Effects of Lidocaine and Verapamil on Early Afterdepolarizations in Isolated Rabbit Sinoatrial Node

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The effects of local anaesthetic anti-arrhythmic agents (lidocaine) and Ca antagonists (Verapamil) have been examined on the early afterdepolarizations (EADs) in isolated rabbit sinoatrial (SA) node. In a nominally calcium free and magnesium free solution, strontium (0.5–4.5 mM) produced an EAD in small pieces isolated from the SA node. The additional presence of 0.02–0.6 mM lidocaine did not abolish the strontium (0.5 mM)-induced EAD. 0.6 mM lidocaine produced an increase in EAD amplitude and then abolished a prolonged action potential (AP) associated with repetitive EADs. On the other hand, the addition of 4 μM verapamil abolished the strontium (0.5 mM)-induced EAD but did not abolish the AP.

It is concluded that under conditions when the AP is not abolished, EAD blockade by lidocaine is less effective than that by verapamil. (Key words: isolated sinoatrial node preparation, strontium, early afterdepolarization, lidocaine, verapamil)

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Arrhythmias due to early afterdepolarizations (EADs) occur in heart muscle before action potential (AP) repolarization is completed[1–3]. EADs are a type of triggered activity[2]. It has been known that sarcolemmal slow Ca channel plays an essential role in the generation of EADs in ferret ventricular muscles[3] and in sheep Purkinje fibers[4]. In sheep cardiac Purkinje fibers, strontium (Sr^{++}) ions are known to induce EADs[5]. It has previously been reported for mammalian myocardial fibers that Sr^{++} can substitute for Ca^{++} as carriers of the slow inward current[6]. On the other hand, lidocaine and verapamil are known to suppress EADs[4]. Until now there has been no detailed observation of the effects of lidocaine and verapamil on the EADs induced by superfusion with Sr^{++}-containing solutions in rabbit sinoatrial (SA) node cells.

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The present experiments on rabbit SA node cells were undertaken to provide information about the effects of lidocaine and verapamil on the Sr^{++}-induced EADs. The effects of lidocaine and verapamil on the EADs are also interesting since similar EADs are found in monophasic action potential recordings from patients with long QT syndrome[7].

Materials and Methods

The experimental procedures were essentially the same as described previously[8–10].

Experiments were performed in small pieces of SA node, obtained from the hearts

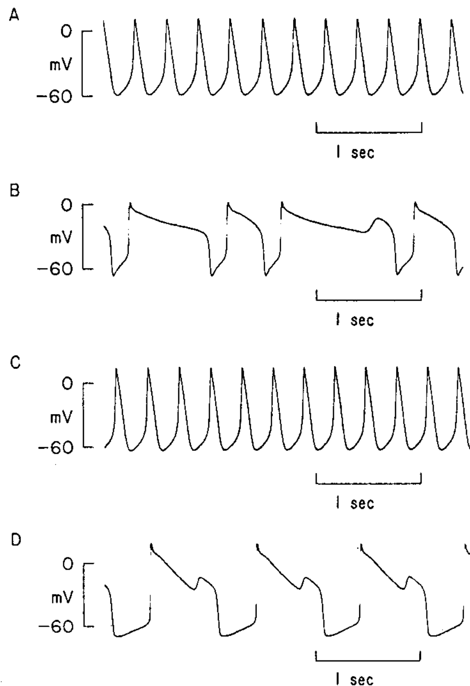

Fig. 1. Effect of superfusing a rabbit SA node preparation with Sr solution on AP repolarization. A and B: APs recorded in one cell immediately before(A) and 10.5 min(B) after the 0.5 mM Sr solution change. C and D: APs recorded in one cell immediately before(C) and 5.5 min(D) after the 4.5 mM Sr solution change. Superfusion with Sr solution produced an EAD. Calibrations apply throughout.

of rabbit (1.5–2.5 kg) anaesthetised with sodium pentobarbitone (40 $mg \cdot kg^{-1}$). Hearts were rapidly excised through a mid-sternal incision and placed in an oxygenated Tyrode solution at 32–35°C. The right atrium was excised, and thin strips about 6–8 mm in length and 0.5 mm in width were prepared by cutting the SA region along lines parallel with the crista terminalis. The SA node tissue was trimmed further to a piece smaller than 0.5 × 0.5 mm. The dominant SA node cells were then pinned in the recording chamber where a normal Tyrode solution was superfused. The atrial muscle within the crista terminalis as well as the subsidiary (latent) SA node tissue were removed with a razor blade. The flow rate of the perfusates through the recording chamber (1 ml) was 2.5 to 3.5 $ml \cdot min^{-1}$. The temperature of the recording chamber was maintained at 37.0 ± 0.5°C by circulation in a thermostatically controlled water bath.

Transmembrane potential was recorded by means of conventional glass microelectrodes filled with 3 M KCl. Resistance of the microelectrode was 30–45 megaohms. The signals were led to an oscilloscope (VC-9 Nihon Kohden) via a high input impedance preamplifier (MEZ-8201 Nihon Kohden) and recorded continuously on a pen recorder (RJG-3024 Nihon Kohden).

The composition of the normal Tyrode was (mM): NaCl 136.9, KCl 3.0, $MgCl_2$ 1.05, $NaHCO_3$ 24.0, NaH_2PO_4 1.2, $CaCl_2$ 2.5, and glucose 5.5. After continuous saturation with a mixture of 95% O_2 and 5% CO_2 gas, the pH was maintained at 7.3 to 7.4. In nominally calcium free and magnesium free (Ca-free, Mg-free) solutions, $CaCl_2$ and $MgCl_2$ were omitted. Ca-free, Mg-free solutions containing strontium (Sr solutions) were prepared by adding 0.5–4.5 mM Sr^{++} to Ca-free, Mg-free solutions. Lidocaine and verapamil were added to the bathing media in appropriate amounts.

Each series of APs shown was obtained during a single penetration and a whole series of extracellular solution changes was completed with continuous recording from one cell. AP characteristics were obtained under these conditions. When arrhythmias due to EADs occurred, the AP and EAD parameters were measured from an example of a maximum EAD arising during repolarization phase. Amplitude of the AP was measured as the potential difference between the peak of the AP and the maximum diastolic potential. AP duration was measured at 50% of AP amplitude with or without an EAD. Cycle length was defined as the time required to complete one full cycle and was measured at the peak of the AP. Potential (EAD-MP) before initiation of the EAD was measured at the level of the membrane potential of the repolarization interrupted by a depolarization of an EAD. Amplitude of the EAD was measured as the potential difference between the EAD-MP and the peak of the EAD.

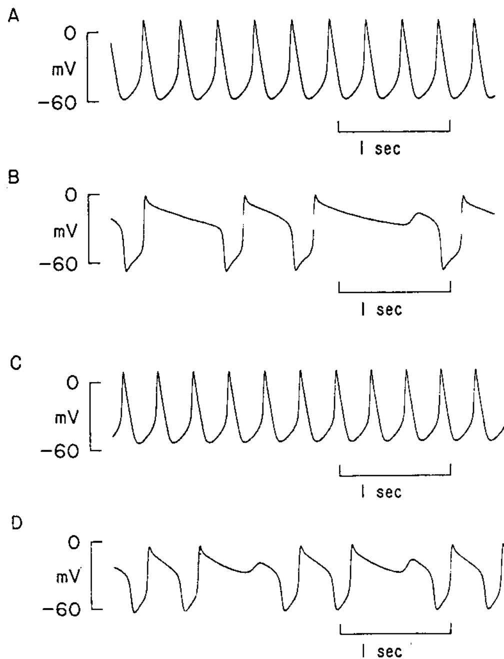

Fig. 2. Effect of lidocaine (0.02 and 0.1 mM) on Sr (0.5 mM)-induced EADs. A and B: APs recorded in the same cell immediately before(A) and 10 min(B) after changing from normal Tyrode to 0.5 mM Sr solution containing 0.02 mM lidocaine. C and D: APs recorded in the same cell immediately before(C) and 5.7 min(D) after changing from normal Tyrode to 0.5 mM Sr solution containing 0.1 mM lidocaine. The additional presence of lidocaine (0.02 and 0.1 mM) did not affect the EAD. Calibrations apply throughout.

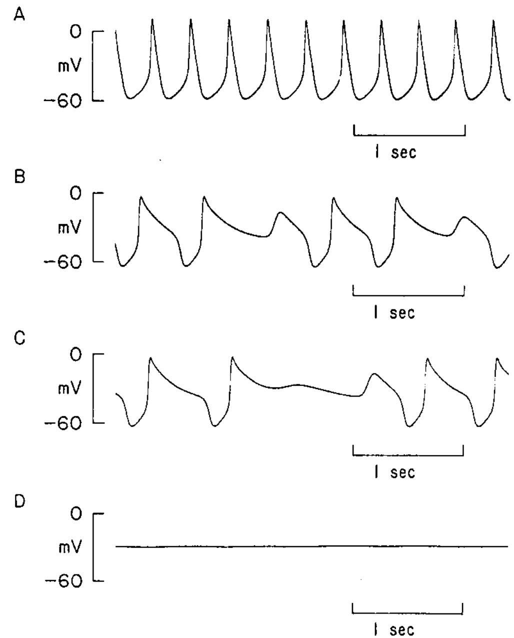

Fig. 3. Effect of 0.3 mM lidocaine on Sr (0.5 mM)-induced EADs. A, B, C and D were recorded in a cell immediately before(A), and 6.5(B), 6.8(C), and 8.3 min(D) after changing to 0.5 mM Sr solution containing 0.3 mM lidocaine. The addition of 0.3 mM lidocaine abolished the AP(D). Calibrations apply throughout.

Experimental values were presented as mean ± SEM. Data were analyzed using Student t test. P values less than 0.05 were considered statistically significant.

Results

In the present experiments, both extracellular calcium and magnesium were replaced by strontium as magnesium is known to have a stabilizing effect similar to that of calcium in frog skeletal muscle[11] and to compensate for the lack of calcium in frog heart[12]. Figure 1 shows the effect of Ca-free, Mg-free solution containing 0.5 mM Sr^{++} (A and B) and 4.5 mM Sr^{++} (C and D) on AP repolarization. The APs shown in figure 1A and B were recorded in the same cell immediately before(A) and 10.5 min(B) after changing from normal Tyrode to 0.5 mM Sr solution which produced a prolonged AP and an EAD reaching 13 mV. The APs shown in figure 1C and D were recorded in the same cell immediately before(C) and 5.5 min(D) after changing from normal Tyrode to 4.5 mM Sr solution which produced an EAD reaching 11 mV. No clear difference was observed between the EADs obtained with the two Sr solutions.

Figure 2 shows the effect of lidocaine (0.02 mM and 0.1 mM) on the EADs induced by superfusion with 0.5 mM Sr solution. The APs shown in figure 2A and B were recorded in a cell before(A) and 10 min(B) after changing from normal Tyrode to 0.5 mM Sr solution containing 0.02 mM lidocaine which produced an EAD reaching 10 mV. The APs shown in figure 2C and D were recorded in a cell before(C) and 5.7 min(D) after changing from normal Tyrode to 0.5 mM Sr solution containing 0.1 mM lidocaine which produced

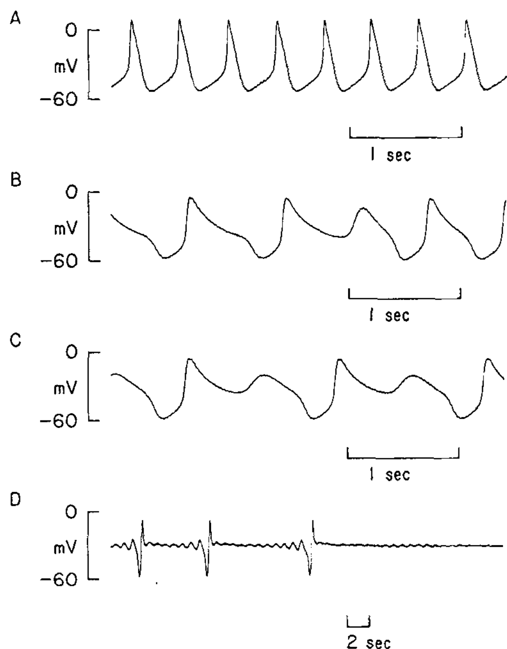

Fig. 4. Effect of 0.6 mM lidocaine on Sr (0.5 mM)-induced EADs shown at two different time scales. The APs were recorded in a cell immediately before(A), and 4.4(B), 5.0(C), and 6.2 min(D) after changing to 0.5 mM Sr solution containing 0.6 mM lidocaine. The addition of 0.6 mM lidocaine abolished the prolonged AP associated with repetitive EADs.

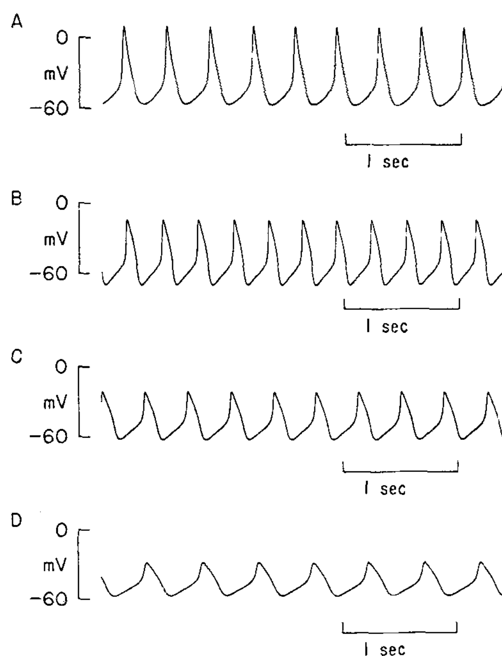

Fig. 5. Effect of 4 μM verapamil on Sr (0.5 mM)-induced EADs. APs were recorded in the same cell immediately before(A), and 5(B), 9(C), and 13 min(D) after changing to 0.5 mM Sr solution containing 4 μM verapamil. The addition of verapamil abolished an EAD. Calibrations apply throughout.

an EAD reaching 10 mV.

Figures 3 and 4 show the effect of lidocaine (0.3 mM and 0.6mM) on the EADs induced by exposure to 0.5 mM Sr solution. Figure 3A shows the control in which SA node cells were superfused with normal Tyrode solution. Figures 3B, 3C and 3D were recorded 6.5(B), 6.8(C) and 8.3 min(D) after superfusion with 0.5 mM Sr solution containing 0.3 mM lidocaine was initiated. The additional presence of 0.3 mM lidocaine produced an EAD reaching 21 mV(B) and abolished AP(D). Figure 4A shows the control. Figures 4B, 4C and 4D were recorded 4.4(B), 5.0(C) and 6.2 min(D) after superfusion with 0.5 mM Sr solution containing 0.6 mM lidocaine was initiated. The addition of 0.6 mM lidocaine produced an EAD reaching 25 mV(B) and abolished AP including EADs(D).

Figure 5 shows the influence of verapamil on the EADs. The APs shown were recorded in the same cell before(A) and 5(B), 9(C) and 13 min(D) after changing from normal Tyrode to 0.5 mM Sr solution containing 4 μM verapamil. The additional presence of verapamil abolished the EADs induced by superfusion with 0.5 mM Sr solution but did not abolish the AP.

The parameters of an EAD and an AP of spontaneously beating SA node cells in the various solutions are summarized in table 1. T in table 1 illustrates the time (min) of measurement of an EAD and an AP parameters.

Discussion

The present experiments showed that superfusion with Sr^{++} solution induced an EAD (fig. 1), that additional presence of 4 μM verapamil caused blockade of the EAD (fig. 5), and that addition of 0.3–0.6 mM

Table 1. Parameters of early afterdepolarization (EAD) and action potential (AP) in various solutions

Solution	Amplitude (mV)	MDP or MP (mV)	AP-duration (msec)	Cycle length (msec)	T (min)	n
normal Tyrode	62 ± 2	−57 ± 1	74 ± 4	354 ± 23		8
0.5 mM Sr	61 ± 4	−58 ± 3	758 ± 113	986 ± 107	8 - 11.5	5
EAD	11 ± 1*	−27 ± 2				
4.5 mM Sr	85 ± 2	−67 ± 1	626 ± 47	992 ± 61	3.1 - 6	5
EAD	11 ± 1	−21 ± 1				
0.5 mM Sr + 0.02 mM lidocaine	65 ± 2	−64 ± 1	930 ± 71	1146 ± 69	8 - 10	5
EAD	8 ± 1	−24 ± 2				
0.5 mM Sr + 0.1 mM lidocaine	62 ± 3	−63 ± 3	774 ± 47	990 ± 53	5.5 - 11.5	5
EAD	12 ± 1	−27 ± 2				
0.5 mM Sr + 0.3 mM lidocaine	55 ± 3	−60 ± 2	736 ± 48	1008 ± 50	4.3 - 6.9	5
EAD	19 ± 1	−37 ± 2				
0.5 mM Sr + 0.6 mM lidocaine	53 ± 2	−61 ± 1	892 ± 76	1266 ± 60	4.4 - 5.7	5
EAD	25 ± 1**	−42 ± 2				
0.5 mM Sr + 4 μM verapamil 5 min	43 ± 3	−57 ± 3	100 ± 9	334 ± 17		5
13 min	19 ± 3	−46 ± 3	254 ± 67	559 ± 68		5

Values are expressed as the mean ± SEM. n = number of experiments. T shows the time (min) of measurement of an EAD and an AP parameters, and is measured at the time of superfusion of the preparation with the various solutions. *$P < 0.001$, as compared to **. MDP = maximum diastolic potential. MP = membrane potential before initiation of depolarization of EAD.

lidocaine did not block the EAD but AP blockade by the addition of lidocaine did not elicit the EAD (fig. 3 and fig. 4). Lidocaine (0.02–0.1 mM) exposure did not affect the EAD (fig. 2). In addition, superfusion with normal Tyrode solution containing Sr^{++} did not induce an EAD. Further, 0.1 μM verapamil did not block the EAD. Since 0.6 mM lidocaine exposure produced a blockade of an AP associated with EADs (fig. 4D), it is plausible that lidocaine is not an advantageous inhibitor of the EAD. The inhibitory effect of 0.3 mM lidocaine on the AP associated with EADs was quite similar to that of 0.6 mM lidocaine. From these experiments it is concluded that verapamil is the optimum inhibitor of the EAD under conditions when the AP is not blocked. This hypothesis is strengthened by the observation that 0.6 mM lidocaine exposure caused a marked increase in EAD amplitude (table 1).

There is evidence indicating that EAD is the cause of clinically important arrhythmias seen in the hearts of patients with long

QT intervals and torsades de points[7,13,14]. It is well established that the activity of the SA node pacemakers gives rise to normal or abnormal sinus rhythm and during sinus rhythm the propagation of the cardiac impulse is an orderly process[15]. Therefore, it may be possible that the abnormal impulse in the SA node pacemakers gives rise to the abnormal atrial or ventricular rhythms. Long QT intervals and torsades de points or ventricular fibrillation exhibit characteristics associated with abnormal prolongation of AP duration[16]. From the above view, the aim of the present paper is to observe, in isolated rabbit SA node cells, EADs and prolonged APs similar to the abnormal APs with EADs seen in atrial or ventricular muscle fibers. In the present experiments, it is unclear whether Sr^{++}-induced EADs and prolonged APs can be associated with APs from the hearts of patients with long QT intervals and torsades de points or ventricular fibrillation. However, the possibility to be considered is that Sr^{++}-induced APs with apparent EADs can produce the abnormal atrial or ventricular rhythms.

In the clinical administration of lidocaine, it has been described that 50–150 mg of 2 percent lidocaine is given intravenously to each patient[17]. The concentration of lidocaine utilized is about 1–3 μM. Further 17 μM lidocaine is known to abolish EADs in sheep Purkinje fibers[4]. Therefore the present experiments were initially performed in the presence of 0.02 mM lidocaine (fig. 2). Since 0.02 mM lidocaine did not affect the Sr^{++}-induced EAD, the effect of the high-lidocaine concentrations on the EAD was tested. The results revealed that high-lidocaine (0.1–0.6 mM) did not inhibit the EAD. This indicates that the action of lidocaine on the Sr^{++}-induced EAD in rabbit SA node cells has different properties from that of lidocaine on the EAD induced with Bay K 8644 in sheep Purkinje fibers[4]. There is no obvious explanation for this discrepancy. However, histological or methodological differences may have contributed. In contrast, the inhibitory effect of 4 μM verapamil on the Sr^{++}-induced EAD in SA node cells (fig. 5) was quite similar to that observed in 2.2 μM verapamil, because 2.2 μM verapamil is known to abolish the EAD induced with Bay K 8644 in sheep Purkinje fibers[4]. When the action of verapamil on the Sr^{++}-induced EAD in SA node cells is compared to that of lidocaine, it seems that verapamil is important for the effective regulation of the EAD seen in SA node cells. In addition, 5 μM lidocaine did not affect the Sr^{++}-induced EAD in SA node cells. Also, superfusion of the rabbit SA node cells with normal Tyrode solution containing 5–20 μM lidocaine had no significant influence on the AP. Further in normal Tyrode solution 0.1–0.2 mM lidocaine produced a decrease in AP amplitude and a slow AP. 0.3–0.6 mM lidocaine produced a large decrease in AP amplitude or an AP blockade.

Sr^{++} has been shown to carry inward current in Ca channels[18]. Superfusion with solution containing Sr^{++} substituted for Ca^{++} has been found to induce Sr^{++}-action potential, generated by the slow inward Sr^{++} current[19,20]. On the other hand, verapamil has been shown to block the slow inward current through Ca channels[21]. In the present experiments, superfusion of SA node cell with Sr solution induced both EAD and prolonged AP. Further addition of verapamil abolished the EAD and caused a large decrease in AP amplitude. Therefore the main cause of the depolarization of the EAD is likely to be a slow inward Sr^{++} current through Ca channels. This assumption is in agreement with the theory that EADs arise as a direct consequence of slow inward current through sarcolemmal Ca channels[3,4].

Lidocaine is known to block slow inward Ca^{++} current and inwardly-rectifying K^+ current[22]. The present experiments showed that 0.3–0.6 mM lidocaine blocked a prolonged AP associated with repetitive EADs (fig. 4D). However, verapamil blocked an EAD without complete inhibition of the AP (fig. 5). Since it is not indicated that verapamil produces inhibition of K^+ current, the possibility to be considered is that a decrease in K^+ current produced by lidocaine blocks an AP associated with repetitive EADs. It

is reported that all that is needed to cause repetitive firing from an EAD is a sufficient decrease in K^+ conductance relative to the conductance for inward current[2]. Therefore the increase in EAD amplitude produced by 0.6 mM lidocaine is likely to be due to the decrease in K^+ current. Lidocaine and verapamil are widely used in the treatment of cardiac arrhythmias. However, a difference was observed between the results obtained with lidocaine and verapamil.

Membrane potential (EAD-MP) before initiation of the EAD was −21 - −42 mV (table 1). It has been shown that the slow inward current is activated on depolarization to about −40 mV or more positive potentials[23]. These results are strongly suggestive against the assumption of considering the slow inward current carried by Sr^{++} through Ca channel as a mechanism of EAD.

In summary, the results are consistent with the idea that the depolarization of EAD is due to the slow inward Sr^{++} current and it is possible that under conditions when the AP is not abolished, EAD blockade by lidocaine is less effective than that by verapamil.

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References

1. Cranefield PF: The conduction of the cardiac impulse. New York, Futura, 1975
2. Hoffman BF, Rosen MR: Cellular mechanisms for cardiac arrhythmias. Circ Res 49:1–15, 1981
3. Marban E, Robinson SW, Wier WG: Mechanisms of arrhythmogenic delayed and early afterdepolarizations in ferret ventricular muscle. J Clin Invest 78:1185–1192, 1986
4. January CT, Riddle JM: Early afterdepolarizations: mechanism of induction and block; a role for L-type Ca^{2+} current. Circ Res 64:977–990, 1989
5. Gonzalez MD, Vassalle M: Electrical and mechanical effects of strontium in sheep cardiac Purkinje fibers. Cardiovasc Res 23:867–881, 1989
6. Kohlhardt M, Herdey A, Kübler M: Interchangeability of Ca ions and Sr ions as charge carriers of the slow inward current in mammalian myocardial fibers. Pflügers Arch 344:149–158, 1973
7. Gavrilescu S, Luca C: Right ventricular monophasic action potentials in patients with long QT syndrome. Br Heart J 40:1014–1018, 1978
8. Miyamae S, Goto K: The effects of extracellular calcium removal on sinoatrial node cells treated with potassium-depleted solutions. Jpn J Physiol 36:403–409, 1986
9. Miyamae S, Goto K: Effects of ethylene glycol-bis-(β-aminoethyl ether)-N, N'-tetraacetic acid on rabbit sinoatrial node cells treated with cardiotonic steroids. J Pharmacol Exp Ther 245:706–717, 1988
10. Miyamae S: Influence of magnesium and extracellular calcium reduction on ouabain-treated sinoatrial node cells in rabbit heart. Pharmacol Toxicol 65:192–197, 1989
11. Dörrscheidt-Käfer M: The action of Ca^{2+}, Mg^{2+} and H^+ on the contraction threshold of frog skeletal muscle. Pflügers Arch 362:33–41, 1976
12. Miller DJ, Mörchen A: On the effects of divalent cations and ethylene glycol-bis-(β-aminoethyl ether) N,N,N', N'-tetraacetate on action potential duration in frog heart. J Gen Physiol 71:47–67, 1978
13. Brachmann J, Scherlay BJ, Rosenstraukh LV, Lazzara R: Bradycardia-dependent triggered activity: relevance to drug-induced multiform ventricular tachycardia. Circulation 68:846–856, 1983
14. Roden DM, Hoffman BF: Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibers. Relationship to potassium and cycle length. Circ Res 56:857–867, 1985
15. Hoffman BF, Dangman KH: Mechanisms for cardiac arrhythmias. Experientia 43:1049–1056, 1987
16. Kupersmith J, Hoff P, Duo GS: In vitro characteristics of repolarization abnormality – a possible cause of arrhythmias. J Electrocardiol 19:361–370, 1986
17. Rosen KM, Lau SH, Weiss MB, Damato AN: The effect of lidocaine on atrioventricular and intraventricular conduction in man. Am J cardiol 25:1–5, 1970
18. Hagiwara S, Byerly L: Calcium channel. Annu Rev Neurosci 4:69–125, 1981
19. Vereecke J, Carmeliet E: Sr action potentials in cardiac Purkinje fibers. I. Evidence for a regenerative increase in Sr conduc-

tance. Pflügers Arch 322:60–72, 1971
20. Sakai T, Fujii S, Hirota A, Kamino K: Optical evidence for calcium-action potentials in early embryonic precontractile chick heart using a potential-sensitive dye. J Membrane Biol 72:205–212, 1983
21. Lee KS, Tsien RW: Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. Nature 302:790–794, 1983
22. Josephson IR: Lidocaine blocks Na, Ca and K currents of chick ventricular myocytes. J Mol Cell Cardiol 20:593–604, 1988
23. Trautwein W: Membrane currents in cardiac muscle fibers. Physiol Rev 53:793–835, 1973