AUTOMATICITY INDUCED BY Ca++ CHELATING AGENTS IN ISOLATED RABBIT LEFT ATRIA

Noboru TODA
Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan
Accepted July 20, 1974

Abstract—Transmembrane potentials were recorded from single cells of isolated rabbit left atria. Action potentials generated by electrical stimulation were markedly depressed or completely abolished by Ca++ chelating agents, EGTA and EDTA (4 and 5 mM), in association with a decrease in the resting membrane potential. Automaticity was initiated. EGTA-induced automaticity was abolished by the addition of 2 mM Ca++. Changes in the membrane potential induced by 5 mM EGTA were reversed by 3 to 5 mM Ca++. Reduction in [Na+]_o from 162 mM to 74 or 52 mM delayed the initiation of automaticity, slowed the automatic rate, shortened the duration of automaticity and decreased the action potential amplitude. Magnesium ions (0.1 to 0.2 mM) abolished automaticity induced by EGTA. The resting potential was not significantly changed. Increasing concentrations of Mg++ to 0.5 to 2 mM made preparations unresponsive to electrical stimulation. The addition of Mn++ in concentrations higher than 2 mM abolished EGTA-induced automaticity in association with increased resting potential. Electrical stimulation generated action potentials, which were not abolished by increasing concentrations of Mn++ but by tetrodotoxin. Atrial contractions abolished by EGTA were temporarily restored by 3 mM Mn++. Automaticity induced by these chelating agents may be associated with increased resting permeability for Na+, permitting membranes to depolarize to a level at which action potentials generate spontaneously. It appears that upon excitation Mg++ decreases the membrane permeability for Na+.

Calcium chelating agent, EDTA (ethylenediamine tetraacetic acid), produces automaticity in isolated canine Purkinje fibers and ventricles (1) and rabbit ventricles (2) in association with a decrease in the resting membrane potential and a prolongation of the duration of action potentials. It has been demonstrated that Ca++ and Na+ are antagonistic with regard to their effects on the action potential (3) and that prolongation of action potential duration induced by Ca++ chelating agents (4) are related directly to extracellular concentrations of Na+. The membrane effect of Ca++ chelating agents may thus be due to removal of Ca++ from a variety of sites on and within the muscle cell, permitting Na+ to move easily across membranes upon excitation and during diastole. However, removal of both Ca++ and Mg++ from bathing media fails to produce automaticity and changes in the membrane potential such as are caused by EDTA (1), and EDTA-induced changes are not totally reversed by Ca++ (2). Thus, it may be postulated that membrane effects of the chelating agent are associated not only with removal of Ca++ and Mg++ from the cell but also with another mechanism of action.

Magnesium ions decrease the threshold potential and the slope of diastolic depolarization in S-A nodal pacemaker fibers, resulting in bradycardia (5); this effect is anta-
gonized by Ca$^{2+}$ (6). There are data which suggest direct competition between Ca$^{2+}$ and Mg$^{2+}$ for binding sites on the cell surface (7, 8). Such being the case, it may be possible that preferential chelation of Ca$^{2+}$ to Mg$^{2+}$ by EGTA (ethyleneglycol bis-(β-aminoethylether)-N, N'-tetraacetic acid) causes changes in the membrane potential which are different from those obtained when EDTA is applied.

It has been reported that Mn$^{2+}$ inhibits slow inward current carried by Ca$^{2+}$ and Na$^+$ when membranes of cardiac muscle cells are depolarized to approximately -40 mV (9) and also that Mn$^{2+}$ enters into cells when preparations are exposed to Ca$^{2+}$-free media (10).

The present study was undertaken to investigate the effect of Ca$^{2+}$ chelating agents on the transmembrane potential of isolated rabbit left atria and to analyze quantitatively effects of Ca$^{2+}$, Mg$^{2+}$ and Mn$^{2+}$ in atria showing EGTA-induced automaticity.

**MATERIALS AND METHODS**

Sixty-one albino rabbits of both sexes, weighing 1.8 to 2.3 kg, were sacrificed under ether anesthesia by bleeding from common carotid arteries. The heart was rapidly removed and left atrial preparations were prepared as described in an earlier report (11). The specimen was fixed horizontally between hooks under a resting tension of 300 to 450 mg, endocardial surface uppermost, in the nutrient solution, which was maintained at 30 ± 0.5°C and aerated with a mixture of 95% O$_2$ and 5% CO$_2$. Hooks anchoring the cut end of the atrium were connected to an electronic stimulator and those fixing the atrial appendage were to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo). The composition of the bathing solution was as follows (mM): Na$^+$, 162.1; K$^+$, 5.4; Ca$^{2+}$, 2.2; Cl$^-$, 157.0; HCO$_3^-$, 14.9; dextrose, 5.6. The pH of the solution was 7.2 to 7.4. Osmotic adjustment was not made when Ca$^{2+}$, Mg$^{2+}$ or Mn$^{2+}$ was added to the solution. Preparations were allowed to equilibrate for 60 to 120 min with the normal solution and for 20 to 30 min with experimental solutions before experiments were commenced.

Left atrial preparations were driven by a train of 1 msec electrical pulses of about twice threshold intensity, applied at a frequency of 60/min, unless automaticity was induced. Stimulus pulses were provided by an electronic stimulator (Type MSE-3R, Nihonkoden Kogyo Co.).

Four rabbits were pretreated with intravenous injections of reserpine, 0.5 mg/kg, for 2 successive days and sacrificed 16 to 20 hr after the 2nd injection. In order to confirm the depletion of functional noradrenaline, the chronotropic response of right atrial preparations to tyramine was tested. The average rate before tyramine was 92 ± 1.8 beats/min, and the rates after tyramine in concentrations of 5 · 10$^{-6}$ and 2.5 · 10$^{-5}$ M were 93 ± 2.7 and 100 ± 6.1 beats/min (N = 4), respectively, in reserpine-pretreated atria. These values obtained in normal atria were 105 ± 3.4, 122 ± 8.8 and 152 ± 8.6 beats/min (N = 6), respectively.

Intracellular recordings were effected by the use of microelectrodes having resistances of 10 to 30 megohms. The transmembrane potential was recorded from a VC-7 oscil-
oscilloscope (Nihonkohden Kogyo Co.) on films moving at speeds of 5 and 10 cm/sec for the measurement of parameters of the membrane potential except the maximum rate of depolarization. Speeds from 0.1 to 2 cm/msec were used for the measurement of the maximum rate of rise. Parameters of the membrane potential measured were: (a) resting potential or maximal diastolic potential; (b) threshold potential when automaticity was induced; (c) overshoot (action potential amplitude—resting potential; when the action potential was less than the resting potential, the difference was expressed as ‘minus’ overshoot); (d) durations at the level of 10% and 90%, action potential amplitude which will be termed 10% and 90% duration in this report; and (e) maximum rate of depolarization which was estimated from a straight line inscribed through the portion clearly representing the period of most rapid depolarization. Transmembrane potential and either atrial contraction or extracellular recording with bipolar silver electrodes placed on the atrial appendage were also displayed on a two-channel oscillograph (Sanei Sokki Co., Tokyo). Absolute values obtained in control and experimental solutions were compared statistically. Automatic rates were calculated from mean values of ten measurements of the cycle length between spontaneously-appearing action potentials. Results shown in the text, figures and tables are presented as mean values ± standard errors of the means. Significance of differences were estimated by the Student’s t test.

Concentrations of Ca++ in the bathing medium were determined spectrophotometrically by the use of orthocresolphthalein complexone (12).

Drugs were added directly to the bathing medium, and the agents used in this study included ethyleneglycol bis-(β-aminoethyl ether)-N, N'-tetraacetic acid (EGTA), ethylenediamine tetraacetic acid disodium salt (EDTA), tetrodotoxin (Sankyo), tyramine hydrochloride, atropine sulfate and reserpine (Serpasil, Ciba-Geigy). The EGTA was dissolved in 1 N NaOH.

RESULTS

Automaticity induced by EGTA and EDTA

In left atria driven electrically at a frequency of 60/min the addition of EGTA and EDTA in a concentration of 2 mM caused a marked reduction in the amplitude of action potentials and a moderate decrease in the resting potential (significant difference from control, P<0.01) (Fig. 1). The 10% duration was significantly prolonged (P<0.01). The estimated maximum rate of depolarization was reduced; the mean values in control and EGTA (2 mM)-added solutions were 72.5±2.8 V/sec (N=37) and 25.1±1.5 V/sec (N=32), respectively. When the concentration of the chelating agents was raised to 5 mM, action potentials generated by electrical stimulation were markedly depressed or completely abolished in association with a further decrease in the resting potential. In addition, automaticity occurred (Fig. 2). The rate of automatic activities was stabilized after 30 to 40 min exposure to the chelating agent. In 8 out of 15 atria electrical activity recorded from “A” region (Table 1) corresponded to that from “D” (extracellular recording), whereas in the remaining 7 activities from “A” and “D” were independent of
Fig. 1. Modification by EGTA and EDTA of parameters of the membrane potential from left atria. OS = overshoot; ThP = threshold potential when automaticity was induced; MDP = maximal diastolic potential or resting potential; 10% D = 10% duration; 90% D = 90% duration. Vertical bars represent standard error of means. Figures in parentheses indicate the number of penetrations obtained from 3 to 25 preparations. *values obtained 3 to 10 min after automaticity was induced. † values obtained 15 to 30 min after automaticity was induced.

Fig. 2. Effects of EGTA on the membrane potential and contractility of a left atrium. Tracings from top to bottom: time scale (1 sec); membrane potential; contraction. Downward deflections in membrane potential recordings represent stimulation artifacts.
each other. In order to exclude re-entrant impulses from other regions, the preparations were separated into 4 (approx. 2 × 2 mm) as shown in the figure in Table 1, and the incidence of automaticity and the mean automatic rate in each preparation were compared. Results are summarized in Table 1. As the incidence of automaticity and the rate were the highest in "A", recordings were obtained from this region in the whole left atrium in the subsequent experiments. When activities from "A" and "D" regions actually did correspond, action potentials were recorded only from cells of "A" which depolarized on more than 40 msec in advance of excitation of the "D" region.

In preparations showing spontaneity, the maximum rate of rise averaged 3.2 ± 0.2 V/sec (N=46), which was similar to the mean value of the maximum rate of rise obtained from S-A nodal pacemaker action potentials (3.3 ± 0.2 V/sec, N=15). Mean values of parameters of action potentials obtained from atria exposed to EGTA and EDTA are presented in Fig. 1. EDTA produced a more marked prolongation of action potential durations than EGTA.

Ectopic automaticity was induced in left atria by EGTA and EDTA in concentrations higher than 4 mM. Latency for inducing the automaticity, automatic rate under steady state conditions obtained after 25 to 40 min exposure to the agents and duration of the automaticity are summarized in Table 2. The latency was shortened and the rate was accelerated by increasing concentrations of EGTA from 4 to 5 mM. When EDTA (5 mM) was applied, the automatic rate was approx. as fast as that following 5 mM EGTA within 10 min, but thereafter gradually slowed in association with development of action potential prolongation. Automaticity induced by EGTA and EDTA was not influenced
TABLE 2. Automaticity induced by EGTA and EDTA in left atria

<table>
<thead>
<tr>
<th>Solution</th>
<th>Treatment</th>
<th>N</th>
<th>Latency (min)</th>
<th>Rate (beats/min)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+ 162 mM</td>
<td>EGTA 4 mM</td>
<td>3</td>
<td>10.3±2.4</td>
<td>31.7±7.9</td>
<td>30&lt;#</td>
</tr>
<tr>
<td></td>
<td>EGTA 5 mM</td>
<td>18</td>
<td>3.6±0.6</td>
<td>47.9±2.1</td>
<td>60&lt;</td>
</tr>
<tr>
<td>Na+ 162 mM †</td>
<td>EGTA 5 mM</td>
<td>6</td>
<td>3.2±0.5</td>
<td>43.0±4.8</td>
<td>60&lt;</td>
</tr>
<tr>
<td>Na+ 74 mM</td>
<td>EGTA 5 mM</td>
<td>3</td>
<td>7.5±1.3*</td>
<td>18.5±3.5*</td>
<td>40&lt;</td>
</tr>
<tr>
<td>Na+ 52 mM</td>
<td>EGTA 5 mM</td>
<td>4</td>
<td>6.5±1.2**</td>
<td>11.8±3.1*</td>
<td>26.5±3.1</td>
</tr>
<tr>
<td>Na+ 162 mM</td>
<td>EDTA 5 mM</td>
<td>7</td>
<td>1.9±0.6</td>
<td>39.0±2.7</td>
<td>60&lt;</td>
</tr>
</tbody>
</table>

N, number of preparations used.
† atria from rabbits pretreated with reserpine.
* significant difference from the value obtained following 5 mM EGTA in normal atria exposed to control media (162 mM Na+), P<0.01.
** P<0.05.
# Automaticity was not abolished during an observation period of 30 min.

FIG. 3. Effects of Ca++ on the membrane potential of left atria showing EGTA (5 mM)-induced automaticity. OS=overshoot; ThP=threshold potential when automaticity was induced; MDP=maximal diastolic potential or resting potential. *values obtained after 10 to 20 min exposure to 3 mM Ca++. † values obtained after 30 to 45 min exposure to 3 mM Ca++. Penetrations, 24 to 46. Preparations used, 4.
by tetrodotoxin in concentrations of $3 \times 10^{-6}$ to $10^{-5}$ M, which were sufficient to abolish action potentials generated by electrical stimulation in normal solutions.

In left atria from 4 rabbits pretreated with reserpine, the addition of EGTA in concentrations of 4 and 5 mM induced automaticity. The latency for inducing automaticity and the automatic rate were not significantly different from those obtained in atria from normal rabbits (Table 2).

Reversal by Ca$^{++}$ of changes induced by EGTA

The effect of Ca$^{++}$ in cumulative concentrations up to 5 mM on the membrane potential was investigated in the 4 left atria showing EGTA (5 mM)-induced automaticity. Results are summarized in Fig. 3. The addition of 1 mM Ca$^{++}$ to the bathing medium slowed the automatic rate from $42.5 \pm 5.3$ to $33.1 \pm 2.8$ beats/min but did not abolish automaticity. Parameters of the membrane potential were not significantly altered. Increasing Ca$^{++}$ to 2 mM abolished automaticity within 5 min without significant alterations in the resting membrane potential. Action potentials were not initiated by electrical stimulation during an observation period of 30 min. Calcium ions at 3 mM significantly increased the resting potential ($P < 0.01$) but no action potential was initiated in response to the electrical stimulation within 20 min. After 30 min exposure to the 3 mM Ca$^{++}$, the preparations began to respond to electrical stimuli applied at a frequency of 60/min. Further increases in the resting potential and in the action potential amplitude were observed when concentration of Ca$^{++}$ added to the bathing medium were raised to 4 and 5 mM. Action potential durations varied inversely with increasing concentrations of Ca$^{++}$. Atrial contractility was also restored by 3 mM Ca$^{++}$ in 2 out of the 4 atria and by 4 mM Ca$^{++}$ in the remaining 2. Mean values of contractions observed in these preparations after adding Ca$^{++}$ in concentrations of 3, 4 and 5 mM were $32 \pm 28$, $171 \pm 91$ and $254 \pm 120$ mg respectively, whereas that before the addition of EGTA was $736 \pm 182$ mg.

EGTA-induced automaticity in relation to extracellular concentrations of Na$^{+}$

Reduction in [Na$^{+}$]$_o$ by replacing NaCl with isotonic sucrose from 162 mM to 74 or 52 mM delayed the initiation of automaticity following 5 mM EGTA and slowed the automatic rate (Table 2). In all 4 preparations exposed to 52 mM Na$^{+}$ the rate of induced automaticity was gradually slowed until automatic activities were completely abolished within 40 min. In contrast, in the other bathing media used in this study the induced automaticity was never abolished spontaneously during an observation period of more than 40 min.

Lowering Na$^{+}$ to 74 and 52 mM decreased the overshoot and the $10^\%$ duration of action potentials from atria driven electrically: mean values of the resting potential, overshoot and $10^\%$ duration were $73.0 \pm 0.5$ mV, $14.8 \pm 0.2$ mV and $14.0 \pm 0.2$ msec ($N=123$), respectively, at 162 mM Na$^{+}$ (control solution), whereas those were $69.0 \pm 1.3$ mV, $1.9 \pm 1.0$ mV and $10.9 \pm 0.2$ msec ($N=19$), respectively, at 74 mM Na$^{+}$ and $68.5 \pm 0.9$ mV, $18.3 \pm 1.9$ mV and $10.0 \pm 0.7$ msec ($N=43$), respectively, at 52 mM Na$^{+}$. In atria in which automaticity was induced by 5 mM EGTA, the maximum diastolic potential
and the threshold potential were not significantly influenced by reducing Na⁺; however, the size of the overshoot related directly to [Na⁺]₀ (Fig. 4). The 10% duration decreased with reducing Na⁺.

Reduction of [Na⁺]₀ to 52 mM by replacing NaCl with choline chloride produced similar decreases in the resting potential and the overshoot to those obtained when NaCl was replaced with sucrose. Mean values of these parameters were 70.8 ± 1.3 mV and -28.3 ± 2.3 mV (N = 41), respectively. In 3 out of 4 atria exposed to reduced Na⁺, EGTA (5 mM) initiated automatic activities: mean values of the latency and the automatic rate were 18.3 ± 4.1 min and 40.0 ± 1.7 beats/min (N = 3), respectively. The induced automaticity persisted for 10 and 58 min in 2 atria and for longer than 60 min in the remaining one. Changes in parameters of the membrane potential in atria showing EGTA-induced automaticity by lowering Na⁺ are shown in Fig. 4.

**Effects of Mg⁺⁺ on the electrical activity of atria showing automaticity**

The addition of Mg⁺⁺ in concentrations of 0.05 and 0.1 mM caused a dose-related decrease in the rate of automaticity induced by 5 mM EGTA; the mean rate before Mg⁺⁺ was 46.6 ± 5.6 beats/min (N = 7) and the rates after 0.05 and 0.1 mM Mg⁺⁺ were 37.5 ± 7.4 (N = 6) and 21.7 ± 7.1 beats/min (N = 7), respectively. The automaticity was abolished by 0.1 mM Mg⁺⁺ in 2 out of 7 atria, at 0.2 mM in 4 atria and at 1 mM in the remaining one. When the atria were separated into 4 (Table 1), the automaticity was also abolished by 0.1 mM Mg⁺⁺ in 4 out of 9 “A” and at 0.2 mM in the remaining 5. The time required to abolish the automaticity was approx. the same in “A” (1.6 ± 0.6 min, N = 5, at 0.2 mM Mg⁺⁺) and “D” (2.2 ± 1.0 min). In left atria in which automaticity was
FIG. 5. Effects of Mg"' and Mn++ on action potential durations in left atria driven electrically in control media. Preparations used, 4 to 6 (25 control preparations). Figures in parentheses indicate the number of penetrations.

FIG. 6. Effects of Mg"' on the membrane potential of atria exposed to 5 mM EGTA. Preparations used, 3 to 5 (15 control preparations).

completely abolished by low concentrations of Mg"', action potentials were generated in response to electrical stimulation. However, increasing concentrations of Mg"' to 0.5 to 2 mM made preparations unresponsive to electrical stimulation. Increase in the stimulus strength to 20 to 30 volts (about 4 to 7 times threshold) restarted action potentials only for 1 to 5 min. Pretreatment of atria for 10 to 20 min with 3 mM Mg"' prevented
initiation of automaticity following 5 mM EGTA (in 2 out of 2 atria) and EDTA (in 4 out of 4 atria).

The addition of Mg\(^{2+}\) in a concentration of 3 mM to normal left atria driven electrically shortened the action potential duration (Fig. 5) but did not cause significant changes in the other parameters of the membrane potential. Modification by Mg\(^{2+}\) of parameters of the membrane potential from atria treated with EGTA is shown in Fig. 6. Magnesium ions did not significantly alter the resting potential and the overshoot. Action potential durations were shortened in preparations in which automaticity was abolished and electrical activities were generated by electrical stimuli. These activities were completely abolished by higher concentrations of Mg\(^{2+}\) at the resting potential which was almost identical with that in active atria. The addition of 3 mM Ca\(^{2+}\) made these atria excitable to electrical stimulation in association with an increase in the resting potential to -55 to -65 mV.

**Effects of Mn\(^{2+}\) on electrical and mechanical activities**

Manganese ions in concentrations ranging from 1 to 15 mM did not cause significant changes in the resting potential and the overshoot of action potentials recorded from normal atria driven electrically at a frequency of 60/min but elicited a dose-related decrease in the 10% duration of action potentials (Fig. 5). Atrial contractions (300 to 550 mg) were markedly reduced to 30 to 70 mg by 1 mM Mn\(^{2+}\) and completely abolished at 5 mM or higher.

The rate of automaticity induced by 5 mM EGTA was slowed by 1 mM Mn\(^{2+}\) to 36.4 ± 4.6 beats/min (N = 9) from the control rate of 46.6 ± 3.6 beats/min (N = 9). Further increase in concentrations of Mn\(^{2+}\) to 2 mM abolished automaticity within 4 min in all 8 atria and in all 4 "A" (Table I). All the 8 atria responded to electrical stimulation at a frequency of 60/min. The induced electrical activity persisted for longer than 20 min.

![Graph](image_url)

**Fig. 7. Effects of Mn\(^{2+}\) on the membrane potential of atria exposed to 5 mM EGTA. Preparations used, 3 and 5 (15 control preparations).**
Comparison of parameters of action potentials recorded from atria showing automaticity and those made active by electrical pulses after the addition of Mn\(^{++}\) are shown in Fig. 7. The resting potential was significantly increased by Mn\(^{++}\) (P<0.01). Action potential durations were markedly reduced with increasing Mn\(^{++}\). Thus, the configuration of action potentials following Mn\(^{++}\) in EGTA-treated atria resembled that in normal atria (Fig. 8). The maximum rate of rise in EGTA (5 mM) and Mn\(^{++}\) (3 mM)-treated atria averaged 24.6±1.9 V/sec (N=30), which was almost identical with that obtained from atria soaked in 2 mM EGTA (25.1±1.5 V/sec). These action potentials were shortened but not suppressed by increasing concentrations of Mn\(^{++}\) to 5 to 10 mM in EGTA-treated atria, however, were completely abolished by 3×10^{-8} M tetrodotoxin in 2 out of 2 preparations.

Atrial contractions were restored within 8 min after the addition of 3 mM Mn\(^{++}\) in 6 out of 8 atria exposed to 5 mM EGTA (Fig. 8). The mean values of the maximum contraction in EGTA (5 mM)- and Mn\(^{++}\) (3 mM)-treated atria were 63±11 mg (N=6), whereas the value before adding EGTA was 477±84 mg (N=8). The restored contractions were spontaneously suppressed after the maximum force was attained at 5 to 20 min.
In order to determine whether or not Ca\(^{2+}\) trapped by EGTA was released by Mn\(^{2+}\) (stability constant of Ca\(^{2+}\) and Mn\(^{2+}\) as applied to EGTA: 11.00 and 12.30, respectively), EGTA-containing solutions were replaced with Ca\(^{2+}\)-free media after preparations had shown automaticity for 15 to 30 min. Two to 5 min after replacement with Ca\(^{2+}\)-free media, the rate was accelerated to 46.9±3.7 beats/min from the rate of 37.9±2.6 beats/min prior to the replacement (N=7), in association with shortening of the action potential duration. In 3 out of the 7 atria automaticity persisted for longer than 20 min. The addition of Mn\(^{2+}\) at 1 mM abolished the automaticity within 1 min. Electrical activity was restored in response to artificial stimuli in preparations treated with 2 mM Mn\(^{2+}\). In the remaining 4, the atrial rate was slowed until automaticity was spontaneously abolished (average time to atrial arrest: 11.9±3.4 min). Electrical stimulation failed to evoke action potentials. However, after treatment for 5 to 20 min with 2 to 3 mM Mn\(^{2+}\), excitability to electrical stimuli was restored. The regenerated action potentials persisted for longer than 30 min without deterioration; mean values of the resting potential, overshoot, 10% duration and 90% duration were 69.2±0.9 mV, 0.5±1.7 mV, 4.2±0.3 msec and 90.0±4.3 msec (N=20), respectively. Tetrodotoxin at 3×10\(^{-6}\) M completely abolished the action potential. Contraction were not restored, although in 3 out of 7 atria the resting tension increased by 320±37 mg after 15 to 20 min exposure to 3 mM Mn\(^{2+}\).

Mean concentrations of Ca\(^{2+}\) in the bathing medium determined spectrophotometrically were: 2.122 mM in control media, 1.068 mM after 1 mM EGTA, 1.032 mM after 1 mM EGTA plus 1 mM Mn\(^{2+}\), 0.017 mM after 2 mM EGTA and 0.020 mM after 2 mM EGTA plus 2 mM Mn\(^{2+}\) (N=2). Calcium ions could not be detected in the presence of 4 and 5 mM EGTA even when Mn\(^{2+}\) in concentrations up to 4 mM was added.

**DISCUSSION**

**Automaticity induced by EGTA and EDTA**

The present study revealed that Ca\(^{2+}\) chelating agents, EGTA and EDTA, abolished action potentials recorded from electrically driven atria and generated spontaneous activity in association with a reduction in the resting potential. Incidence of automaticity was higher in areas close to the interatrial septum than in the atrial appendage. The maximum rate of rise in action potentials induced by electrical stimulation at the resting level of -70 to -75 mV was more than 20 times as rapid as the rate of the automatic action potential, which was almost identical with that observed in S-A nodal pacemaker action potentials. According to Rougier et al. (9), in frog atrial muscles there are two different channels allowing the flow of distinct inward currents; one a fast channel through which Na\(^{+}\) rapidly enters into cells, and the other a slow channel for slow movements of Na\(^{+}\) and Ca\(^{2+}\). The fast channel is activated at a membrane potential lower than -60 mV and the activation relates inversely to the take-off level in a range from -70 to -90 mV (13). The slow channel is activated when the membrane is depolarized to -35 mV (14, 15) or approx. -40 mV (10, 16). These data are similar to the average take-off level
for spontaneous excitation obtained in the present study (34 and -32 mV in atria treated with EGTA and EDTA, respectively). Findings that automaticity induced by the Ca\(^{++}\) chelating agents was not influenced by tetrodotoxin but was abolished by Mn\(^{++}\) suggest the involvement of Na\(^{+}\), which carries charges through a slow channel, in the genesis of the automaticity.

In atria treated with 5 mM EGTA the contractility was only partially restored by Ca\(^{++}\). Similar results have also been obtained in isolated perfused rabbit heart (17). On the other hand, EGTA-induced changes in the membrane potential were completely reversed by Ca\(^{++}\) in a concentration of 5 mM. It has been demonstrated that removal of Ca\(^{++}\) and Mg\(^{++}\) from the bathing medium fails to produce changes in the membrane potential such as are observed with EDTA (1). Cellular Ca\(^{++}\) is decreased only slightly when Ca\(^{++}\) and Mg\(^{++}\) are removed from the bathing media, but is rapidly removed by the chelating agent. Thus, it appears that automaticity and changes in the membrane potential induced by Ca\(^{++}\) chelating agents are mainly associated with removal of Ca\(^{++}\), which is possibly responsible for the membrane permeability for Na\(^{+}\), from sites on and within the cell.

Lowering [Na\(^{+}\)]\(_0\) did not prevent the membrane-depolarizing effect of EGTA. However, latency for inducing automaticity was prolonged and duration of automaticity was shortened by reducing [Na\(^{+}\)]\(_0\). The size of the overshoot was related directly to [Na\(^{+}\)]\(_0\). Sodium ions appear to play an important role in generating automaticity in left atria, as suggested in S-A nodal pacemakers (18).

It has been suggested that under normal conditions myocardial catecholamines may be released in small quantities in order to increase the rate of pacemaker and contractility and serve as humoral agents for the regulation of normal cardiac function (19, 20). However, reserpine-pretreatment which markedly depleted functional catecholamines from atrial muscles did not alter the ability of EGTA and Ba\(^{++}\) (11) to induce ectopic automaticity. It may be concluded that myocardial catecholamines do not participate in inducing automaticity in isolated left atria.

Membrane effects of Mg\(^{++}\)

Prolongation of action potential durations by EDTA was appreciably more marked than that by EGTA. EDTA chelates both Ca\(^{++}\) and Mg\(^{++}\) (stability constants for these ions: 10.59 and 8.69, respectively), whereas EGTA is more effective in chelating Ca\(^{++}\) than Mg\(^{++}\) (the constants: 11.00 and 5.21, respectively). Thus, the difference in the effect of EGTA and EDTA on action potential durations would be attributable to different chelating properties. Magnesium ions very effectively shortened action potentials and suppressed automatic activities induced by these chelating agents. Surawicz et al. (21) demonstrated that when Mg\(^{++}\) was completely absent, solutions free of Ca\(^{++}\) caused a progressive prolongation of action potentials and Q-T intervals of electrocardiograms in isolated rabbit hearts. The addition of Mg\(^{++}\) reversed these changes. These findings suggest that Mg\(^{++}\) behaves like Ca\(^{++}\) in stabilizing membranes, contrasting to known
antagonistic actions of Mg\(^{2+}\) to Ca\(^{2+}\) responsible for the release of neurotransmitters (5, 22, 23) and for cardiac acceleration (6). Since the resting membrane potential was not increased in EGTA-treated atria in which the automatic activity and electrical excitability were suppressed by Mg\(^{2+}\), it appears that Mg\(^{2+}\) decreases the permeability of membranes for Na\(^+\) upon excitation but not during diastole.

**Effects of Mn\(^{2+}\) on electrical and mechanical activities**

Manganese ions abolished automaticity induced by Ca\(^{2+}\) chelating agents in association with a significant hyperpolarization of the membranes. Electrical stimulation re-generated action potentials, which were completely abolished by tetrodotoxin. Thus, it would be considered that inward currents carried by Na\(^+\) through fast channels are restored by Mn\(^{2+}\). Since EGTA chelates Mn\(^{2+}\) more easily than Ca\(^{2+}\) (stability constant for Mn\(^{2+}\): 12.30), part of Ca\(^{2+}\) trapped by the chelating agent may be released. However, this is not a likely explanation for the Mn\(^{2+}\) action, since even when EGTA-containing media were replaced with Ca\(^{2+}\)-free solutions, Mn\(^{2+}\) restored excitability to electrical stimuli, and the addition of Mn\(^{2+}\) to EGTA-added solutions did not increase the amount of ionized Ca in the medium. It may be possible that Mn\(^{2+}\) acts as a substitute of Ca\(^{2+}\) responsible for maintaining atrial excitability and for decreasing the permeability of diastolic membranes for Na\(^+\), or that Mn\(^{2+}\) increases the K\(^+\) conductance. However, in frog skeletal muscles the possibility that Mn\(^{2+}\) reduces the K\(^+\) conductance has been demonstrated (24).

The present study demonstrated that Mn\(^{2+}\) restored atrial contractility abolished by EGTA; however, after replacement of EGTA-containing solutions with Ca\(^{2+}\)-free media, Mn\(^{2+}\) failed to restore contractions but increased the resting tension in 3 out of 7 preparations. It is not known whether Mn\(^{2+}\) substitutes for Ca\(^{2+}\) as a contributor of contractile mechanisms or releases intracellular Ca\(^{2+}\) in cardiac muscles. Frank (25) suggested that Mn\(^{2+}\) released Ca\(^{2+}\) from intracellular binding sites of skeletal muscles but could not take the place of Ca\(^{2+}\) in excitation-contraction coupling. Inward movements of Mn\(^{2+}\) across cell membranes have been postulated in isolated guinea pig ventricles, as a slow inward current is obtained in a Ca\(^{2+}\)- and Na\(^+\)-free solution when Mn\(^{2+}\) is added (10). Thus, the release of cellular Ca\(^{2+}\) by Mn\(^{2+}\) could be a reason for the restoration of mechanical activities. The mechanism underlying the restoration of mechanical activities appears to be different from that for electrical activities, since contractions were gradually depressed and spontaneously abolished, whereas action potentials persisted long without deterioration.

**REFERENCES**

6) SEIFEN, E.: Pflügers Arch. ges. Physiol. 304, 46 (1968)
11) TODA, N.: Circulation Res. 27, 45 (1970)
16) PEUTER, H.: J. Physiol. 192, 479 (1967)
18) TODA, N.: J. Physiol. 196, 677 (1968)
20) LEE, W.C. AND SHIDEMAN, F.E.: Science 129, 967 (1959)