Regulation of ANP secretion by cardiac Na\(^+\)/Ca\(^{2+}\) exchanger using a new controlled atrial model

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The myocardial interstitium plays an important role in the regulation of cardiac function. In the intermediate space, nutrients, bioactive substances, and metabolites are exchanged between cardiac myocytes and the bloodstream. Fluid exchange between the interstitial space and blood depends on the balance of hydraulic and oncotic pressures between these compartments. The hydraulic pressure caused by the continuous beating of the heart exerts a dynamic pressure on the interstitial space, and this pressure is dependent on myocardial hemodynamics. It is well known that the heart maintains its water content within a narrow critical range. The heart is supplied by a rich lymphatic network, and the interstitial fluid (ISF) of the heart is washed down directly into the atrial lumen. Therefore, the interstitial space is an important route for the regulation of interstitium, venous flow, and lymphatics. We have reported the existence of transmural pathways between the atrial lumen and the pericardial space (5). ISF movement through these pathways is one of the main driving forces in the secretion of atrial natriuretic peptide (ANP) from the interstitial space into the atrial lumen (5). Various models have been proposed for fluid transport in the myocardium, but some questions remain unanswered (5). It is uncertain how ISF translocation from the interstitial space into the luminal space is regulated by each component of atrial hemodynamics. Using isolated perfused rabbit atria, we developed a new method whereby specific physical parameters, such as atrial rate, stroke volume (SV), and atrial end-systolic volume (AESV), can be controlled. Using this perfusion model, we attempted to elucidate the role of each physical parameter on the translocation of ISF and ANP.

In cardiac muscle cells, three primary transport systems are responsible for intracellular Ca\(^{2+}\) homeostasis (2, 11, 24). In particular, the Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) is the primary mechanism responsible for removing Ca\(^{2+}\) from the cells (17). The NCX can function in “forward mode” or “reverse mode” (15). The direction of this transport is governed by the magnitude and orientation of the transmembrane electrical and chemical gradients for Na\(^+\) and Ca\(^{2+}\) (17). NCX activity directly influences intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), which causes cardiac hemodynamic change followed by the secretion of ANP. However, the effect of...
[Ca\(^{2+}\)]; on ANP secretion is controversial, despite extensive studies (6, 9, 14, 19–21). Additionally, there are few reports about the relationship between NCX and ANP secretion, possibly because of difficulties in dissociating ANP secretion from hemodynamic changes. Therefore, the aim of the present study was to characterize the physiological role of cardiac NCX in ANP secretion by depleting the concentration of extracellular Na\(^{+}\) ([Na\(^{+}\)]\text{o}). The effects of gradual decreases in [Na\(^{+}\)]\text{o} on ANP secretion and [Ca\(^{2+}\)] were studied with and without KB-R7943, diltiazem, mibebradil, caffeine, Ni\(^{2+}\), amiloride, 5-(N-methyl-N-isobutyl)-amiloride (MIA), and monensin.

**MATERIALS AND METHODS**

**Animals**

New Zealand White rabbits, weighing ~1.5 kg, were used. All animal experimentation was conducted in accord with the American Association for the Accreditation of Laboratory Animal Care (1).

**Isolated Perfused Atrial Preparation**

New Zealand White rabbits were anesthetized with thiopental sodium and killed by exsanguination. A perfusion catheter was inserted into the atrium by a method described previously, with some modifications (5, 7, 23). Briefly, the heart was rapidly removed and placed in oxygenated saline. The right atrium was dissected along the tricuspid valve annulus, and then the area above the sinoatrial node was cut away. A double-barreled cannula (5 mm OD, 4 mm ID) was inserted into the atrium and secured with ligatures. The inner barrel (1.5 mm ID) was used for outflow, and the outer barrel was used for inflow of perfusate. The cannulated atrium was immediately transferred and fitted into a constant-temperature (36°C) organ chamber containing HEPES buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 10 mM HEPES, 10 mM glucose, and 0.1% BSA). Air bubbles trapped in the pericardial space were removed through polyethylene tubing located on the water-tight silicone rubber cap of the organ chamber. Atrial luminal and pericardial pressures were recorded on a physiograph (model MK IV, Narco Bio-Systems, Houston, TX) via a pressure transducer throughout the experiment. Per fusate was continuously oxygenated and infused into the atrial lumen with a peristaltic pump. The chamber space was oxygenated through silicone tubing coils located inside the organ chamber.

**Control of Physical Parameters**

The experimental system was made by some modifications of our nonbeating model (8, 23) and consisted of three major components: a perfusion system, a rate control unit, and an SV control unit (Fig. 1). The circular movement of the motor (Con-Torque, Eberbach, Ann Arbor, MI) was converted to the linear movement of the SV syringe through a ball joint. The chamber space was completely closed, except for the connection to the SV control unit. Thus the repetitive, push-and-pull motion of chamber fluid appeared a positive-and-negative transmural pressure, which caused passive, cyclic changes in atrial volume. The amount of SV was regulated by adjusting distance. One rotation of the adjustment knob of the SV control motor was 15 μl of SV. Flow through the atrial lumen was maintained only in the forward direction by placing one-way check balls on inflow and outflow lines. Basal distension of the atrium (AESV) was adjusted by pull-and-fix of chamber fluid by means of an AESV syringe.

**Experimental Protocols**

The atria were perfused for 30 min to stabilize ANP secretion and equilibrate the extracellular space with a marker at a steady state. \(^{51}\)Cr-EDTA (10 μCi/ml) was introduced into the pericardial fluid.

**Protocol 1:** effect of SV on ISF translocation and ANP secretion. SV was changed from 65 to 110 and 150 μl/cycle for 10 min in a stepwise pattern and returned to 65 μl/cycle. AESV was fixed at 0 μl for all atria used in this protocol. Thus the ejection fraction (EF) was always 100%, and the atrial end-diastolic volume (AEDV) was the same as SV. Atrial rate was fixed at 30 cycles/min (AR30).

**Protocol 2:** effect of basal volume (AESV) on ISF translocation and ANP secretion. SV was fixed at 60 μl for all atria used in this protocol, and AESV was increased from 0 μl to 55 and 110 μl by withdrawal of pericardial fluid using the AESV syringe. AESV/AEDV changed from 0/60 μl (EF = 100%), 55/115 μl (EF = 52.2%), and 110/170 μl (EF = 35.3%) for 10 min in a stepwise pattern and returned to 0/60 μl. Atrial output was constant, because atrial rate (AR30) and SV (60 μl) were fixed.

**Protocol 3:** effect of atrial rate on ISF translocation and ANP secretion. Atrial rate was changed from 30 to 60, 120, 200, and 280 cycles/min for 10 min in a stepwise pattern and returned to AR30 to check the effect of the time sequence of the experiments. In some experiments, the order of changing atrial rate was performed downward to rule out changes due to the order of changes on the atrial rate. The other parameters were fixed as follows: SV = 65 μl, AESV = 0 μl, and AEDV = 65 μl.

**Protocol 4:** effect of Na\(^{+}\) depletion on ANP secretion. Atrial rate and AESV were fixed at 23 cycles/min and 0 μl, respectively. SV was gradually increased from 50 to 100 and 200 μl, respectively. In another protocol, SV was also fixed at 100 μl.
To determine the effect of NCX on ANP secretion, the Na⁺ gradient between the extracellular and intracellular space was gradually decreased by extracellular Na⁺ depletion. N-methyl-D-glucamine (NMG) or LiCl was used instead of NaCl. The atrium was perfused with HEPES buffer containing 140 mM [Na⁺], and stabilized for 30 min. A sample was collected every 2 min for 10 min as a control, and then the buffer on the luminal and pericardial sides was changed to HEPES buffer containing 70 mM [Na⁺]. After 10 min of stabilization, samples were collected for 10 min, and then buffer was continuously changed to HEPES buffer containing 28, 14, and 0 mM [Na⁺], continuing the same protocol as above. To test the effect on Na⁺ depletion-induced ANP secretion, samples were pretreated with KR-B7943 (10⁻⁵ M), diltiazem (10⁻⁶ M), mibebradil (10⁻⁶ M), caffeine (10⁻³ M), Ni²⁺ (10⁻³ M), amiloride (10⁻⁴ M), MIA (10⁻⁶ M), or monensin (10⁻⁷ M).

Measurement of ISF Translocation

To estimate ISF translocation, the transmural atrial clearance of ⁵¹Cr-EDTA was measured with a gamma counting system (Auto Gamma 500 C, Packard, Downers Grove, IL), as described previously (5). Radioactivity in the atrial perfusate and pericardial buffer solution was measured, and the amount of ISF translocated through the atrial wall was calculated as follows:

\[
\text{ISF translocated (µl/min • g atrial wet wt⁻¹)} = \frac{\text{total radioactivity in perfusate (cpm/min) × 1,000}}{\text{radioactivity in pericardial reservoir (cpm/µl) × atrial wet wt (mg)}}
\]

Total ISF translocation was expressed as microliters of ISF per gram of tissue per minute after calculation of ISF translocated by measuring ⁵¹Cr-EDTA in perfusate for every 2-min fraction.

Radioimmunoassay of Immunoreactive ANP

Immunoreactive ANP in the perfusate was directly measured by radioimmunoassay, as described previously (5). The radioimmunoassay was performed on the same day of the experiment, and all samples in an experiment were analyzed in a single assay. Nonspecific binding was <3%. The 50% intercept was at 26.6 ± 2.9 pg/tube. The molar concentrations of immunoreactive ANP released were calculated as follows:

\[
\text{ANP released (pM)} = \frac{\text{immunoreactive ANP (pg • min⁻¹ • g⁻¹)}}{\text{ISF translocated (µl • min⁻¹ • g⁻¹) × 3,060}}
\]

Measurement of [Ca²⁺]i in Single Atrial Myocytes

Single atrial myocytes were isolated according to a previously described technique with minor modifications (18). Changes in the [Ca²⁺]i of single atrial myocytes were measured with a fluorescence digital imaging microscopy system (Atto Instruments, Rockville, MD), as described elsewhere (18). Isolated myocytes were incubated in HEPES buffer containing 2 µM fura 2-AM (Molecular Probes, Eugene, OR) and 0.02% Pluronic F-127 (Molecular Probes) for 20 min at room temperature and then washed with fresh HEPES buffer to remove extracellular dye. Cells were attached on a perfusion chamber coated with matrix gel and placed on the stage of an inverted fluorescence microscope (Axiovert 135, Karl Zeiss, Jena, Germany) attached to an Attofluor digital fluorescence microscope system. Cells were stimulated at 1 Hz (60 V for 0.6 ms) and perfused with HEPES buffer containing 1 mM Ca²⁺ for 5 min at 0.7 ml/min. Buffer was changed to Na⁺-depleted HEPES buffer. Cells were imaged with excitation wavelengths of 338 and 380 nm and an emission wavelength of 520 nm. The fluorescence images were captured with an intensified charge-coupled device camera and analyzed with Attofluor image processing software. Changes in [Ca²⁺]i are presented as a ratio of fluorescence at 340 nm to fluorescence at 380 nm. Background was subtracted. Experiments were performed at room temperature.

Statistical Analysis

Values are means ± SE. Statistical assessment of the data was made by Student's t-test or ANOVA followed by Duncan's multiple range test when appropriate. Linear regressions were calculated by the least-squares method. P < 0.05 was taken as significant.

RESULTS

Effect of SV on ISF Translocation and ANP Secretion

To evaluate the effect of SV on ISF translocation and ANP secretion, SV was changed from 65 to 110 and 150 µl/cycle at fixed atrial rate (AR30) and AESV (0 µl/cycle; Fig. 2A; n = 6). When SV was increased from 65 to 150 µl/cycle and returned to 65 µl/cycle, ANP secretion and ISF translocation were proportionately increased and returned to basal level. Figure 2B (top) shows the average of each parameter in terms of SV. The ISF translocation and ANP secretion increased in proportion to SV. Therefore, ISF ANP concentration, which represents the release rate of ANP from atrial myocytes, was increased by increasing SV from 65 to 110 µl/cycle.

To evaluate possible differences of ISF response to SV at different atrial rates, we performed two additional series of experiments at AR30 and 180 cycles/min (AR180). There were no significant differences in ISF translocation between AR30 and AR180 (45.18 ± 6.98 vs. 39.11 ± 14.24 µl/min at 65 µl/cycle, n = 6). However, ANP secretion was significantly higher at AR180 than that at AR30 (179.09 ± 29.17 vs. 54.08 ± 7.74 pg/min at 65 µl/cycle, P < 0.001). Figure 2B (bottom) shows the comparison of responsiveness of ISF translocation and ANP secretion in terms of SV between AR30 and AR180. The response of ISF translocation to low SV was similar in both groups, but the response to higher SV was accentuated at AR180. However, the response of ANP secretion to SV was markedly attenuated at AR180.

Effect of AESV on ISF Translocation and ANP Secretion

In these experiments, AESV/AEDV was changed from 0/60 µl (EF = 100%), 55/115 µl (EF = 52.2%), and 110/170 µl (EF = 35.3%) at fixed SV (65 µl/cycle) and atrial rate (AR30, n = 6). Therefore, atrial output was maintained constantly. When AESV was increased from 0 to 55 µl, neither ISF translocation nor ANP release was changed. When AESV was increased to 110 µl, ISF translocation and ANP release were increased, indicating that the response of ISF translocation and ANP secretion to AESV was accentuated.
secretion changed (Fig. 3). By increasing AESV from 55 to 110 μl, ISF translocation and ANP secretion increased significantly. When AESV was decreased from 110 to 0 μl, ISF translocation and ANP secretion increased markedly, but ANP concentration did not change (fraction 16 in Fig. 3A). The response of ISF translocation and ANP secretion to AESV at AR180 (n = 6) was similar to that at AR30 (Fig. 3B, bottom).
**Effect of Atrial Rate on ISF Translocation and ANP Secretion**

Surprisingly, total ISF translocation remained relatively constant over the range of atrial rates. ANP secretion was markedly increased from AR30 to 120 cycles/min and then increased slowly (Fig. 4; *n* = 6). Rate dependency is more prominent in ANP secretion than in ISF translocation. Therefore, ISF ANP concentration gradually increased with increasing atrial rate and reached a peak at 120 cycles/min.

Figure 5 shows the relationship between atrial output, ANP secretion, and ISF translocation in groups with changing SV and atrial rate. Atrial output was obtained by multiplying SV by atrial rate. Atrial output is positively correlated with ANP secretion in the SV-changed group (Fig. 5A; *y* = 4.76*x* + 235, *r* = 0.55, *P* < 0.001) and the atrial rate-changed group (y = 7.34*x* + 126.12, *r* = 0.79, *P* < 0.001). A positive correlation between atrial rate and ISF translocation was observed in the SV-changed group (Fig. 5B; *y* = 1.72*x* + 35.5, *r* = 0.49, *P* < 0.05) but not in the atrial rate-changed group (*y* = 1.25*x* + 60.9, *r* = 0.3, *P* = 0.2). There were also close relationships between extracellular fluid translocation and ANP secretion in both groups (Fig. 5C).
Effect of Gradual Decreases in $[\text{Na}^+]_o$ on ANP Secretion

To determine the effect of NCX on ANP secretion using this model, $[\text{Na}^+]_o$ was decreased from 140 to 0 mM at fixed atrial rate (23 cycles/min) and AESV (0 µl/cycle), while SV was gradually increased from 50 to 100 and 200 µl (Fig. 6A). ANP secretion was markedly increased by the replacement of extracellular Na+ with NMG; ANP was particularly accentuated at higher SV ($n = 9$). An increase in ANP secretion by Na+ replacement with NMG was similar to that by Na+ replacement with Li+ (Fig. 6B; $n = 10$). This effect was blocked by Ca2+-free conditions (Fig. 6B; $n = 5$), but not by pretreatment with mibefradil (10⁻⁵ M, $n = 8$) or diltiazem (10⁻⁶ M, $n = 5$; Fig. 6C).

$[\text{Na}^+]_o$ was gradually decreased from 140 to 70, 28, 14, and 0 mM at fixed atrial rate (23 cycles/min), SV (100 µl/cycle), and AESV (0 µl/cycle), and ANP secretion was measured (Fig. 7A). We also measured the ratio of increase in ANP secretion by Na+ depletion to 140 mM $[\text{Na}^+]_o$ (Fig. 7B). The secretion of ANP was markedly increased by decreasing $[\text{Na}^+]_o$ to 70 mM and then gradually increased further as $[\text{Na}^+]_o$ decreased to 0 mM. Increases in ANP secretion by Na+ depletion from 140 mM to 70, 28, 14, and 0 mM were 1.41 ± 0.20-, 1.71 ± 0.25-, 2.29 ± 0.46-, and 3.04 ± 0.51-fold, respectively (Fig. 7B).

To define the mechanism involved in $[\text{Na}^+]_o$ depletion-induced ANP secretion, we used KB-R7943 and Ni²⁺, an NCX blocker. KB-R7943, a selective inhibitor of the reverse mode of NCX, did not cause any significant changes in $[\text{Na}^+]_o$ depletion-induced accentuation of ANP secretion ($10^{-5}$ M, $n = 5$). Increases in ANP secretion by $[\text{Na}^+]_o$ depletion were completely blocked by pretreatment with a high dose of Ni²⁺ ($10^{-3}$ M, $n = 5$; Fig. 7B). An increase in ANP secretion by $[\text{Na}^+]_o$ depletion was partially or completely blocked by pretreatment with amiloride ($10^{-4}$ M, $n = 5$) or MIA ($10^{-6}$ M, $n = 6$), which are known Na+/H+ exchange (NHX) inhibitors (Fig. 7, B and C). Amiloride is also an Na+ channel blocker. Therefore, we used an Na+ channel ionophore monensin, which did not show any significant effect on $[\text{Na}^+]_o$ depletion-induced ANP secretion (10⁻⁶ M, $n = 5$; Fig. 7C). Pretreatment with diltiazem (10⁻⁶ M) and mibefradil (10⁻⁵ M) together did not significantly change $[\text{Na}^+]_o$ depletion-induced accentuation of ANP secretion (Fig. 7D; $n = 5$). Caffeine (10⁻⁴ M) tended to attenuate the response of ANP secretion by $[\text{Na}^+]_o$ depletion, but not to a significant extent (Fig. 7D; $n = 6$). KB-R7943, Ni²⁺, MIA, amiloride, and monensin alone did not cause any significant changes in ANP secretion in 140 mM $[\text{Na}^+]_o$ (13.25 ± 3.45, 15.20 ± 2.37, 11.42 ± 1.97, 15.83 ± 5.90, and 8.17 ± 0.41 vs. 12.12 ± 1.85 ng·min⁻¹·g⁻¹).

Effect of Gradual Decreases in $[\text{Na}^+]_o$ on $[\text{Ca}^{2+}]_i$

Decreasing $[\text{Na}^+]_o$ from 140 to 70 mM did not cause any significant changes in $[\text{Ca}^{2+}]_i$ in single beating

Fig. 5. Relationship between atrial output and other parameters. AR, atrial rate. A: atrial output and ANP secretion. B: atrial output and ISF translocation. C: ISF translocation and ANP secretion.
atrial myocytes. However, gradual decreases in $[\text{Na}_\text{i}]$ from 70 to 42, 28, 14, and 0 mM caused increases in $[\text{Ca}^{2+}]_i$, which were abolished in $\text{Ca}^{2+}$-free conditions (Fig. 8A) and in the presence of Ni$^{2+}$. However, pre-treatment with MIA, diltiazem, and mibefradil or caffeine did not affect $[\text{Na}_\text{i}]$ depletion-induced increases in $[\text{Ca}^{2+}]_i$ (Fig. 8B).

**DISCUSSION**

The myocardial interstitium plays an important role in the regulation of cardiac function under physiological and pathological conditions. It is believed that exchanges of substances between atrial tissue and blood are dependent on their metabolic rates and the transport rate of ISF. Using a new experimental model that can control the physical parameters of atrial hemodynamics, we showed that the translocation of ISF from

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**Fig. 6.** Effect of extracellular Na$^+$ depletion on ANP secretion at fixed atrial rate (AR30) and AESV (0 μl). A: SV was changed from 50 μl to 100 and 200 μl before (stippled bars) and after (open bars) exposure to Na$^+$-free buffer. B: ANP secretion was increased in response to Na$^+$ replacement by N-methyl-D-glucamine or Li$^+$, which was blocked in $\text{Ca}^{2+}$-free buffer (NMG/Ca free). C: Na$^+$ depletion-induced accentuation of ANP secretion was not changed by $10^{-5}$ M mibefradil (Mibe) or $10^{-6}$ M diltiazem (Dilt). Dotted line, perfusion of atria with Na$^+$-free buffer. Cont, control.

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**Fig. 7.** Effect of gradual decrease in extracellular Na$^+$ concentration ($[\text{Na}_\text{o}]$) on ANP secretion. Atrial rate (AR30), AESV (0 μl), and SV (100 μl) were fixed. A: ANP secretion was gradually increased by decreasing $[\text{Na}_\text{o}]$ from 140 mM to 70, 28, 14, and 0 mM. B–D: modification of accentuation of ANP secretion by gradual decreases in $[\text{Na}_\text{o}]$, by treatment with $10^{-6}$ M 5-(N-methyl-N-isobutyl)-amiloride (MIA), $10^{-3}$ M, Ni$^{2+}$, $10^{-6}$ M monensin (MONEN), $10^{-4}$ M amiloride, $10^{-4}$ M caffeine, or mibefradil + diltiazem. Pretreatment with MIA, Ni$^{2+}$, and amiloride markedly attenuated an accentuation of ANP secretion by Na$^+$ depletion; pretreatment with monensin, mibefradil + diltiazem, and caffeine did not. Significantly different from control: *$P < 0.05$ and **$P < 0.01$. 

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atrial tissue is differently regulated by each physical factor. The translocation of ISF increased more by increasing SV than by increasing atrial rate or basal volume. Interestingly, the translocation of ISF was maintained relatively constant across the range of atrial rates by abruptly decreasing the amount of ISF translocation in each cycle. The maintenance of total ISF translocation, regardless of atrial rates, appears to be the ideal way for the atrium to maintain a constant interstitial environment. However, ISF translocation in terms of SV was decreased by increasing SV, similar to ISF translocation in terms of atrial rate by increasing atrial rate. These decrements caused an attenuation of total ISF translocation per unit time by changing SV and atrial rate. These results suggest that total ISF movement across the atrial wall through paracellular pathways may be more dependent on cardiac output than on atrial rate or SV.

Degrees of contraction (AESV) and distension (AEDV) of atrial muscles are different in physiological and pathological conditions of the heart. The contraction of cardiac muscle is severely disturbed in the distended atrium of congestive heart failure. There was no accumulation of atrial ISF when atria were working with 100% EF, but significant amounts of ISF accumulated in the interstitial space when EF was severely disturbed. This stagnated ISF was translocated into the atrial lumen by the release of basal volume distension (shortening of muscle length), as clearly shown in fraction 16 of Fig. 3A. The accumulation of ISF by atrial distension was dependent on the degree of distension volume (2, 24), but not on the duration of distension. These data suggest that significant amounts of metabolites may accumulate in the ISF during atrial fibrillation or congestive heart failure, where atria are severely distended and SV is decreased SV (decreased EF). These metabolites may further aggravate atrial function. Our data clearly show that changes of muscle length and frequency play a unique role in ISF translocation during atrial cycles. It is not clear whether paracellular pathways in cardiac atria are important for the exchange of molecules through ISF translocation.

The patterns of ANP release observed in this model provided interesting facts about the exchange patterns of molecules between atrial tissue and blood. First, ANP molecules are released from myocytes into the interstitial space in a unique pattern by each physical factor, and then these molecules are transported into the atrial lumen concomitantly with unique patterns of ISF translocation for each physical factor (5). One example is the relationship between heart rate and plasma ANP concentration. Tachycardia generally raises plasma ANP concentration (18, 22), but this relationship was not linear over all ranges of heart rate and showed a significantly blunted response at higher heart rate. There are many hypotheses related to this pattern. There is a relationship between heart rate and plasma ANP concentration. Tachycardia generally raises plasma ANP concentration (18, 22), but this relationship was not linear over all ranges of heart rate and showed a significantly blunted response at higher heart rate. There are many hypotheses related to this pattern. Our study clearly showed that a lesser increase in plasma ANP in tachycardia resulted from the function of unique characteristics of ANP release from myocytes and of ISF regulation by atrial rate. Patterns of decreasing unit ISF translocation caused by atrial rate and SV resulted in a lower release of total ANP into the atrial lumen concomitantly with unique patterns of ISF translocation for each physical factor (5). Our hypothesis suggests that the relationship between heart rate and plasma ANP concentration is under fine control by each hemodynamic factor. This fine control provides maximum response when atrial rate and SV are increased (e.g., exercise), moderate response when only one factor is increased (e.g., palpitation), and minimal response when one factor is increased but the other is decreased (e.g., hemorrhagic hypotension). Therefore, we believe that this kind of ANP regulation may help atria differentiate various stimuli and may help properly regulate the body fluid through the blood vessels and kidneys.

Using this model to characterize the physiological role of cardiac NCX on ANP secretion, we studied the effects of gradual decreases in [Na\(^{+}\)]\(_o\) on ANP secretion under conditions of fixed atrial rate, SV, and AESV. A decrease in [Na\(^{+}\)]\(_o\) to 70 mM caused a twofold increase in ANP secretion without a change in [Ca\(^{2+}\)]. Further decreases in [Na\(^{+}\)]\(_o\), to 28, 14, and 0 mM caused gradual increases in [Ca\(^{2+}\)], and ANP secretion, which were

**Fig. 8.** Changes in intracellular Ca\(^{2+}\) concentration by extracellular Na\(^{+}\) depletion in beating rabbit atrial myocytes. Extracellular Na\(^{+}\) depletion caused marked increases in intracellular Ca\(^{2+}\) concentration, which was completely blocked by pretreatment with 10 \(\mu\)M Ni\(^{2+}\) (A), but not by 10 \(-\mu\)M 5-(N-methyl-N-isobutyl)-amiloride, 10 \(-4\) M caffeine, or mibefradil + diltiazem (B). Significantly different from control group: # \(P<0.05\) and ## \(P<0.01\).
blocked under Ca\(^{2+}\)-free conditions. These data suggest that [Na\(^{+}\)]\(_o\) depletion-induced increases in ANP secretion may be a direct effect, rather than due to changes in SV and AESV. To determine the mechanism involved in [Na\(^{+}\)]\(_o\) depletion-induced increase in ANP secretion, Ca\(^{2+}\) channel modulators such as dil-tiazem and/or mibebradil or caffeine were used. However, they did not show any significant effects on [Na\(^{+}\)]\(_o\) depletion-induced increase in ANP secretion or on [Ca\(^{2+}\)]\(_i\). Therefore, we used KB-R7943, a selective inhibitor of the reverse mode of NCX. KB-R7943 had no effect on basal ANP secretion and accentuation of ANP secretion by [Na\(^{+}\)]\(_o\) depletion, which caused an inhibition of the forward mode of NCX. The reverse mode of NCX seems at least to be not operational in the isolated controlled atria. Heavy metal ions inhibit NCX current when externally applied (13, 15). In particular, Ni\(^{2+}\) has been widely used in investigations of NCX. Ni\(^{2+}\) inhibits nonspecific cation channels (4). Hinde et al. (12) reported that a high concentration of Ni\(^{2+}\) was sufficient to produce maximal inhibition of NCX current in ventricular myocytes. Therefore, we used a high dose of Ni\(^{2+}\) as an NCX inhibitor to block the effect of [Na\(^{+}\)]\(_o\) depletion on ANP secretion. Ni\(^{2+}\) at 10\(^{-3}\) M completely blocked the effect of [Na\(^{+}\)]\(_o\) depletion, but at 10\(^{-4}\) M it did not. From these data, we suggest that an inhibition of NCX by pretreatment with Ni\(^{2+}\) may block the [Na\(^{+}\)]\(_o\) depletion-induced increase in ANP secretion. However, it is difficult to explain why Ni\(^{2+}\) did not show any significant changes in ANP secretion. If Ni\(^{2+}\) inhibits NCX activity, the secretion of ANP as well as [Ca\(^{2+}\)]\(_i\) may be increased. However, because Ni\(^{2+}\) also acts as a nonspecific cation channel inhibitor (4) and Ca\(^{2+}\) channel blocker, its effects may be due to actions at other sites.

To define whether [Na\(^{+}\)]\(_o\) depletion-induced accentuation of ANP secretion may be related to NHX activity, we used MIA. MIA, as well as amiloride, markedly attenuated the effect of gradually decreased [Na\(^{+}\)]\(_o\) on ANP secretion. Amiloride is an Na\(^{+}\) channel blocker as well as an NHX inhibitor. To discriminate between the blocking effects of amiloride, we used monensin, an Na\(^{+}\) channel ionophore. Monensin did not cause any significant changes in [Na\(^{+}\)]\(_o\) depletion-induced increase in ANP secretion. We expected that monensin would cause a decreased Na\(^{+}\) gradient followed by inhibition of NCX activity, whereas amiloride may cause an increased Na\(^{+}\) gradient followed by activation of NCX activity. However, monensin did not influence NCX activity. It has been reported that intracellular acidification clearly inhibits outward NCX current in guinea pig ventricular myocytes (10). Therefore, we suggest that MIA or amiloride may inhibit NCX activity by intracellular acidification through the inhibition of NHX activity.

A decrease in [Na\(^{+}\)]\(_o\) from 140 to 70 mM may cause a decrease in Na\(^{+}\) gradient followed by a decrease in NCX activity and an increase in [Ca\(^{2+}\)]\(_i\). In the present study, decreasing [Na\(^{+}\)]\(_o\) to 70 or 42 mM did not cause any significant changes in [Ca\(^{2+}\)]\(_i\) in single beating atrial myocytes. Further decreases in [Na\(^{+}\)]\(_o\) to 28, 14, and 0 mM caused gradual increases in [Ca\(^{2+}\)]\(_i\). This result can be explained as follows: another Ca\(^{2+}\) extrusion mechanism, such as a Ca\(^{2+}\) pump, may be activated when NCX activity is slightly inhibited by reduction of [Na\(^{+}\)]\(_o\) to 70 mM, and then [Ca\(^{2+}\)]\(_i\) may not be changed. However, a marked inhibition in NCX activity by further decreases in [Na\(^{+}\)]\(_o\) may cause an increase in [Ca\(^{2+}\)]\(_i\), even though the Ca\(^{2+}\) extrusion mechanism may be fully activated. Because there are few specific blockers of NCX, manipulation of ion concentrations is essential for identification of this transporter. To define the mechanisms involved in [Na\(^{+}\)]\(_o\) depletion-induced increase in ANP secretion and [Ca\(^{2+}\)]\(_i\), we used Ni\(^{2+}\) as an NCX blocker. An increase in [Ca\(^{2+}\)]\(_i\) by [Na\(^{+}\)]\(_o\) depletion was attenuated in the presence of Ca\(^{2+}\)-free buffer and Ni\(^{2+}\), but not in the presence of MIA and Ca\(^{2+}\) channel modulators. These results suggest that extracellular Ca\(^{2+}\) may be essential for the [Na\(^{+}\)]\(_o\) depletion-induced increase in [Ca\(^{2+}\)]\(_i\). Through an Ni\(^{2+}\)-sensitive nonselective cation channel, not only through L- or T-type Ca\(^{2+}\) channels, but also a relationship between increased ANP secretion and [Ca\(^{2+}\)]\(_i\). by [Na\(^{+}\)]\(_o\) depletion appears to be independent. Increases in ANP secretion and [Ca\(^{2+}\)]\(_i\), by [Na\(^{+}\)]\(_o\) depletion were blocked by Ca\(^{2+}\)-free buffer and Ni\(^{2+}\), MIA and amiloride inhibited the response of ANP, but not Ca\(^{2+}\), to [Na\(^{+}\)]\(_o\) depletion. However, diltiazem, mibebradil, and caffeine did not affect either response.

In conclusion, we believe that this model may provide many benefits in the study of the mechanism of atrial ANP secretion and ISF translocation. These results also suggest that the increase in ANP secretion in response to [Na\(^{+}\)]\(_o\) depletion may partly involve inhibition of the NCX and NHX but may not involve an increase in [Ca\(^{2+}\)]\(_i\).

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REFERENCES


