leading pacemaker site of this preparation after the application of nifedipine was located on the medial edge of the crista terminalis (1.0 mm lateral to the original leading pacemaker site in control). From there, the excitation spread rapidly in both cranial and caudal directions along the crista terminalis and toward the right atrial appendage. The propagation towards the septum was, however, inhibited and blocked in the intercaval region ~1 mm away from the crista terminalis (Fig. 9A). The treatment with nifedipine resulted in large alterations in the morphology of extracellular potentials (Fig. 9B). The extracellular potentials near the center (site m) and in the periphery of the SA node on the cranial and caudal sides (sites b, e, t) all showed small and slow positive deflections without negative ones like the potentials in the septal side of the SA node showing very slow conduction under control conditions. Transmembrane potentials recorded from these areas showed only electrotonic deflections from the resting level, but no excitation (Fig. 9C). At the new leading pacemaker site (k), the extracellular potentials showed primary negative deflections, and the transmembrane action potential had a prominent pacemaker slope (Fig. 9B, C). In the atrial muscle lateral to the crista terminalis (site j), the extracellular potentials and the transmembrane potentials were essentially unchanged after nifedipine except for an appreciable shortening of action potential duration.

Qualitatively similar changes in extracellular and transmembrane potentials were observed in the remaining two preparations. Thus, the leading pacemaker site was shifted to the medial border of the crista terminalis. The slow negative deflection of the extracellular potentials in the center of the SA node (original leading pacemaker site) changed into slow positive deflections; the changes were accompanied by disappearance of excitation in the transmembrane potentials.

Table 1

| Conduction velocities of spontaneous excitation before and after application of 20 \(\mu\)M tetrodotoxin | Across the crista terminals |
|-----------------|------------------|------------------|
| \(\Theta_1\) (cm.s\(^{-1}\)) | \(\Theta_2\) (cm.s\(^{-1}\)) | \(\Theta_1\) (cm.s\(^{-1}\)) | \(\Theta_2\) (cm.s\(^{-1}\)) |
| Control (\(n=5\)) | 4.5±1.2 | 49.7±10.2 | 3.0±1.1 | 36.3±6.5 |
| TTX (20 \(\mu\)M) | 3.8±0.8 | 6.2±1.1\(*\) | 1.7±1.0\(*\) | – |

The conduction velocity of spontaneous excitation was measured by plotting distances traveled by isochrones in a direction along the crista terminalis toward the superior vena cava, and in a direction across the crista terminalis toward the right atrial appendage. \(n=\)number of preparations. \(\Theta_1\)=slow conduction velocity near the center of the SA node (within 1.5 mm from the leading pacemaker site). \(\Theta_2\)=fast conduction velocity from the periphery of the SA node to the surrounding atrial muscle. \(\Theta_3\) across the crista terminalis was not measured after TTX because the atrial muscle lateral to the crista terminalis was made inexcitable. Values are presented as means±s.e. * , significantly different from control at \(P<0.05\).

### 4. Discussion

The extracellular potential changes associated with the pacemaker activity of the SA node were first described by Cramer et al. [22] in experiments on isolated rabbit atria with use of high-gain direct-coupled amplification. They found a slow diastolic slope (–30 to –90 \(\mu\)V.s\(^{-1}\)) and a
more rapid upstroke slope (−400 to −800 μV/s) closely correlated to phase-4 and phase-0 depolarization of dominant pacemaker cells. These potentials were, however, interrupted by large high-frequency deflections as cells in the surrounding atrium depolarized [22]. Similar extracellular potentials were recorded by the same group of investigators from the dominant pacemaker site of the SA node in dog right atria in vitro as well as in vivo (endocardial and epicardial surface) with the use of low frequency (0.1–50–100 Hz) band pass filters [23]. In endocardial mapping experiments on isolated rabbit right atria including the whole SA node, Haberl et al. [16] confirmed that phase-4 and phase-0 depolarizations at the leading pacemaker site are reflected in a slow diastolic negative slope followed by a more rapid negative slope in the direct coupled extracellular electrograms. They also found a large spectrum of variations of the extracellular SA node potential depending on the length of sinoatrial conduction time. In the sinoatrial conduction time was relatively long (>25 msec), a smooth transition from the diastolic to the upstroke slopes was recognized in the extracellular electrograms. If the sinoatrial conduction time was relatively short (≤25 msec), however, the transition was more rapid, and the upstroke slope was interrupted by subsequent large high-frequency deflections reflecting the atrial activity [16].

The leading pacemaker-type extracellular potentials observed in the present study are consistent in part with these previous reports [16,22,23] in terms of the gradual transition from the diastolic negative slope to the more rapid negative slope during the upstroke phase. The interruption of the upstroke slopes by sharp deflections reflecting the atrial activity was, however, minimal or negligible in our electrograms. This difference may be attributed to different recording techniques. The tip diameter of unipolar electrodes employed in the present study (0.1 mm) is much smaller than that used by previous investigators (0.2–0.5 mm). The indifferent electrode was placed only 1 mm above the recording site in the present study, whereas it was placed far away (≤15 mm) from the recording site in the experiments by Cramer et al. [22,23] and Haberl et al. [16]. A similar interelectrode distance (10–15 mm) is used in clinical electrophysiology to record the SA node potential [1–7]. In the SA node region, the amount of the volume conductor is very small and the electrical coupling between cells is weak compared to those in atrial muscle [20,21]. Accordingly, extracellular potential changes localized to the SA node are expected to

Fig. 9. Spread of spontaneous excitation and the morphology of extracellular potentials after application of 1 μM nifedipine (the same preparation as shown in Fig. 1). The extracellular potentials (ECPs) were recorded consecutively in 0.5–1.0 mm steps. (A) The recording sites of ECPs (open and closed circles) and isoschrones of activation every 5–10 ms are shown. A star indicates the leading pacemaker site before nifedipine (control). No excitation was observed in the shaded area. (B, C) The ECPs recorded from 16 sites (closed circles from a to x) and the transmembrane potentials (TMPs) recorded from 3 sites (j,k and m) are shown.
be easily interrupted or masked by relatively large far-field potentials from the atrial muscle mass surrounding the SA node. In the present experiments, such far-field signals were minimized by a high level of common-mode rejection with a good preservation of local signals.

The negative upstroke of the leading pacemaker-type potentials was normally followed by the second slow negative deflection. The second slow deflection corresponded to the repolarization phase of the action potential (Fig. 2). Because of a large variation of action potential duration from the center through periphery of the SA node to the atrial muscle (Fig. 3), the cells at the leading pacemaker site depolarized first but repolarized last in the right atrial preparation. Consequently, the leading pacemaker site may play a role as a current source not only during depolarization but also during repolarization. This may result in the characteristic dual negative deflections in the extracellular potentials. In the experiments using TTX (Fig. 8), the depolarization in the periphery of the SA node was delayed, and the repolarization in the leading pacemaker site preceded the repolarization in the periphery. This reversal of the repolarization sequence was associated with a positive deflection of the extracellular electrogram following the negative upstroke slope at the leading pacemaker site.

In the experiment with premature atrial stimulation (Fig. 4), the morphology of the extracellular electrograms changed abruptly from the normal leading pacemaker-type potential to a primary positive deflection. Following vagal stimulation (Fig. 5), the morphology of the extracellular electrograms at the leading pacemaker site changed transiently to the peripheral-type in association with a transient change of action potential configuration. Thus, beat-to-beat changes in activation sequence in response to pacemaker shift are well reflected in the extracellular electrograms.

We examined the effects of TTX and nifedipine on the morphology of the extracellular electrograms recorded in and around the SA node. In the presence of 20 μM TTX, the negative deflections corresponding to the upstroke phase of the action potential in the center and periphery of the SA node became much wider in association with extremely slow conduction restricted to a corridor-like area along the crista terminalis (Fig. 6). In addition, the atrial muscle surrounding the area was made inexcitable, resulting in an elimination of negative deflections in the extracellular electrograms. In the presence of 1 μM nifedipine, the leading pacemaker site was shifted to the periphery of the SA node close to the crista terminalis. Along with this pacemaker shift, the negative deflections in the center and periphery (cranial and caudal sides) of the SA node were minimized or nearly abolished reflecting no excitation in these areas. These effects of TTX and nifedipine can be attributed to the regional differences in the role of $I_{Na}$ and $I_{Ca}$ in electrical activity of the SA node.

It was demonstrated in our recent experiments [24] using small ball-like specimens isolated from different regions of rabbit SA node that a high concentration (20 μM) of TTX had no effect on electrical activity in small balls from the center, but it caused a marked decrease in $V_{max}$ and spontaneous activity in association with a decrease of the take-off potential in small balls from the periphery. In contrast, 2 μM nifedipine abolished the spontaneous action potential in small balls from the center, whereas accelerated the rate of spontaneous action potentials in small balls from the periphery near the crista terminalis through a shortening of action potential duration and an increase in the slope of the pacemaker potential [24]. $V_{max}$ and the amplitude of action potential in small balls from the periphery was reduced only a little by the nifedipine treatment [24]. From these findings, it was suggested that $I_{Ca}$ plays an obligatory role in pacemaker activity in the center of the node, but it does not play such a role in the periphery: $I_{Na}$ rather than $I_{Ca}$ may play a major role in pacemaker activity in the periphery. In small balls from the periphery, block of $I_{Na}$ did not lead to the loss of electrical activity despite a marked decrease in $V_{max}$ [24]. This is consistent with the present data; a corridor-like area of intercaval region along the crista terminalis remained excitable in the presence of 20 μM TTX, although the conduction velocity in that area was extremely low (Fig. 6). In the peripheral regions, $I_{Ca}$ may take over the role played by $I_{Na}$ for the tissue excitability after block of $I_{Na}$.

In the SA node, it is generally believed that calcium channel blockers have a negative chronotrophic effect [25–28]. In the present experiments with 1 μM nifedipine, however, the spontaneous cycle length was shortened significantly (by 21% on average) after application of the drug. We reported a similar increase of the spontaneous rate of isolated rabbit right atria after application of 2 μM nifedipine, although the change was statistically insignificant [24]. Boyett et al. [13] also observed in rabbit right atria that 6 μM nifedipine caused a transient increase of rate before complete cessation of the spontaneous excitation. The conflicting findings are possibly due to concentration-dependence of the chronotropic effect of nifedipine.

Cramer et al. [22,23] and Haberl et al. [16] presented the extracellular electrograms of SA node in reversed polarity to facilitate comparison with the configuration of transmembrane action potentials. The polarity of the SA node potential recorded from the endocardial surface of human patients through catheter electrodes is also usually reversed to see the upward going potentials at the time of depolarization [1—7]. The present results suggest that this convention does not make any sense, and may be misleading. Deflections of the extracellular unipolar electrograms are known to be caused by local current through the volume conductor in association with a propagation of excitation; an activation wave front approaching the recording site...
from upstream produces a positive deflection, whereas a wave front going away from the recording site downstream produces a negative deflection. Accordingly, an alteration of activation sequence can produce a fundamental change of extracellular potentials even though a change of transmembrane action potential is minimal as presented in Fig. 4. We propose that the extracellular electrograms recorded in and around the SA node should be presented in the normal polarity to facilitate the translation of their morphology into the two-dimensional propagation patterns of excitation.

The techniques employed in the present study could be applied in clinical electrophysiology for recording of low-frequency extracellular potentials from the SA node; a close setting of the indifferent electrode just above the recording site would result in a better isolation of potentials localized to the SA node. If such a technique is combined with newly developed catheter-based electroanatomical mapping technology using a magnetic field [29,30], morphologies of the SA node electrograms and their spatial distribution could provide quite valuable information to understand the complex pathophysiology of SA node dysfunction. However, the clinical implication might be limited by species differences in the anatomical architecture of the SA node. The rabbit SA node is a superficial structure just beneath the endocardium, whereas the SA nodes of dogs, pigs and humans are embedded within atrial tissue (atrial muscle and transitional cell layers are in between the endocardium and the nodal cells) [17,31–34]. In the latter group of animal species (including humans), the extracellular potentials recorded from the endocardial surface would include breakthrough of the conducted impulse, which may take place at different sites. In experiments using perfused right atria of dogs, Bromberg et al. [33] demonstrated a spatial and temporal discrepancy between dominant pacemaker activity recorded through glass microelectrodes and the earliest extracellular activation estimated by their multi-channel unipolar endocardial mapping system. According to their results, primary negativity does not seem to correlate with the location of the dominant pacemaker, but rather with the exit points of excitation to the atrial myocardium. In the present experiments, the dominant pacemaker area was always well recognized from the characteristic morphology of endocardial extracellular potentials. The discrepancy may be attributed to the species difference of the anatomical architecture of the SA node, or to the different methods employed. In the experiments by Bromberg et al. [33], the dominant pacemaker activity, which is restricted to a small area (~0.5 mm² in dogs), might have been failed to be reflected in the extracellular potentials because of relatively large interelectrode distance (3–5 mm). Different conditions of amplification (gain and filter setting) may have also affected the morphologies of the electrograms. Further in-vivo experimental studies in dogs or pigs will be required before the possible clinical use of the present recording technique can be evaluated.

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