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Na^+-Ca^{2+} Exchange Transport and Pacemaker Activity of the Rabbit SA Node

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Recent electrophysiological data have provided the evidences that background currents such as Na^+-Ca^{2+} exchange can significantly modulate cardiac pacemaker activity. In this study. the effects of extracellular Na^+ and Ca^{2+} concentrations on the pacemaker activity were investigated by measuring the intracellular Na^+ activity (a^i_{Na}) with Na^+-selective microelectrodes and the results are summarized as follows. 1) In the rabbit SA node, a^i_{Na} was 3.2±0.3 mM and mean MDP (maximal diastolic potential) was -63.3±1.4 mV. 2) Graded decreases of external Na^+ concentration resulted in the loss of spontaneous beating, hyperpolariztion and the decrease of a^i_{Na}. 3) An increase in extracelular Ca^{2+} concentraion in low Na^+ solution augmented the transient decrease of a^i_{Na}, about 3 minutes in low Na^+ solution, until a^i_{Na} started to increase. 4) In low Na^+ solution, which had extracellular Ca^{2+} concentration according to the calculation based on the equilibrium state of Na^+-Ca^{2+} exchange, a^i_{Na} was continuously decreased. It was concluded that intracellular Na^+ activity modulated by Na^+-Ca^{2+} exchange could play an important role in the initiation of pacemaker potential.

Key Words: Rabbit, SA node, pacemaker activity, Na^+-Ca^{2+} exchange, Na^+-selective electrodes, intracellular Na^+ activity

The applications of the conventional microelectrode voltage-clamp and whole cell clamp techniques to pacemaker cells of the SA node revealed membrane currents underlying the pacemaker activity (Noma and Irisawa 1976, Hagiwara *et al.* 1988). Among those currents are the potassium current (i_K), the calcium current (i_{Ca}), Hyperpolarization induced inward current (i_h) and inward background current (i_b).

The high membrane resistance in the pacemaker cells, contrary to other cardiac tissue, implies that very tiny net currents could cause large changes in the membrane potential and be important for the generation of the pacemaker potential. Therefore, background currents such as electrogenic Na^+-K^+ pump or Na^+-Ca^{2+} or Na^+-H^+ exchange transports can play a significant role in the modulation of cardiac pacemaker activity (Giles *et al.* 1986; Irisawa 1987).

Recently, inward Na^+-Ca^{2+} exchange currents have been found in single cardiac cells (Kimura *et al.* 1987: Hagiwara and Irisawa 1989). Although there remains the possibility that an ATP-dependent Ca^{2+} pump also plays a role in Ca^{2+} transport across the cell membrane, Na^+-Ca^{2+} exchange appears to be the principle transport system for Ca^{2+} efflux out of the cell. Na^+-Ca^{2+} exchange is a transport system that uses the movement of Na^+ down its electrochemical gradient into the cell to transport Ca^{2+} up its electrochemical gradient out of the cell. The exchange is not neutral, but is electrogenic (i.e., $3Na^+$: $1Ca^{2+}$) (Mullins 1981). There is a significant influx of Ca^{2+} during each cardiac cycle, and it could generate a part of the inward background current (Na^+-Ca^{2+} exchange current) in pacemaker activity of cardiac cells (Brown *et al.* 1984ab; Mechamann and Pott 1986; Giles *et al.* 1986; Irisawa 1987).

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There are many reports about ionic currents during pacemaker activity, and it will be more helpful to understand the pacemaker mechanism if we can directly measure the change of intracellular ionic activites generated with these currents. There is, however, no direct evidences for changes of intracellular ionic activity, related to pacemaker activity of cardiac cells.

In this study, the involvement of Na^+-Ca^{2+} exchange transport in pacemaker activity was investigated by measuring the changes in intracellular Na^+ activity (a^i_{Na}) with a Na^+-selective microelectrode.

METHODS

Rabbits of either sex about 1 Kg in size were sacrificed with an intraperitoneal injection of Na pentobarbital (50 mg/Kg). The heart was rapidly excised and placed in oxygenated (100% O_2) Tyrode solution. The right atrial tissue was dissected free of other tissue, and strips of the SA node, 1~2 mm in width and 1 cm in length, were dissected out along the direction perpendicular or parallel to the crista terminalis. Then strips were transferred to a recording chamber which was continuously perfused with the oxygenated Tyrode solution. Tension was monitored by a Model 400 A Force Transducer System (Cambridge Technology, Inc.) and displayed on a Digital Storage Oscilloscope (Model Philips PM 3305) and traced on a chart recorder (Gould Brush 220). The signal from the differential electrometer for the ion-selective electrode (ISE) was also recorded. The temperature in the recording chamber was maintained at 27±0.2°C. The Tyrode solution had the following composition in millimoles per liter (mM): NaCl 140; KCl 4.0; $CaCl_2$ 1.8; HEPES 5; glucose 5.5; and pH 7.4. For Na^+-deficient solutions, NaCl was isotonically replaced with tetramethylammonium (TMA) chloride. Spring-aided sup-ports of the solution container helped maintained constant solution level and flow rate (about 5 ml/minute) of the superfusion solution.

Transmembrane potentials were recorded between 3 m KCl -3% agar bridge in the bath and a standard microelectrodes, pulled from microfiber capillary tubings (WPI, Inc.) which had a typical resistance in the range of 5~20 megohms and tip diameters of less than 0.5 microns.

The Na^+-selective electrodes (NSE) were manufactured as follows. Glass microelectrodes were pulled from borosillicate glass capillaries (WPI 1B 200F6) which had been cleaned with alcohol, boiled in distilled water, and dried completely. Glass pipettes were inserted, tip upward, into holes drilled into a small Teflon plate. The Teflon plate with the pipettes was placed on the top of a bottle containing a small drop of pure dichlorodi-methylsilane and then placed in a oven at 200°C. The silane vapor was allowed to react with the glass for 30 minutes. The silanized pipettes were filled with a 100 mM NaCl reference solution. A column of exchanger resin (Fluka 71176, Fluka Chemie AG) was forced into the electrodes by means of a partial vacuum. The electrode was filled with resin to the tip.

The e.m.f. (electromotive force) from the NSE was measured with an electrometer (Analog Devices AD515 operational amplifier, Norwood, MA U.S. A.). Membrane potential was measured by RE which was electronically subtracted from the e.m.f. measured with the NSE. Na^+-selective electrodes were calibrated with mixed electrolyte solutions (NaCl 100, KCl 40; NaCl 30, KCl 110; NaCl 10, KCl 130; NaCl 3, KCl 137; NaCl 1, KCl 139; NaCl 0.3, KCl 139.7; in mM). NSE had Nernstian responses in pure NaCl calibrating solutions. In mixed NaCl-KCl

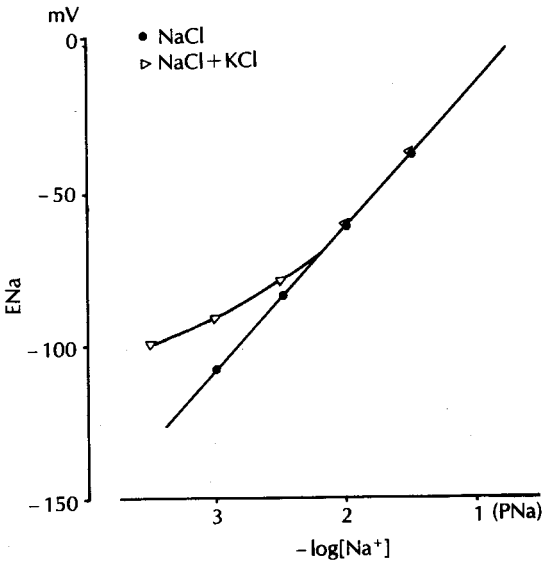

Fig. 1. *Calibration curve for Na^+-Selective electrodes. The closed circles(●) represent the electrode potential measured with single electrolyte solutions of NaCl. The open circles(○) represent the electrode potentials measured with mixed solutions of NaCl and KCl (NaCl 100, KCl 40; NaCl 30, KCl 110; NaCl 10, KCl 130; NaCl 3, KCl 137; NaCl 1, KCl 139; NaCl 0.3, KCl 139.7; in mM).*

solution calibration curve began to deviate from the Nernstian response at the solution containg 30 mM NaCl. We only used those electrodes that had slopes greater than 40mV between 30mM NaCl with 120 mM NaCl with 120 mM KCl and 3 mM NaCl with 147 mM KCl, which indicates a good selectivity for Na^+ over Ca^{2+} (Sheu and Fozzard 1982) (Fig. 1). To improve the slow response time, the negative capacity compensation circuit was connected to the amplifier input of ion-selective microelectrodes.

RESULTS

Membrane potential and intracellular Na^+ activity of the SA node

SA node specimens generate spontaneous action potentials and develop twitch tension. In this study, we measured simultaneously and continously a^i_{Na} and/or twitch tension. Fig. 2 shows the measurements of transmembrane potential of such a specimen.

The mean of maximal diastolic potential (MDP) measured in this study was -63.3 ± 1.4 mV (n=18) at normal Tyrode solution, which was lower than MDP measured in central pacemaker cells ($-40\sim-50$ mV) (Brown 1980). This outcome could be explained by the measurements of not only central pacemaker cells but also latent (peripheral) pacemaker cells.

A cell was then impaled with a Na^+-selective microelectrode as shown in Fig. 2A (b) (E_{Na}) reached a stable level, a cell was impaled with a conventional microelectrode (Fig. 2A (a)) and the measured transmembrane potential (Em) was substracted electronically from the E_{Na} (Fig. 2B). The mean of the intracellular Na ion activity (a^i_{Na}) of the SA node cells was found to be 3.2 ± 0.3 mM (n=18) (Table 1).

Effects of low Na^+ solutions on membrane potential and tension

Simultaneous recording of both membrane potential and muscle tension of SA node specimens showed that hyperpolarization of the resting membrane was accompanied with increase in muscle tension in low Na^+ solution (Fig. 3A).

Table 1. Transmembrane potentials and intracellular Na^+ activity measured from the SA node cells of rabbits.

MDP (mV)	63.3 ± 1.4
$[Na^+]_i$(mM)	4.2 ± 0.4
a^i_{Na} (mM)#	3.2 ± 0.3

MDP: Maximal diastolic potential (n=18)
$[Na^+]_i$: Intracellular Na^+ concentration
a^i_{Na}: Intracellular Na^+ activity
#: A mean activity coefficient for Na^+ of 0.75 was used

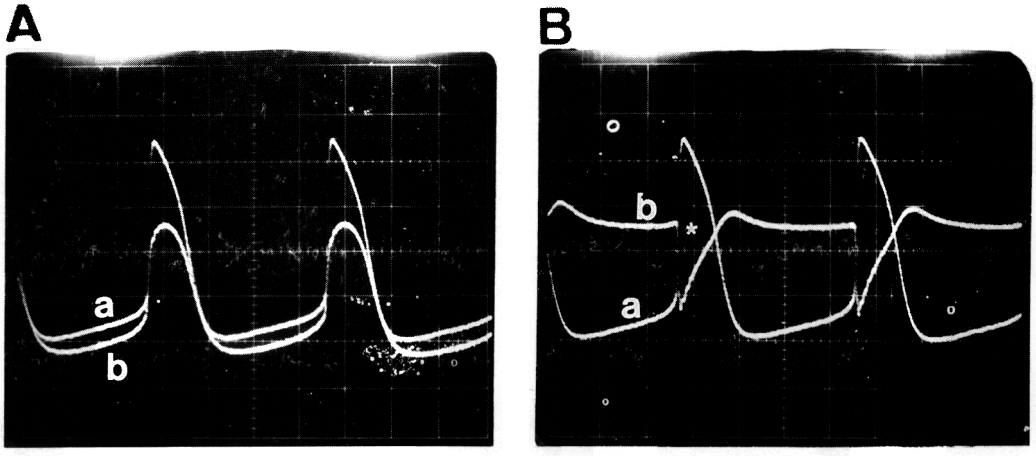

Fig. 2. Measurement of intracellular Na^+ activity. A: The measured transmembrane potential (E_m; a) and the e.m.f. measured with the NSE (E_{Na}; b) were displayed on an oscilloscope. B: The membrane potential measured by the RE (E_m) was electrically subtracted from the E_{Na}-E_m (b) (20 mV/div, 200 msec/div)*

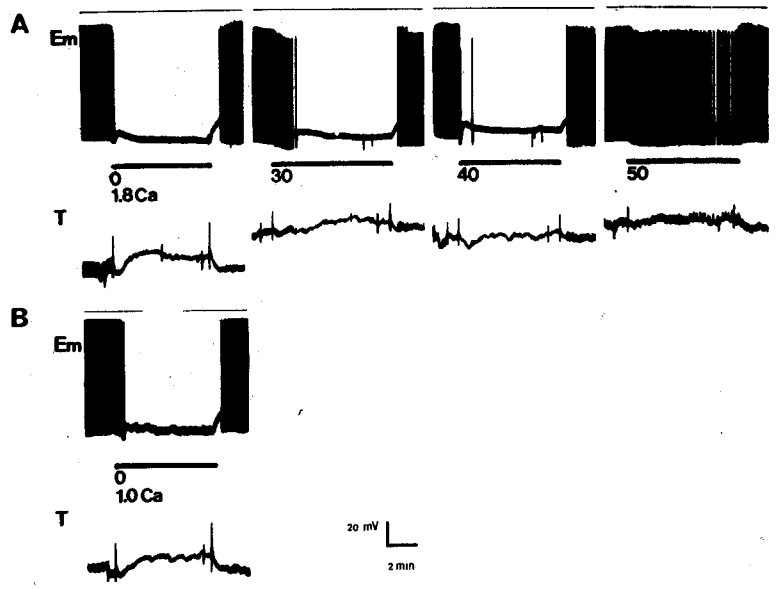

Fig. 3. *Effects of low Na^+ solution on membrane potential and muscle tension. A: During the periods shown by a horizontal bar, low Na^+ solutions (0, 30, 40, 50% Na^+ of that in the normal Tyrode solution) were superfused. B: 100% Na^+, low Ca^{2+} solutions were superfused during the period shown by a bar. Upper traces represent membrane potential (Em) and lower traces represent tension (T). The tension of the strip was monitored by a Model 400A Force Transducer System (Cambridge Technology, Inc.) and displayed on a Philips PM 3305 Digital Storage Oscilloscope and on a Gould Brush 220 recorded along with the signal from the differential electrometer for the ion-selective electrode (ISE).*

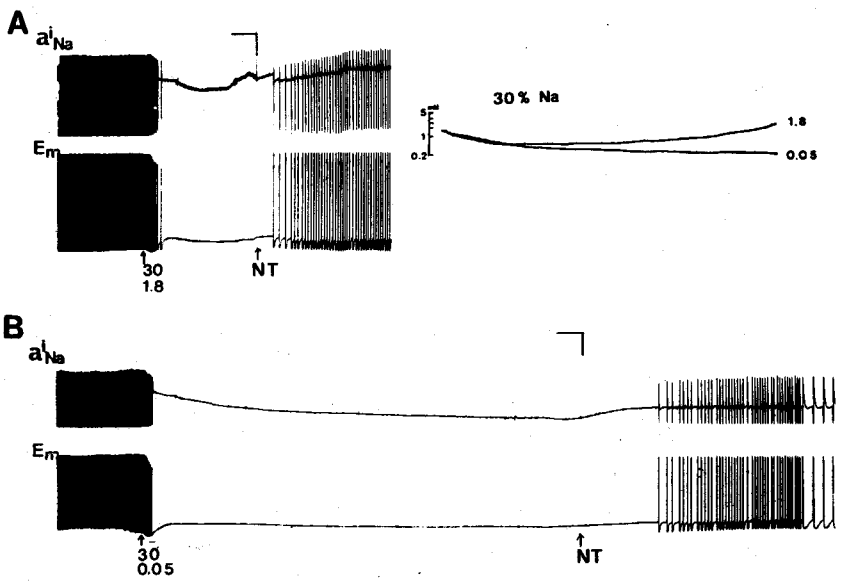

Fig. 4. *Effects of Ca^{2+} concentration on intracellular Na^+ activity during the superfusion of 30% Na^+ solutions. A: Spontaneous beatings of the tissue were stopped several second after the superfusion of 30% Na^+, 1.8 mM Ca^{2+} solution was started, and restarted when the tissue was superfused with the normal Tyrode solution. Horizontal scale, 1 minute; vertical scale, 20 mV. B: spontaneous beatings were stopped with 30% Na^+, 0.05 mM Ca^{2+} solution, and restarted with the normal Tyrode solution. Horizontal scale, 12 seconds; vertical scale, 20 mV.*

Hyperpolarization and contracture in low Na^+ solution decreased in amplitude as $[Na^+]_o$ was increased and $[Ca^{2+}]_o$ was decreased. Contracture was not observed at 50% Na^+ solution. Spontaneous activity ceased after onsets of 0, 30, 40% Na^+ solution perfusion. But in 50% Na^+ solution spontaneous activity did not cease.

Effects of $[Na^+]_o$ on intracellular Na^+ activity of the SA node

Intracellular Na^+ activity was measured continuously in SA node specimens exposed to 30%, 50% and 70% Na^+ solutions with different Ca^{2+} concentration for 3~5 minutes (Fig. 4, 5). Low Ca^{2+} concentrations were determined from the following equation.

$$([Na^+]_i)^3/[Ca^{2+}]_i = ([Na^+]_o)^3/[Ca^{2+}]_o \exp(-VmF/RT)$$

Vm: membrane potential
F: Faraday constant
R: Ideal gas constant
T: Temperature (°K)

This equation is derived from the equation of

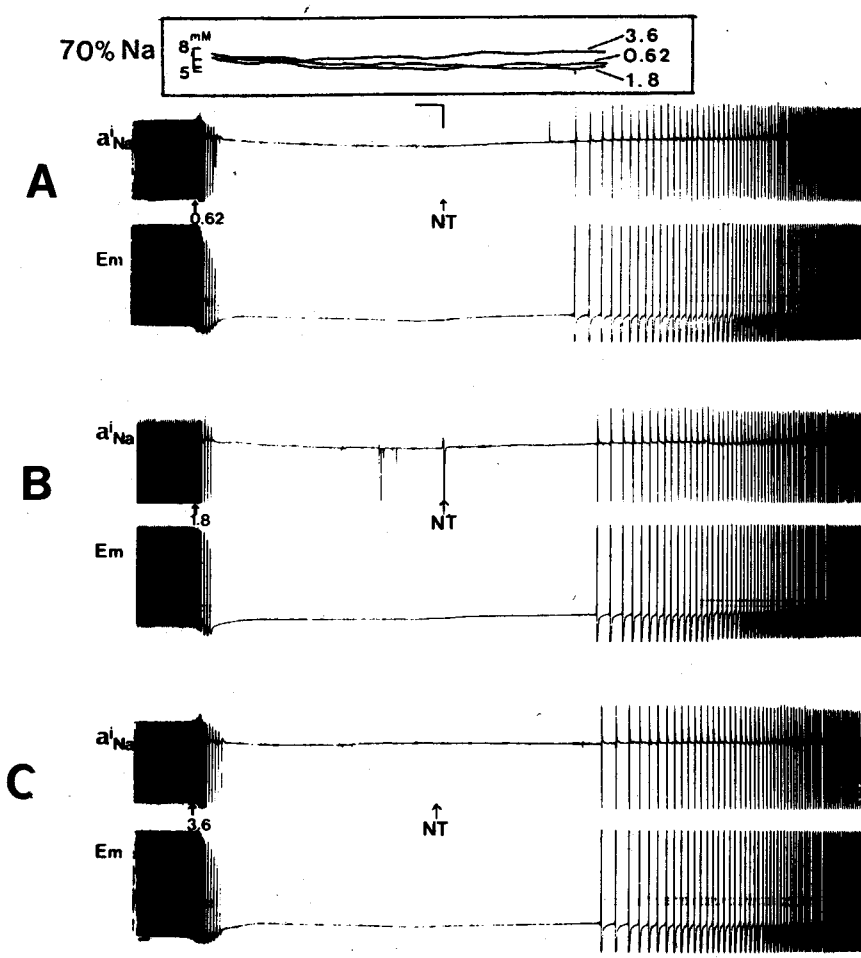

Fig. 5. *Effects of Ca^{2+} concentration on intracellular Na^+ activity during the superfusion of 70% Na^+ solutions, 70% Na^+ solutions with various Ca^{2+} concentration (0.62 mM for A, 1.8 mM for B, and 3.6 mM for C) were superfused for 3 minutes. A: The superfusion of 0.62 mM Ca^{2+} solution stopped spontaneous beatings and caused a steady decline in a^i_{Na} for 3 minutes, from 7.4 mM to 6.2 mM. B. The superfusion of 1.8 mM Ca^{2+} solution caused a steady decline of a^i_{Na} from 7.4 mM to 5.5 mM. C. The superfusion of 3.6 mM Ca^{2+} solution caused a decline of a^i_{Na} for 2 minutes, from 8.7 mM to 7.9 mM, and an increase to 8.8 mM for following 1 minute. Horizontal scale, 12 seconds; vertical scale, 20 mM.*

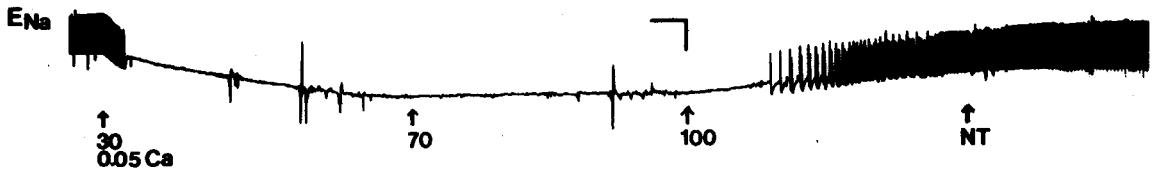

Fig. 6. Effects of extracellular Na^+ on the intracellular Na^+ activity and pacemaker activity. The superfusion of 30% Na^+, 0.05 mM Ca^{2+} solution stopped beatings and intracellular Na^+ activity continuously decreased (from 3.1 mM to 0.4 mM). Intracellular Na^+ activity increased (to 1.0 mM) with 100% Na^+, 0.05 mM Ca^{2+} solution and beating resumed before the superfusion of normal Tyrode solution. It implies that inward current generated by Na^+-Ca^{2+} exchange has a role in generation of pacemaker potential.

Na^+-Ca^{2+} exchange in the equilibrium state. If extracellular Na^+ is decreased, the reverse mode of Na^+-Ca^{2+} exchange become operational. This reverse mode can be prevented if $[Ca^{2+}]_0$ is decreased simul-taneously with $[Na^+]_0$. Therefore,$[Ca^{2+}]_0$ can be determined by the folowing equation.

$$[Ca^{2+}]_{0,2} = [Ca^{2+}]_{0,1} \times ([Na^+]_{0,2}/[Na^+]_{0,1})^3$$

$[Na^+]$ or $[Ca^{2+}]_{0,1}$: 1st state of extracellular concentration of Na^+ or Ca^{2+}

$[Na^+]$ or $[Ca^{2+}]_{0.2}$: 2nd state of extracellular concentration of Na^+ or Ca^{2+}

The 30% Na^+ solutions with 1.8 mM Ca^{2+} and 0.05 mM Ca^{2+} concentration were superfused for 5 minutes (Fig. 4). Spontaneous beatings of the tissue were stopped several seconds after the superfusion of 30% Na^+, 1.8 mM Ca^{2+} solution. The a^i_{Na} decreased from 2.8 mM to 0.9 mM for 3 minutes, and increased to 2.8 mM for the following 2 minutes. The superfusion of 0.05 mM Ca^{2+} solution stopped spontaneous beating and caused a steady decline in a^i_{Na} initilally from 3.5 mM to 0.5 mM for the first 3 minutes. In the following 2 minutes the a^i_{Na} fall to 0.2 mM.

These results indicate that the re-increase of a^i_{Na} during the perfusion of 30% Na^+, 1.8 mM Ca^{2+} solution was due to the forward mode of Na^+-Ca^{2+} exchange caused by intracellular Ca^{2+} loading. But in 0.05 mM Ca^{2+} concentration (according to the calculation from the equilibrium state of Na^+-Ca^{2+} exchange), a^i_{Na} was continuously decreased.

The 70% Na^+ solutions with various Ca^{2+} conceutrations (0.62, 1.8, 3.6 mM)were superfused for 3 minutes (Fig.5). The superfusion of 0.62 mM Ca^{2+} solution stopped spontaneous beatings and caused a steady decline in a^i_{Na} for 3 minutes from 7.4 mM to 6.2 mM. The superfusion of 1.8 mM Ca^{2+} solution caused a steady decline of a^i_{Na}, from 7.4 mM to 5.5 mM. The superfusion of 3.6 mM Ca^{2+} solution caused a decline of a^i_{Na} initially from 8.7 mM to 7.9 mM for the first 2 minutes. In the following 1 minute the a^i_{Na} increased to 8.8 mM. This suggests that the superfusion of 70% Na^+, 3.6 mM Ca^{2+} solution induced intracellular Ca^{2+} loading that caused re-influx of Na^+ from the Na^+-Ca^{2+} exchange.

To show the effect of extracellular Na^+ concentration on the intracellular Na^+ activity and pacemaker activity, 0.05 mM Ca^{2+} solutions with various Na^+ concentrations (30%, 70%, 100%) were perfused in series (Fig. 6). The reason for maintaining Ca^{2+} concentration at 0.05 mM was to prevent intracellular Ca^{2+} loading. The superfusion of 30% Na^+, 0.05 mM Ca^{2+} solution stopped beatings and intracellular Na^+ activity was continuously decreased from 3.1 mM to 0.4 mM. Intracellular Na^+ activity was increased to 1.0 mM with 100% Na^+ with 0.05 mM Ca^{2+} solution, and simultaneous resumption of beating occured before the superfusion of normal Tyrode solution.

DISCUSSION

The ionic basis of cardiac pacemaker activity has been investigated by electrophysiologist for more than 100 years. Information on the pacemaker mechanism in SA node tissue has been greatly enhanced by the development of the single cell isolation technique and the patch clamp technique (Denyer and Brown 1990). Some currents (I_{Ca}, I_K, I_f) were found with the experimented methods mentioned above, and recent data have provided evidences that background currents such as Na^+-Ca^{2+} exchange currents can significantly modulate cardiac pacemaker activity (Irisawa 1987). But there in no evidence for the change of intracellular ionic

activity related to pacemaker activity of cardiac cells. Thus we first measured the intracellulr Na^+ activity in SA node tissues. And then the effects of Na^+ and Ca^{2+} on the intracellular Na^+ activity were studied.

Intracelluar Na^+ activity in the rabbit SA node during spontaneous activity and arrest induced with low Na^+ solution were measured with Na^+-selective electrodes. The mean a^i_{Na} measured during spontaneous activity were 3.2±0.3 mM (Table 1). The a^i_{Na} measured in this study was lower than the value measured from other cardiac myocytes, 5.7 mM in rabbit ventricular muscle, 6.4~7.2 mM in sheep Purkinje fibers (Lee 1981), 6.4±0.6 mM in in sheep ventricular myocytes and 8.0±0.6 mM in sheep Purkinje fibers (Sheu and Fozzard, 1982). This lower measured value might be due to the fact that the Na^+ channels in the SA node cells were inactivated, and, if any, the Na^+ influx was negligible or markedly decreased. (Nakayama *et al.* 1984; Giles *et al.* 1986). And furthermore this lower value of a^iNa in SA node cells might provide a steeper Na^+ concentration gradients for Na^+ influx during pacemaker activity through background currents such as Na^+-Ca^{2+} exchange, Na^+-K^+ pump and Na^+-H^+ exchange.

Because the effect in true pacemaker cells is almost certainly different from that in latent pacemaker cells, the effect of $[Na^+]_o$ on the electrical activity of SA node is variable (Crenefield 1975; Masson-Pevet *et al.* 1982). In some cases the reduction of $[Na^+]_o$ to 30% or 40% of normal, produced the stop of pacemaker activity (Fig. 3A). In some cases pacemaker activity disappeared in 70% Na^+ of normal solution (Fig. 5). Such differences could be explained by electrical signals not only in central pacemaker cells but also in peripheral pacemaker cells.

When extracellular Na^+ was reduced, spontaneous activity stopped and contracture developed con-commitantly with a marked hyperpolarization of the SA node cell membrane (Fig. 3) as reported elsewhere (Reuter and Seitz 1986; Vassort 1973; Chapman 1974; Irisawa and Noma 1976). These results suggested that an increase in intracellular Ca^{2+} induced both responses. Ca^{2+} influx by Na^+-Ca^{2+} exchange (reverse mode) increases with decreasing $[Na^+]_o$ as well as with increasing $[Ca^{2+}]_o$. And intracellular Ca^{2+} loading will finally induce reinflux of Na^+ with Ca^{2+} efflux through the forward mode of Na^+-Ca^{2+} exchange.

Thus, to understand the Na^+-Ca^{2+} exchange transport in pacemaker activity, the dependence of intracellular Na^+ activity on $[Na^+]_o$ and $[Ca^{2+}]_o$ was investigated.

When $[Na^+]_o$ was reduced to 30%, 50%, 70% of normal Na^+, intracellular Na^+ activity was decreased and the decrease was depended on the changes in $[Ca^{2+}]_o$(Fig. 4, 5). This data suggests that during the perfusion of Na^+ depleted solution the reverse mode of Na^+-Ca^{2+} exchange is operated and $[Ca^{2+}]_i$ is increased.

The increase of intracellular Ca^{2+} will be reduced with uptake of free Ca^{2+} by the sarcoplasmic reticulum (SR) and/or transmembranous efflux through the Na^+-Ca^{2+} exchange (Irisawa and Noma 1976). In the SA node cells the amount of SR is fewer than other cardiac myocytes (Masson-Pevet *et al.* 1982), therefore increased $[Ca^{2+}]_i$ might be mainly effluxed through Na^+-Ca^{2+} exchange. This intracellular Ca^{2+} loading and re-influx of Na^+ occured more rapidly during the perfusion of the lower $[Na^+]_o$ and the higher $[Ca^{2+}]_o$ solution. But in low $[Na^+]_o$ solution which had $[Ca^{2+}]_o$ according to the calculation from the equilibrium state of Na^+-Ca^{2+} exchange (see Results), a^i_{Na} was continuously decreased (Fig. 4, 5). This could be due to not only Na^+ efflux through the Na^+-Ca^{2+} exchange but also Na^+ efflux through the Na^+-K^+ pump (Lee and Diagostino 1982).

If membrane potential remains changed, the forward mode of Na^+-Ca^{2+} exchange could be operated not only in high $[Ca^{2+}]_i$ states (Fig. 4, 5) but also in high $[Na^+]_o$ states. But the increase of Na^+ concentration of perfusion solutions was difficult to maintain physiologic osmolarity. Therefore intracellular Na^+ activity was decreased with low Na^+ solution. The change of intracellular Na^+ activity by $Na^+ - Ca^{2+}$ exchange with perfusion of different concentrations of extracellular Na^+ was measured (Fig. 6). That is, after intracellular depletion of Na^+ with 30% Na^+, 0.05 mM Ca^{2+}, a graded increase in external Na^+ concentration (to 70% and 100% Na^+) resulted in the recovery of spontaneous beating and increase of a^i_{Na}.

From these findings, it was concluded that intracellular Na^+ and Ca^{2+} concentrations modulated by the Na^+-Ca^{2+} exchange transport could be an important mechanism for the initiation of pacemaker potential. Futhermore low a^i_{Na} of SA node cells might play an important role in generation of ionic currents under the pacemaker activity.

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