Unaffected Electrogenic Na-K Pump Activity in “Diseased” Human Atrial Fibers, as Assessed by Intracellular K^+ Activity

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Abstract To investigate the role of the electrogenic Na-K pump in the resting membrane of “diseased” or “depolarized” human atrial muscles, intracellular K^+ activity (a_K^i) and resting membrane potential (V_m) were simultaneously measured using double-barreled K^+ -selective micro-electrodes. Under perfusion with normal Tyrode’s solution (37°C) containing 5.4 mM $[K]_o$, V_m averaged -43.9 ± 1.4 mV, and a_K^i was 99.7 ± 1.3 mM (mean $\pm$ S.E., $n=33$). The a_K^i was comparable to that of atrial muscles obtained from other intact mammalian species. In 5.4 mM $[K]_o$, dihydro-ouabain (DHO) at concentrations of 10^{-6} and 10^{-5} M significantly decreased a_K^i and depolarized V_m . Similar decreases in a_K^i were observed when $[K]_o$ was decreased from 5.4 to 0.5 mM or when the temperature of the perfusing solution was decreased from 37 to 22°C. Upon returning $[K]_o$ from 0.5 to 5.4 mM at 37°C, a_K^i increased, V_m hyperpolarized markedly for about 3 min, and this was followed by less marked levels of hyperpolarization in the steady state. The high $[K]_o$ -induced increases in a_K^i were inhibited in the presence of DHO, and at low temperature (22°C). Isoproterenol (10^{-7} M) increased a_K^i and hyperpolarized V_m . Acetylcholine (10^{-5} M) hyperpolarized V_m with no change in a_K^i . The rate of reduction of Na^+ -efflux during application of DHO (10^{-5} M) was calculated based on the change in a_K^i and surface-to-volume ratio of the cell measured electronmicroscopically in the same tissue, and estimated to be 2.6 to 3.8 pmol/(cm²·s), close to the value reported for Purkinje fibers excised from intact animals. We conclude that the Na-K pump functions normally even in “diseased” human atrial muscles, thereby keeping a_K^i within a physiological range.

Key words: human atrial fiber, K^+ -selective microelectrode, electrogenic Na-K pump, dihydro-ouabain, low temperature.

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The Na-K pump operates against the electrochemical gradient of Na^+ and K^+ to maintain intracellular Na^+ and K^+ concentration homeostasis. The pump extrudes Na^+ from the cell to a greater degree than it takes up K^+ and thus generates a net outward current (electrogenic Na^+ pump current). This pump current, in conjunction with the ionic gradient and ionic membrane conductance, affects the membrane potential (GLITSCH, 1979).

Diseased human atrial fibers are characterized by low, non-uniform levels of the resting membrane potential (TEN EICK and SINGER, 1979; MARY-RABINE *et al.*, 1983; IMANISHI and ARITA, 1987). Such changes have been attributed to non-homogeneous decreases in the resting K^+ conductance (TEN EICK and SINGER, 1979), increases in the resting Na^+ conductance (IMANISHI and ARITA, 1987), and impairment of the electrogenic Na-K pump (LEE, 1986). However, IMANISHI and ARITA (1987) emphasized the role of electrogenic Na-K pump current in maintaining the resting potential at relatively high levels, which counteracted the depolarization due to the altered ionic membrane conductance. However, their conclusion was derived from alterations of the membrane potential measured before and after inhibition of the Na-K pump by various procedures such as ouabain or low external K^+ concentration, which inevitably affects the membrane conductance. To evade this problem and clarify the functional role of the Na-K pump in diseased human atrial preparations, we attempted to measure the intracellular K^+ activity, a_{K}^i as a quantitative index of Na-K pump activity, by using double-barreled K^+ -selective microelectrodes. The main purposes of the study are to determine: 1) whether or not the Na-K pump activity is impaired in depolarized or diseased human atrial preparations; and 2) the functional effect of the Na-K pump current on the resting potential of those preparations.

MATERIALS AND METHODS

Preparations and procedures. Specimens of right atrial appendage were obtained from 33 patients undergoing corrective cardiac surgery for various congenital and acquired heart diseases. The patients were selected consecutively in order of the operation and ranged in age from 3 to 72 years (Table 1). No patient had been treated with digitalis or antiarrhythmic agents within 2 months prior to surgery. Their electrocardiogram revealed no atrial fibrillation and examination of cardiac catheterization disclosed the mean right atrial pressure to be less than 8 mmHg.

As a part of the routine atriotomy in open heart surgery, a small specimen was removed from the appendage of the right atrium. Immediately after excision, the tissue was immersed in cold oxygenated Tyrode's solution ($5-6^\circ\text{C}$) and taken to the laboratory. A few trabeculae, 0.8–1.5 mm long and 0.3–0.8 mm wide, were dissected from the specimen under a dissecting microscope. The preparation was mounted in a Lucite chamber (0.8 ml) and superfused (flow rate, 3–4 ml/min) with warmed ($37 \pm 0.2^\circ\text{C}$) Tyrode's solution. Specimens were allowed to equilibrate in

Table 1. Number of patients classified by clinical diagnoses.

Congenital heart disease		16
Atrial septal defect	10	
Ventricular septal defect	6	
Acquired heart disease		17
Valvular disease	9	
Coronary artery disease	7	
Unspecified	1	
Total		33

Tyrodé's solution for 1 h prior to study. To measure a_K^i , multiple impalements were made on the surface of the preparation with a double-barreled K^+ -selective microelectrode. Only stable records lasting for more than 5 min were used as data. When the impalement was successful for more than 20–30 min, various test solutions were introduced into the chamber to alter the Na-K pump activity and to check changes in a_K^i . The procedure included change of external K^+ concentration ($[K]_o$), change of the temperature of the perfusing solution, and application of dihydro-ouabain, isoproterenol, or acetylcholine.

Solutions. The control Tyrodé's solution contained (in mM): NaCl, 136.7; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHCO_3$, 11.9; NaH_2PO_4 , 0.42; glucose, 5.0. Solutions with 0.5 mM K^+ were made by equimolar substitution of Na^+ for K^+ . In some experiments, dihydro-ouabain (DHO), isoproterenol (Iso), or acetylcholine (ACh), were added directly to the Tyrodé's solution to give final concentrations of 10^{-6} – 10^{-5} , 10^{-7} , and 10^{-5} M, respectively. All solutions were equilibrated with 97% O_2 –3% CO_2 , making the pH 7.35 to 7.40, and kept at $37 \pm 0.2^\circ C$. For cooling experiments, cold Tyrodé's solution ($22^\circ C$) was introduced into the perfusion chamber. The temperature of the bath solutions could be changed from 37 to 22, or 22 to $37^\circ C$ within 3 min by passing the Tyrodé's solution through coiled polyethylene tube immersed in chilled water.

Fabrication of K^+ -selective microelectrodes. The method of preparing K^+ -selective microelectrodes (KISEs) was essentially the same as that described by HOTOKEBUCHI *et al.* (1987). In brief, two capillaries (Narishige, GD-1) were mounted in parallel on a horizontal micropipette puller (Narishige, PW-77), and heat was applied. The capillaries were then twisted 180° and pulled. The tip diameter of these capillaries was smaller than $0.5 \mu m$. After one barrel was siliconized, it was back-filled with potassium ionophore (Fluka, 60031), until the ionophore column became approximately $100 \mu m$ in length from the tip to the base. The remaining portion of this barrel was filled with 0.5 M KCl. The other barrel (reference) was back-filled with 1 M NaCl. The DC-resistance of the K^+ -selective barrel and the reference barrel were 10–100 G Ω and 20–60 M Ω , respectively. The KISE, thus prepared, was stable for more than 12 h. The "response time" (HOTOKEBUCHI *et al.*, 1987) of the

Fig. 1. A: calibration curve for a double-barreled K⁺-selective microelectrode. V_K , potential measured by the K⁺-selective electrode; a_K , activity of K-ion. V_K (ordinate) is plotted against the logarithm of a_K (abscissa) in solutions containing various combinations of concentrations of K⁺ and Na⁺ (see METHODS). B: an example of the measurement of intracellular K⁺ activity (a_K^i). Bottom trace, membrane potential from reference barrel (V_m); top trace, potential difference between the K⁺-selective barrel and reference barrel reflecting a_K^i ($V_K - V_m$). Downward arrow, cell-impalement; upward arrow, withdrawal of electrode.

KISE was measured to be in less than 10 s ($\tau = 4$ s). The a_K^i was measured only at resting condition of the preparation, because of its slow response time. If spontaneous activity was induced in low $[K]_o$ or in the presence of DHO, a_K^i was measured after cessation of the spontaneous activity.

Calibration. All KISEs were calibrated before and after each experiment in solutions consisting of mixtures of KCl and NaCl with the total concentration fixed at 150 mM (5 mM KCl + 145 mM NaCl; 10 mM KCl + 140 mM NaCl; 20 mM KCl + 130 mM NaCl; 50 mM KCl + 100 mM NaCl; 100 mM KCl + 50 mM NaCl; and 150 mM KCl) at 37°C, and when necessary, at 22°C. Figure 1A represents the calibration curve of a KISE, which shows potential response of the microelectrode filled with potassium ionophore (V_K) versus K⁺ activity of the test solution. The activity coefficient of K⁺ was regarded as to be 0.75. The average slope of the electrodes was 54.3 ± 0.4 mV/decade. The selectivity coefficient (k_{K-Na}) was calculated

Table 2. Morphologic features of human atrial cells ($n=81$).

Length (μm)	Diameter (μm)	Surface area (μm^2)	Volume (μm^3)	S/V ratio (cm^{-1})
76.6 ± 26.3	17.7 ± 4.3	$3,157 \pm 1,299$	$10,480 \pm 6,306$	$3,335 \pm 790$

Values are means $\pm$ S.D. S/V ratio: surface area divided by volume. Each parameter measured assuming smooth cell surface and each cell was a solid of revolution around the central longitudinal axis.

by the Nicolsky equation, for the mixed electrolyte solution (see LEE, 1981). The calculated values were less than 0.01.

Recording techniques. Double-barreled microelectrodes, with one barrel containing potassium ionophore, and the other NaCl, were connected to each input probe of a high input-impedance amplifier (WPI FD 223-D). An Ag-AgCl electrode embedded in 3% agar-3M KCl was used to ground the bath. To measure the intracellular potassium activity, a_{K}^i , potential from the NaCl-filled reference barrel (V_{m}) was subtracted from that of the K^+ selective barrel (V_{K}). All outputs were monitored with digital voltmeters built-in the amplifier, and a digital memory oscilloscope (Kikusui, DSS-5020A), and were displayed on a pen chart recorder (Nihon Kohden, WT645G).

Estimations of cell volume and cell surface area. The atrial tissue blocks excised from 4 patients were fixed in a mixed solution of glutaraldehyde and osmium tetroxide at the slack length, dehydrated in ethanol, and embedded in epoxy resin. Thin sections were examined in a JEM-100x electron microscope, and pictures were taken at an original magnification of 660 and then enlarged as desired. The cells, when sectioned horizontally to the long axis over the entire length with the cut end of the nucleus in the center, were accepted for measurement. Assuming that the human atrial cell was a solid of revolution, we calculated the volume and the surface area of the cell, by marking the contour of the cell and measuring the radius in every section over the entire length of the cell, with the help of a digitizer (Kd4030, Graphtec, Tokyo). Every cross-section area thus obtained was integrated over the whole cell length by a computer (PC9801, NEC, Tokyo), to provide cell volume and cell surface area. These values, including the average length and diameter of the cells, are listed on Table 2. The length and diameter of human atrial cells obtained here were compatible with those reported previously in similar specimens (TRUEX, 1972; ESCANDE *et al.*, 1987).

Statistical analysis. The significant difference of mean values was analyzed using the paired Student's *t*-test, and the difference was considered significant at a *p* value less than 0.05.

RESULTS

Intracellular K^+ activity (a_K^i) and resting membrane potential (V_m) in diseased human atrial preparations

After 60-min incubation in normal Tyrode's solution (5.4 mM $[K]_o$), a_K^i and V_m in non-driven atrial fiber were recorded simultaneously using double-barreled K^+ -selective microelectrodes (KISEs). In Fig. 2, the a_K^i (log-scale) is plotted against V_m in 33 preparations. Each point represents the averaged value of all (3–10) impalements in one preparation. The a_K^i averaged 99.7 ± 1.3 mM (mean $\pm$ S.E., $n=33$, range 80.8 to 111.2 mM). This value was much the same as values reported for heart muscles from intact animals (rabbit, 87.0 mM reported by SKINNER and KUNZE, 1976; guinea pig, 102.1 mM by BAUMGARTEN *et al.*, 1984). In contrast, V_m was partially depolarized (-43.9 ± 1.4 mV, $n=33$), and distributed over a wide range (-75.0 to -32.5 mV). The dashed line shows the regression line between a_K^i and V_m estimated using the least-squares method ($V_m = 15.6 \log(a_K^i) - 75.1$, $r=0.06$), thereby indicating no significant correlation between a_K^i and V_m . Such findings suggest that: 1) the partial depolarization was not due to a decrease in $[K]_i$; and 2) Na-K pump in the resting membrane appears to be normally operating, despite considerable decreases in V_m . Substitution of the measured values of intra- and extracellular K^+ activity into the Nernst equation gave the V_m of -85.6 mV, which

Fig. 2. Relations between a_K^i and V_m in 33 preparations. Each point, average values obtained from multiple impalements of a single specimen. Dashed line, regression line, estimated using least-squares method; $V_m = 15.6 \log(a_K^i) - 75.1$ (coefficient of correlation, $r=0.06$).

approximately doubled the actual V_m (-43.9 mV).

Effect of dihydro-ouabain (DHO) on a_K^i and V_m

The threshold concentration of DHO to increase the a_{Na}^i by inhibiting the Na-K pump is about 10^{-7} M (DEITMER and ELLIS, 1978) and the effect disappeared at a rate 10 times faster than that of ouabain after washout (BACH and REITER, 1964). Thus, we studied the effects of DHO at concentrations of 10^{-6} and 10^{-5} M on a_K^i and V_m , followed by washout. As shown in the upper trace of Fig. 3A, DHO (10^{-5} M) produced a gradual decrease in a_K^i from its initial value of 84.8 mM to a steady state level of 80.2 mM in approximately 20 min. Figure 3B summarizes the results of the changes in a_K^i obtained from 4 similar experiments, in which DHO significantly decreased a_K^i from 95.3 ± 3.6 to 91.5 ± 3.0 mM within 5 min, and then to 88.3 ± 2.8 mM in 20 min. The low concentration of DHO (10^{-6} M) had less effect but still significantly decreased a_K^i by 3.0 ± 0.3 mM ($n=4$) within 20 min (not shown in Fig. 3). In contrast, the V_m was typically affected in biphasic manner after application of DHO, as shown in Fig. 3A (bottom trace). In the first 5 to 7 min after application, DHO (10^{-5} M) decreased V_m by 3.9 ± 0.5 mV on average, i.e., from -36.6 ± 0.8 to -32.7 ± 1.0 mV ($n=4$, $p<0.01$), while the lower DHO concentration (10^{-6} M) produced much less but still significant depolarization by 0.8 ± 0.1 mV ($n=4$, $p<0.05$). After this depolarization, however, the V_m tended to hyperpolarize with time. Actually, in 4 of 8 preparations tested in the presence of DHO at 10^{-6} or 10^{-5} M, the V_m did hyperpolarize by 1.4 ± 1.0 mV ($n=4$), compared to the V_m just prior to DHO application. These changes in a_K^i and V_m could be reversed within 15 min after washout of the drug.

Effects of altered extracellular K^+ concentrations ($[K]_o$) on a_K^i and V_m

If the Na-K pump is operating in the resting membrane of human atrial preparations, changes in a_K^i and V_m should be expected when the pump is inhibited by introduction of low $[K]_o$. Figure 4A represents one such experiment. Reduction of $[K]_o$ from 5.4 to 0.5 mM, decreased a_K^i by 3.8 mM and decreased V_m by 3.9 mV. On return to 5.4 mM $[K]_o$, the a_K^i recovered to control level and V_m was transiently increased to a maximum level -48.1 mV, which exceeded, by 8.2 mV, the V_m prior to introduction of 0.5 mM $[K]_o$ (-39.9 mV). This maximum hyperpolarization (-48.1 mV) was then followed by a gradual return to the steady state V_m at 5.4 mM $[K]_o$ (-40.2 mV). Figure 4B summarizes the results of changes in a_K^i in 11 similar experiments, in which reduction of $[K]_o$ from 5.4 to 0.5 mM significantly decreased a_K^i from 104.0 ± 2.6 to 95.1 ± 2.6 mM. The resumption of a_K^i to 104.0 ± 2.4 mM, very close to the control value, occurred within 10 min after return to 5.4 mM $[K]_o$. This recovery of a_K^i along with transient hyperpolarization of V_m is considered to be due to activation of the electrogenic Na-K pump upon exposure to high $[K]_o$ (5.4 mM) after being partially suppressed by the perfusion with low $[K]_o$ (0.5 mM).

Fig. 3. Effects of dihydro-ouabain (DHO) on a_K^i and V_m . A: simultaneous recording of a_K^i (upper trace) and V_m (lower trace), when exposed to 10^{-5} M DHO during period indicated by horizontal bar at the top (20 min). B: summarized effects of DHO on a_K^i . Each solid line, different preparation tested. Solid circles connected by heavy solid lines, mean value obtained from 4 individual experiments. Vertical bars, S.E. Asterisk, significant differences from the control (before application of DHO).

Inhibition of high $[K]_o$ -induced increases in a_K^i and V_m

1) *Effects of DHO.* Figure 5A and B show an experiment in which a single impalement was maintained throughout the procedures. In the absence of DHO (Fig. 5A), the elevation of $[K]_o$ from 0.5 to 5.4 mM increased a_K^i by 5.1 mM and produced transient hyperpolarization of V_m , a finding identical to that in the right half of Fig. 4A. In the presence of DHO (Fig. 5B), increase in a_K^i was abolished

Fig. 4. Changes in a_K^i and V_m in response to step changes of $[K]_o$. A: top trace, experimental protocol (changes of $[K]_o$). The a_K^i and V_m were simultaneously recorded. B: steady state a_K^i (ordinate) plotted against different $[K]_o$ (abscissa) for 11 preparations. Solid circles connected by heavy solid lines, averaged values. Vertical bars, S.E. Asterisk, significant difference; N. S., not significant.

and the initial transient hyperpolarization of V_m disappeared as well, but steady hyperpolarization of V_m remained. It must be noted that the steady state V_m at 5.4 mM $[K]_o$ in the presence of DHO (-46.4 mV) was 3.8 mV more negative than the steady state V_m at 5.4 mM $[K]_o$ in the absence of DHO (-42.6 mV). Figure 6 summarizes the changes in a_K^i in 5 similar experiments. Upon changing $[K]_o$ from 0.5 to 5.4 mM in the absence of DHO, a_K^i increased significantly from 94.8 ± 1.7 to

Fig. 5. Effects of DHO on high $[K]_o$ -induced increase in a_K^i and hyperpolarization of V_m . Preparation superfused with 0.5 mM $[K]_o$ -Tyrode's solution switched to 5.4 mM $[K]_o$ in the absence (A) and presence (B) of 10^{-6} M DHO. Entire procedure completed during a single cell impalement. Note that transient hyperpolarization of V_m and increase in a_K^i (panel A) were abolished in the presence of DHO (panel B).

104.3 ± 2.3 mM. In the presence of DHO, the increase in a_K^i was not significant, i.e., 90.3 ± 1.8 mM at 0.5 mM $[K]_o$ and 91.3 ± 2.2 mM at 5.4 mM $[K]_o$.

2) *Effects of low temperature.* To study the effect of temperature on high $[K]_o$ -induced increase in a_K^i and V_m , $[K]_o$ was increased from 0.5 to 5.4 mM at low temperature (22°C). As shown in Fig. 7A, the a_K^i (66.8 mM) and V_m (-20 mV) measured at low temperature and low $[K]_o$ seemed fairly depressed compared to the values measured at normal temperature and low $[K]_o$ (cf. Fig. 5A, a_K^i 97.9 mM; V_m -36.4 mV). At low temperature (22°C), elevation of $[K]_o$ from 0.5 to 5.4 mM did not appreciably change a_K^i or V_m , but increasing temperature from 22 to 37°C markedly increased a_K^i (from 67.1 to 109.9 mM) and hyperpolarized V_m . At the same time, spontaneous action potentials were elicited in 2 of 4 preparations (Fig. 7A). Figure 7B summarizes the temperature-induced changes in a_K^i in 4 similar experiments. The a_K^i was significantly increased (from 88.6 ± 4.4 to 99.9 ± 3.9 mM) by changing $[K]_o$ from 0.5 to 5.4 mM at 37°C (left 2 columns in Fig. 7B, results similar to those shown in the left 2 columns of Fig. 6). At low temperature (22°C),

Fig. 6. Summary of effects of DHO on a_K^i as obtained from experiments as shown in Fig. 5. Each bar, mean value of a_K^i with S.E. (ordinate) in the absence (-) and presence (+) of DHO ($10^{-6}M$). Open bars, data at 0.5 mM $[K]_o$; shaded bars, at 5.4 mM $[K]_o$. Asterisk, significant difference between the values joined by bracket; N. S., no significant difference.

however, neither a_K^i nor V_m was changed by the same increase of $[K]_o$ (from 0.5 to 5.4 mM). In contrast, subsequent increase of temperature from 22 to 37°C with 5.4 mM $[K]_o$ significantly increased a_K^i ($p < 0.01$, right 2 columns in Fig. 7B).

Effects of isoproterenol (Iso) and acetylcholine (ACh) on a_K^i and V_m

Figure 8A shows an example of effect of $10^{-7}M$ Iso on a_K^i and V_m in a preparation perfused in control Tyrode's solution. After application of Iso, a_K^i slightly but significantly increased from 97.5 ± 3.6 to 99.6 ± 3.8 mM ($n=4$, $p < 0.01$), and V_m hyperpolarized by 0.5 to 3.1 mV in all preparations tested. Figure 8B shows a typical effect of ACh on a_K^i and V_m . ACh ($10^{-5}M$) did not produce a detectable change in a_K^i , but it significantly hyperpolarized the membrane potential, i.e., V_m changed from -39.0 ± 4.7 to -43.1 ± 5.1 mV ($n=5$, $p < 0.01$). These effects produced by Iso and ACh were completely reversible within 3 min after washing out of the agents.

Estimation of Na^+ -efflux rate in the steady state

It is possible to calculate the rate of reduction of K^+ net influx (pmol/(cm²·s)) produced by $10^{-5}M$ DHO from the following equation:

$$\Delta[K]_i V / (S \cdot t) \quad \text{or} \quad \Delta[K]_i / \{(S/V) \cdot t\}$$

Fig. 7. Effects of cooling and rewarming on high $[K]_o$ -induced increase in a_k^i and transient hyperpolarization of V_m . A: top row, bath temperature; the second row, $[K]_o$ (mM). Preparation exposed to 0.5 mM $[K]_o$ solution at 22°C for 20 min; then $[K]_o$ was increased to 5.4 mM at this temperature. Thereafter, in 5.4 mM $[K]_o$, bath temperature was raised to 37°C. Traces at extreme right of both a_k^i and V_m show evolution of automatic activity. B: each bar, mean value of a_k^i ($n=4$) with S.E. in response to altered change of $[K]_o$ (0.5 mM, open; 5.4 mM, shaded) at altered temperature as indicated. Asterisk, significant difference between values joined by brackets.

where $\Delta[K]_i$ denotes decrease of $[K]_i$ attained by the first 5 min application of DHO; S and V are surface area and volume of the cell, respectively; and t is the period of application of DHO (5 min or 300 s). Using this equation, the DHO-induced rate of reduction of K^+ net influx was calculated to be approximately

Fig. 8. Effects of isoproterenol (A) and acetylcholine (B) on a_K^i and V_m . Preparation exposed to drug as indicated at top of each panel.

5.1 pmol/(cm²·s), for $\Delta[K]_i$ equal to 5.1 ± 0.8 mM (Fig. 3), t equal to 300 s, and S/V equal to 3,335 cm⁻¹ (Table 2). The value of surface to volume ratio, S/V , was somewhat less than that reported in sheep Purkinje fibers (4,600 cm⁻¹, MOBLEY and PAGE, 1972). Since the photographically measured S may be half to one third of the actual S , due to existence of T-tubules and sarcolemmal caveolae (GADSBY *et al.*, 1985), the true rate of reduction of K⁺ net influx per unit membrane area could be half to one third of 5.1, or 1.7 to 2.6 pmol/(cm²·s). Therefore, the rate of reduction of Na⁺-efflux during a 5-min application of $10^{-5} M$ DHO is estimated to be 2.6–3.8 pmol/(cm²·s), since Na⁺ and K⁺ are likely to exchange at a coupling ratio of 3 to 2 in cardiac muscle.

DISCUSSION

The resting membrane potential of the right atrial muscle excised from the patients with congenital or acquired heart disease ("diseased atrial muscle") is generally decreased, probably due to chronic hemodynamic overload or ischemia that might have been imposed on the atrium for a long period of time (MARY-RABINE *et al.*, 1983; LEE, 1986; IMANISHI and ARITA, 1987). In the present study using such "diseased right atrial muscles," we measured a_K^i and V_m in non-driven conditions and found the a_K^i to be 99.7 ± 1.3 mV and the V_m , -43.9 ± 1.4 mV. This a_K^i is comparable to those estimated for spontaneously active human atrial fibers (98.1 mV

in 4.0 mM $[K]_o$, McCULLOUGH *et al.*, 1987) and intact atrial muscle preparations of other species (rabbit, 87.0 mM in 3.6 mM $[K]_o$, SKINNER and KUNZE, 1976; guinea pig, 102.1 mM in 5.0 mM $[K]_o$, BAUMGARTEN *et al.*, 1984). It was reported that the V_m of "diseased" human atrial fibers is partially depolarized and is distributed over a wide range (MARY-RABINE *et al.*, 1983; LEE, 1986; IMANISHI and ARITA, 1987). This was also true in the present study (Fig. 2). Since there is no significant correlation between V_m and a_K^i (Fig. 2), variation of depolarization may not be attributed to variation of a_K^i . In fact, we found that a_K^i of a certain well-polarized cell ($V_m = -75.0$ mV) was 100.0 mM, while that of a markedly depolarized cell ($V_m = -32.5$ mV) had much higher a_K^i (110.0 mM). Therefore, the mechanism of V_m decrease should be attributed to some factor(s) other than lowered a_K^i , that is, decrease in membrane K^+ conductance (MARY-RABINE *et al.*, 1983; IMANISHI and ARITA, 1987) and increase in Na^+ conductance (IMANISHI and ARITA, 1987).

The finding that a_K^i remained at physiological levels suggests that the Na-K pump is unaffected and functions normally, even in diseased human atrial muscles, and that the outward current caused by electrogenic property of the Na-K pump may affect the V_m . However, measurement of changes in V_m is not relevant to assess the pump function, because "diseased" human atrial fibers had remarkable alteration in the membrane conductance (TEN EICK and SINGER, 1979; MARY-RABINE *et al.*, 1983; IMANISHI and ARITA, 1987). Therefore, direct measurement of intracellular K^+ activity seems to be a more appropriate approach to investigate Na-K pump function in such preparations.

Exposure to 10^{-5} M DHO produced a significant decrease in a_K^i and some depolarization of V_m in the early phase of drug application (Fig. 3). These effects are considered to be due to inhibition of the Na-K pump (DEITMER and ELLIS, 1978). Similarly, a decrease in $[K]_o$ from 5.4 to 0.5 mM also produced a significant reduction in the a_K^i and a slight depolarization of V_m (Fig. 4A and B). However, in the latter case, depolarization of V_m may be also attributed to a decrease in K^+ conductance, because lowered $[K]_o$ decreases K^+ conductance (TEN EICK and SINGER, 1979; IMANISHI and ARITA, 1987). In contrast, an increase in $[K]_o$ from 0.5 to 5.4 mM produced a significant increase in a_K^i and transient hyperpolarization which exceeded the steady state levels of V_m at 5.4 mM $[K]_o$ by about 8 mV. Furthermore, both the increase in a_K^i and initial hyperpolarization induced by this increase in $[K]_o$ were inhibited in the presence of 10^{-6} M DHO or at low temperature (22°C) (Figs. 5–7). This finding is in keeping with the idea that DHO or low temperature inhibits the activation of the Na-K pump that otherwise should be activated by an increase in $[K]_o$. The Na-K ATPase is highly dependent on the temperature (PALATINI *et al.*, 1977) and the low temperature decreases the activity of the Na-K pump (SPERELAKIS and LEE, 1971). In fact, Na-K ATPase activity of bovine heart preparation measured at 22°C was markedly depressed, to be about 5% of that measured at 37°C (Fig. 3 in PALATINI *et al.*, 1977). Lowering the temperature from 37 to 20°C also decreased the outward pump current, that was comparable to the effect produced by 2×10^{-5} M DHO (ISENBERG and TRAUTWEIN,

1975). Such high dependency of Na-K pump function on the temperature may explain relatively marked decreases in a_K^i and V_m produced by low temperature (Fig. 7), as compared to the effects produced by DHO (Fig. 3) or low $[K]_o$ (Fig. 4). Therefore, it is not surprising that the decreases in a_K^i and V_m attained at low temperature in physiologic $[K]_o$ (5.4 mM) were dramatically reversed after rewarming the preparation (Fig. 7).

Isoproterenol (10^{-7} M) caused a significant increase in a_K^i and slight hyperpolarization in all preparations tested (Fig. 8A), a finding compatible with the stimulation of the electrogenic Na-K pump activity. Catecholamine stimulates the electrogenic pump in cardiac muscles of bullfrog (AKASU *et al.*, 1978) and human (IMANISHI and ARITA, 1987), and in rabbit cardiac myocytes (DÉSILETS and BAUMGARTEN, 1986). There is an alternative explanation that isoproterenol increases the membrane K^+ conductance (GADSBY, 1983; NETO and SPERELAKIS, 1989) to increase the V_m . However, it is difficult to explain the increase in a_K^i by this mechanism. Recently, it was reported that β -adrenergic stimulation increases a Cl^- conductance, and thereby depolarizes the V_m (BAHINSKI *et al.*, 1989; HARVEY and HUME, 1989). However, in our preparations Iso (10^{-7} M) did not depolarize the V_m but tended to hyperpolarize it. The resting membrane potential of diseased human atrial muscle was about -44 mV as mentioned, which was close to alleged reversal potential of the Cl^- current (-40 to -50 mV, cf. HARVEY and HUME, 1989). Therefore, the depolarization would be minimal, if any, or it would have been set off by the pump-induced hyperpolarization, in the present study.

Acetylcholine (10^{-5} M) significantly hyperpolarized V_m with no detectable change in a_K^i (Fig. 8B). This may be attributed to increases in membrane K^+ conductance (g_K) via activation of ACh-stimulated K^+ channels (IJIIMA *et al.*, 1985).

The biphasic hyperpolarization found after increasing $[K]_o$ from 0.5 to 5.4 mM (Fig. 5A) may be explained as follows: 1) The initial, transient component of hyperpolarization is mainly due to activation of the Na-K pump current, associated partly with increased membrane K^+ conductance produced by elevated $[K]_o$; and 2) The late, sustained hyperpolarization is most likely due to continued increase in the membrane K^+ conductance, in that the latter effect might have exceeded the depolarization due to decreased K^+ equilibrium potential (IMANISHI and ARITA, 1987).

It is worth noting that the steady state V_m at 5.4 mM $[K]_o$ in the presence of DHO (-46.4 mV) exceeded that in the absence of DHO (-42.6 mV) (Fig. 5). The phenomenon seems to be related to the finding that the depolarization induced by DHO at 5.4 mM $[K]_o$ was not progressive but, after some period (about 5 min), turned toward re- or hyper-polarization, in spite of continuous reduction of a_K^i (Fig. 3A). The first effect of the drug (depolarization of V_m) seemed to be due to inhibition of electrogenic (outward) Na-K pump current by DHO, while the late effect, i.e., change of V_m in the hyperpolarizing direction, may be due to long-lasting application of DHO, which increased intracellular Na^+ and Ca^{2+} concentrations, both of which secondarily enhanced membrane K^+ conductance (ISENBERG, 1976; EISNER *et al.*,

1984; KAMEYAMA *et al.*, 1984; NETO and SPERELAKIS, 1989). Thus, in Fig. 5, the steady state V_m in the presence of DHO exceeded, regardless of slight decreases in a_K^i , the steady state V_m in the absence of DHO.

All of these data support the idea that the electrogenic Na-K pump is not impaired and operates well even in "diseased" human atrial muscles. To analyze the role of Na-K pump more quantitatively, we estimated DHO-induced decrease in the Na^+ -efflux rate, using the rate of decrease in a_K^i occurring during the first 5 min exposure to DHO. The DHO-induced decrease in Na^+ -efflux was estimated to be 2.6–3.8 pmol/(cm²·s) (see SHEU *et al.*, 1981). This value is in keeping with the estimation for cardiac Purkinje fibers in the presence of 10⁻⁵ M DHO (about 3 pmol/(cm²·s), ISENBERG and TRAUTWEIN, 1974), suggesting again that the Na-K pump functions normally in the "diseased" or "depolarized" human preparations that we used in the present study.

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REFERENCES

- AKASU, T., OHTA, Y., and KOKETSU, K. (1978) The effect of adrenaline on the electrogenic Na^+ pump in cardiac muscle cells. *Experientia*, **34**: 488–490.
- BACH, E. J. and REITER, M. (1964) The difference in velocity between the lethal and inotropic action of dihydrodigoxin. *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol.*, **248**: 437–449.
- BAHINSKI, A., NAIRN, A. C., GREENGARD, P., and GADSBY, D. C. (1989) Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes. *Nature*, **340**: 718–721.
- BAUMGARTEN, C. M., SINGER, D. H., and FOZZARD, H. A. (1984) Intra- and extracellular potassium activities, acetylcholine and resting potential in guinea pig atria. *Circ. Res.*, **54**: 65–73.
- DEITMER, J. W. and ELLIS, D. (1978) The intracellular sodium activity of cardiac Purkinje fibers during inhibition and re-activation of the Na-K pump. *J. Physiol. (Lond.)*, **284**: 241–259.
- DESILETS, M. and BAUMGARTEN, C. M. (1986) Isoproterenol directly stimulates the Na^+ - K^+ pump in isolated cardiac myocytes. *Am. J. Physiol.*, **251**: H218–H225.
- EISNER, D. A., LEDERER, W. J., and VAUGHAN-JONES, R. D. (1984) The electrogenic Na pump in mammalian cardiac muscle. In: *Electrogenic Transport: Fundamental Principles and Physiological Implications*, ed. by BLAUSTEIN, M. P. and LIEBERMAN, M., Raven Press, New York, pp. 193–213.
- ESCANDE, D., COULOMBE, A., FAIVRE, J. F., DEROUAIX, E., and CORABOEUF, E. (1987) Two types of transient outward currents in adult human atrial cells. *Am. J. Physiol.*, **252**: H142–H148.
- GADSBY, D. C. (1983) β -Adrenoceptor agonists increase membrane K^+ conductance in cardiac Purkinje fibers. *Nature*, **306**: 691–693.

- GADSBY, D. C., KIMURA, J., and NOMA, A. (1985) Voltage dependence of Na-K pump current in isolated heart cells. *Nature*, **315**: 63-65.
- GLITSCH, H. G. (1979) Characteristics of active Na^+ transport in intact cardiac cells. *Am. J. Physiol.*, **236**: H189-H199.
- HARVEY, R. D. and HUME, J. R. (1989) Autonomic regulation of a chloride current in heart. *Science*, **244**: 983-985.
- HOTOKEBUCHI, N., YANO, T., NISHIZONO, Y., and NISHI, K. (1987) Changes in intra- and extracellular potassium and intracellular sodium activities induced by repetitive stimulation and their relation to membrane potential in guinea-pig papillary muscle. *Jpn. J. Physiol.*, **37**: 797-819.
- IJIMA, T., IRISAWA, H., and KAMEYAMA, M. (1985) Membrane currents and their modification by acetylcholine in isolated single atrial cells of the guinea-pig. *J. Physiol (Lond.)*, **359**: 485-501.
- IMANISHI, S. and ARITA, M. (1987) Factors related to the low resting membrane potentials of diseased human atrial muscles. *Jpn. J. Physiol.*, **37**: 393-410.
- ISENBERG, G. (1977) Cardiac Purkinje fibers, $[\text{Ca}^{2+}]_i$ controls steady state potassium conductance. *Pflügers Arch.*, **371**: 71-76.
- ISENBERG, G. and TRAUTWEIN, W. (1974) The effect of dihydro-ouabain and lithium-ions on the outward current in cardiac Purkinje fibers. *Pflügers Arch.*, **350**: 41-54.
- ISENBERG, G. and TRAUTWEIN, W. (1975) Temperature sensitivity of outward current in cardiac Purkinje fibers. Evidence for electrogenicity of active transport. *Pflügers Arch.*, **358**: 225-234.
- KAMEYAMA, M., KAKEI, M., SATO, R., SHIBASAKI, T., MATSUDA, H., and IRISAWA, H. (1984) Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. *Nature*, **309**: 354-356.
- LEE, C. O. (1981) Ionic activities in cardiac muscle cells and application of ion-selective microelectrodes. *Am. J. Physiol.*, **241**: H459-H478.
- LEE, Y. S. (1986) Pathophysiological mechanisms of altered transmembrane potentials in diseased human atria. *J. Electrocardiol.*, **19**: 41-50.
- MARY-RABINE, L., ALBERT, A., PHAM, T. D., HORDOF, A., FENOGLIO, J. J., JR., MALM, J. R., and ROSEN, M. R. (1983) The relationship of human atrial cellular electrophysiology to clinical function and ultrastructure. *Circ. Res.*, **52**: 188-199.
- MCCULLOUGH, J. R., BAUMGARTEN, C. M., and SINGER, D. H. (1987) Intra- and extracellular potassium activities and the potassium equilibrium potential in partially depolarized human atrial cells. *J. Mol. Cell Cardiol.*, **19**: 477-486.
- MOBLEY, B. A. and PAGE, E. (1972) The surface area of sheep cardiac Purkinje fibers. *J. Physiol. (Lond.)*, **220**: 547-563.
- NETO, F. R. and SPERELAKIS, N. (1989) Analysis of the hyperpolarizing effect of catecholamines on canine cardiac Purkinje fibers. *Br. J. Pharmacol.*, **96**: 591-598.
- PALATINI, P., DABBENI-SALA, F., PITOTTI, A., BRUNI, A., and MANDERSLOOT, J. C. (1977) Activation of $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase by lipid vesicles of negative phospholipids. *Biochim. Biophys. Acta*, **466**: 1-9.
- SHEU, S. S., KORTH, M., and FOZZARD, H. A. (1981) Intracellular K^+ activity of cardiac Purkinje fibers during temperature change. In: *Progress in Enzyme and Ion-Selective Electrodes*, ed. by LUBBERS, D. W., ACKER, H., BUCK, R. P., EISENMAN, G., KESSLER M., and SIMON, W., Springer-Verlag, Berlin, pp. 183-188.
- SKINNER, R. B. and KUNZE, D. L. (1976) Changes in extracellular potassium activity in response to decreased pH in rabbit atrial muscle. *Circ. Res.*, **39**: 678-683.

- SPERELAKIS, N. and LEE, E. C. (1971) Characterization of (Na⁺, K⁺)-ATPase isolated from embryonic chick hearts and cultured chick heart cell. *Biochim. Biophys. Acta*, **233**: 562–579.
- TEN EICK, R. E. and SINGER, D. H. (1979) Electrophysiological properties of diseased human atrium. *Circ. Res.*, **44**: 545–557.
- TRUEX, R. C. (1972) Myocardial cell diameters in primate hearts. *Am. J. Anat.*, **135**: 269–280.