Distinct roles for L- and T-type Ca\textsuperscript{2+} channels in regulation of atrial ANP release

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Departments of \textsuperscript{1}Physiology and \textsuperscript{2}Urology, Institute for Medical Sciences, Jeonbug National University Medical School, Jeonju 561-180; and \textsuperscript{3}Department of Physiology, College of Oriental Medicine, Wonkwang University Medicinal Resources Research Center, Iksan 570-749, Korea

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Wen, Jin Fu, Xun Cui, Jin Sub Ahn, Suhn Hee Kim, Kyung Hwan Seul, Sung Zoo Kim, Young Kyung Park, Ho Sub Lee, and Kyung Woo Cho. Distinct roles for L- and T-type Ca\textsuperscript{2+} channels in regulation of atrial ANP release. Am J Physiol Heart Circ Physiol 279: H2879–H2888, 2000.—Atrial secretion of atrial natriuretic peptide (ANP) has been shown to be regulated by atrial workload. Although modulating factors for the secretion of ANP have been reported, the role for intracellular Ca\textsuperscript{2+} on the secretion of ANP has been controversial. The purpose of the present study was to define roles for L- and T-type Ca\textsuperscript{2+} channels in the regulation of ANP secretion in perfused beating rabbit atria. BAY K 8644 (BAY K) increased atrial stroke volume and pulse pressure. BAY K suppressed ANP secretion and ANP concentration in terms of extracellular fluid (ECF) translocation concomitantly with an increase in atrial dynamics. BAY K shifted the relationship between ANP secretion and ECF translocation downward and rightward. These results indicate that BAY K inhibits myocytic release of ANP. In the continuous presence of BAY K, diltiazem reversed the effects of BAY K. Diltiazem alone increased ANP secretion and ANP concentration along with a decrease in atrial dynamics. Diltiazem shifted relationships between ANP secretion and atrial stroke volume or ECF translocation leftward. The T-type Ca\textsuperscript{2+} channel inhibitor mibefradil decreased atrial dynamics. Mibefradil inhibited ANP secretion and ANP concentration in contrast with the L-type Ca\textsuperscript{2+} channel inhibitor. These results suggest that activation of L- and T-type Ca\textsuperscript{2+} channels elicits opposite effects on atrial myocytic release of ANP.

atria; atrial natriuretic peptide; secretion
8644 (BAY K) increased ANP secretion in perfused hearts (29, 31), isolated beating atria (33, 34), and contracting cultured atrial myocytes (22). On the same line, L-type \( \text{Ca}^{2+} \) channel blocker inhibited ANP secretion in perfused hearts (31), isolated beating atria (33, 34), and contracting atrial myocytes (22). An increase in extracellular \( \text{Ca}^{2+} \) also resulted in an increase in ANP secretion in beating atria (18). In contrast, \( \text{Ca}^{2+} \) has also been reported to be a negative regulator for ANP secretion in perfused hearts (16, 30) and isolated beating atria (10). It was also suggested that \( \text{Ca}^{2+} \) is not involved in the regulation of ANP secretion (14, 15, 37).

Two types of voltage-activated \( \text{Ca}^{2+} \) channels have been shown to be important in the atrial or ventricular myocytes: high-voltage-activated L-type and low-voltage-activated T-type channels (1, 25). Several studies (26, 35, 38) have suggested that the T-type \( \text{Ca}^{2+} \) channel has been involved in the pathophysiology of hypertrophied cardiac dysfunction in which the ANP secretion was accentuated. The purpose of the present study was to explore roles for L- and T-type \( \text{Ca}^{2+} \) channels in the regulation of the mechanically stimulated ANP secretion. The pathway of the secretion of ANP was dissected according to the two-step sequential mechanism for the ANP secretion (4, 5, 20). Experiments have been done to define the effects of BAY K and diltiazem, selective L-type \( \text{Ca}^{2+} \) channel opener and blocker, respectively, and mibefradil, a selective T-type channel blocker (13, 23, 24), in isolated perfused beating rabbit atria.

**METHODS**

**Preparation of beating perfused rabbit atria.** Isolated perfused rabbit atria were prepared by the method described previously (5, 20), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), transmural ECF translocation, atrial pulse pressure, and ANP secretion.

**Experimental protocols.** The atria were perfused for 60 min to stabilize ANP secretion. \(^{[3]}\text{H} \text{inulin} \) was introduced into the pericardial fluid 20 min before the start of the sample collection (5, 20). We collected the perfusate for analysis at 4°C and at 2-min intervals. Atrial pacing at 0.8, 1.1.3, 1.6, and 2 Hz was performed consecutively for 2 min at each frequency and repetitive frequency change. Repetitive frequency changes were separated by 2 min of 0.8–2 Hz pacing. Experiments were carried out by using seven groups of atria (Fig. 1). BAY K \([10^{-5} \text{ M}]\) was introduced into the perfusate just after the control cycle. Diltiazem \([10^{-5} \text{ M}, n = 9]\) or nifedipine \([3 \times 10^{-6} \text{ M}, n = 6]\), another L-type \( \text{Ca}^{2+} \) channel blocker, was introduced in the continuous presence of BAY K after the third cycle of BAY K (group 1, see Fig. 2). The effect of BAY K alone was observed for six cycles as a control for BAY K plus diltiazem (group 2, \( n = 9 \); see Fig. 3). For group 3, diltiazem \([3 \times 10^{-6} \text{ M}]\) was introduced after the control cycle \((n = 10); \) see Fig. 5). For group 4, mibefradil \([10^{-5} \text{ M}]\) was introduced \((n = 11), \) see Fig. 6). For the time-control experiments, the atrium was stimulated with repetitive frequency change, and vehicle only was introduced (group 5, \( n = 5 \)). In another series of experiments, the atrium was stimulated with a fixed-pacing frequency at 0.8 Hz. The effect of BAY K \([10^{-7} \text{ M}]\) was observed continuously during periods corresponding to three cycles of the other groups (group 6, \( n = 9 \); see Fig. 5). For the time-control experiment, only the vehicle was introduced (group 7, \( n = 6 \)). BAY K \([\text{SI}−\text{BAY K}], \) nifedipine, and diltiazem hydrochloride were obtained from RBI (Natick, MA). Mibefradil dihydrochloride (Ro-40–5967) was a gift from Hoffmann-LaRoche (Basel, Switzerland).

**Radioimmunoassay of ANP.** Immunoreactive ANP in the perfusate was measured by a specific radioimmunoassay, as described previously (5). The secreted amount of immunoreactive ANP was expressed as nanograms of ANP per minute per picogram (pg) of atrial tissue. The molar concentration of immunoreactive ANP in terms of ECF translocation, which reflects the concentration of extracellular ANP of the atrium and, therefore, indicates the rate of myocytic release of ANP into the surrounding paracellular space (4, 5), was calculated as ANP released \((\mu \text{M})\) = immunoreactive ANP (in pg·min\(^{-1}·g\text{-1}·\text{ECF translocated}) \((\mu l·\text{min}^{-1}·g^{-1}·3.063)\) [mol/wt, ANP-(1–28)]. Most of the ANP secreted is processed ANP (7).

**Statistical analysis.** Significant differences were compared with the use of a two-way ANOVA analysis for repeated measures (Figs. 2, 3, 6, and 7; E-G). Significant differences between paired data for a given pacing frequency (Figs. 2, 3, 6, and 7; A-D) and also the data of Fig. 5 were analyzed by repeated measures ANOVA, followed by Bonferroni's multi-
ple-comparison test. Differences in ratio increments (values obtained at control cycle of Figs. 3, 6, and 7; C) were compared with the use of Duncan’s multiple-range test. Statistical significance was defined as $P < 0.05$. The results are given as means $\pm$ SE.

RESULTS

**BAY K inhibits myocytic release of ANP.** The stepwise increase in atrial pacing frequency from 0.8 to 2 Hz resulted in an increase in the secretion of ANP concomitantly with translocation of the ECF (Fig. 2, A and B). Increases in the secretion of ANP and translocation of the ECF in response to incremental changes in pacing frequency waned at higher atrial rate. The concentration of ANP in perfusate in terms of the ECF translocation, which reflects the concentration of released ANP in paracellular space of the atrium (5, 20), showed a peak at 1 Hz (Fig. 2C). Atrial stroke volume increased in response to stepwise increase in atrial rate (Fig. 2D). Because increases in the secretion of ANP and translocation of the ECF coincide with changes in atrial dynamics, as shown in the present and previous experiments (5, 20), relationships between these observations were examined. Changes in the secretion of ANP and translocation of the ECF showed positive relationships with the change in atrial stroke volume (Fig. 2, E and F). Increase in the secretion of ANP was a function of the change in translocation of the ECF (Fig. 2G).

BAY K suppressed the secretion of ANP ($P < 0.05$ at 1.3–2.0 Hz, Fig. 2A). At a low atrial rate (0.8 Hz), however, BAY K increased an apparent secretion of ANP slightly but without significance. BAY K showed dual effects on the translocation of the ECF, an increase at a low atrial rate ($P < 0.05$ at 0.8 Hz), and a decrease without significance at a higher atrial rate (Fig. 2B). BAY K ($P < 0.05$, Fig. 2C) significantly...
decreased the concentration of ANP in perfusate in terms of the ECF translocation. BAY K \((P < 0.01\), Fig. 2D) accentuated the increase in atrial stroke volume in response to incremental pacing frequency. BAY K suppressed changes in the secretion of ANP and translocation of the ECF in terms of atrial stroke volume. BAY K shifted relationships between ANP secretion or ECF translocation and atrial stroke volume downward and rightward \((P < 0.001;\) Fig. 2, E and F). BAY K shifted the relationship between ANP secretion and ECF translocation rightward, which indicated an inhibition of a myocytic release of ANP \((P < 0.05,\) Fig. 2G).

Administration of diltiazem in the continuous presence of BAY K restored the suppressed secretion of ANP by BAY K to the control levels \((P < 0.001\) between BAY K and BAY K plus diltiazem at 1.6 and 2.0 Hz; Fig. 2A). The difference in the secretion of ANP between control and BAY K plus diltiazem was not significant. Translocation of the ECF was suppressed by diltiazem at a low atrial rate \((P < 0.05\) between BAY K and BAY K plus diltiazem at 0.8 and 1 Hz, Fig. 2B), and this coincided with a decrease in atrial stroke volume (Fig. 2, B and D). The difference in translocation of the ECF between control and BAY K plus diltiazem was not significant. Diltiazem treatment in the presence of BAY K increased the concentration of ANP \((P < 0.001\) between BAY K and BAY K plus diltiazem, Fig. 2C) and returned the suppressed concentration of ANP to the control levels \((P > 0.05\) between control and BAY K plus diltiazem). Return to the control levels by diltiazem of the BAY K-induced suppression of ANP secretion, and ECF translocation was accompanied by a coincidental change in atrial stroke volume. Diltiazem returned relationships between the secretion of ANP or translocation of the ECF and atrial stroke volume and also between the secretion of ANP.

Fig. 3. Sustained effect of BAY K \((10^{-7}\) M) on ANP secretion (A), ECF translocation (B), ANP concentration (C), and atrial stroke volume (D) in perfused beating rabbit atria (0.8, 1, 1.3, 1.6, 2.0 Hz) \((n = 9)\). Relationships between ANP secretion and atrial stroke volume (E), ECF translocation and atrial stroke volume \((P)\), and ANP secretion and ECF translocation \((G)\) were examined. Values are means ± SE.
and translocation of the ECF to the control levels (Fig. 2, E-G). Nifedipine also showed similar antagonistic effects (data not shown). The effects of BAY K on ANP secretion, the ECF translocation, the ANP concentration, and atrial stroke volume were similar during the third and sixth cycles of treatment (Fig. 3). The relationships between variables were also reproducible. For the time control, changes in secretion of ANP and translocation of the ECF in response to repetitive changes in pacing frequency were constant and stable. The responses of the parameters were reproducible during the periods corresponding to the control and experimental observations (differences between periods were not significant, n = 5). Incremental changes in pacing frequency resulted in an increase in atrial pulse pressure (Fig. 4). BAY K accentuated the response in atrial dynamics and diltiazem inhibited the effect.

During a low pacing frequency (0.8 Hz), BAY K tended to increase ANP secretion with a concomitant change in ECF translocation (Fig. 2, A and B). This may be related to a washout of the ECF with ANP released in the extracellular space of the atrium. To clarify the effect of washout by BAY K on ANP secretion, an alternative protocol was used (Fig. 1). The atrium was paced at a low and fixed frequency. As shown in Fig. 5, BAY K first increased without significance and then decreased ANP secretion (P < 0.01 during the late part of third cycle, A). BAY K increased translocation of the ECF during the late part of first cycle and early part of second cycle (P < 0.05; Fig. 5B). BAY K increased atrial dynamics 6 min after administration (P < 0.001; Fig. 5D). The slight increase in ANP secretion coincided with increases in atrial dynamics and ECF translocation. In this phase the concentration of ANP in terms of the ECF translocation was not significantly changed (Fig. 5C). The decrease by BAY K of ANP secretion coincided with a decrease in ANP concentration, i.e., inhibition of myocytic ANP release (P < 0.01 during the third cycle; Fig. 5, C and E). As shown in Fig. 5E, the positive ANP secretion-ECF translocation relationship during the early phase (control period and first cycle of BAY K) of the BAY K treatment indicated that the increase in ANP secretion is related to an increase in ECF translocation, i.e., washout of the ECF with ANP released. After the steady point (second cycle, Fig. 5E) the relationship shifted to the downward, i.e., inhibition of myocytic release of ANP (third cycle, Fig. 5E). For the time

Fig. 4. Representative trace showing an increase in atrial pulse pressure in response to incremental pacing frequency (0.8, 1, 1.3, 1.6, and 2.0 Hz) in perfused beating rabbit atria. BAY K (10^{-7} M) accentuated the response and Dilt (10^{-6} M) inhibited the effect of BAY K.

Fig. 5. Effect of BAY K (10^{-7} M) on ANP secretion (A), ECF translocation (B), ANP concentration (C), and atrial stroke volume (D) in perfused atria paced at a fixed frequency (0.8 Hz, n = 9). Relationship between ANP secretion and ECF translocation was examined (E). Fraction number, serial 2-min sample collections in 12-min cycle intervals. Values are means ± SE.
control, changes in secretion of ANP, ECF translocation, and atrial dynamics were constant and stable. The differences between control observations and periods corresponding to experimental observations were not significant ($n = 6$).

**Diltiazem increases myocytic release of ANP.** The antagonism between activator and inhibitor of the L-type Ca$^{2+}$ channel in the secretion of ANP observed in beating atria suggests a tonic inhibition by Ca$^{2+}$ influx in the regulation of atrial myocytic ANP release. As shown in Fig. 6A, diltiazem elicits dual effects on the secretion of ANP. Treatment with diltiazem resulted in a slight but not significant inhibition and accentuation in ANP secretion ($P < 0.05$ at 2.0 Hz) at low and high atrial rates, respectively. The slight inhibition of ANP secretion coincided with a decrease in ECF translocation (both $P < 0.01$ at 1.0 and 1.3 Hz; Fig. 6B), and the latter was related to a decrease in atrial stroke volume (all $P < 0.001$; Fig. 6D). Diltiazem increased the concentration of ANP in perfusate in terms of the ECF translocation ($P < 0.01$ at 1.3 Hz; Fig. 6C). Diltiazem shifted relationships between the secretion of ANP or translocation of the ECF and atrial stroke volume upward and leftward (both $P < 0.001$; Fig. 6, E and F). Diltiazem tended to shift the relationship between secretion of ANP and translocation of the ECF upward, although not significantly. These findings indicate that L-type Ca$^{2+}$ channel inhibition with diltiazem increases myocytic release of ANP into the surrounding extracellular space.

**Mibefradil inhibits myocytic release of ANP.** To define the role of T-type Ca$^{2+}$ channel in the regulation of ANP release, the effect of mibefradil on the secretion of ANP was investigated. As shown in Fig. 7, A, C, and D, mibefradil inhibited the secretion of ANP (both $P < 0.05$ at 1.3 and 1.6 Hz) and the concentration of ANP in

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**Fig. 6.** Effect of Dilt (3 × 10$^{-6}$ M) on ANP secretion (A), ECF translocation (B), ANP concentration (C), and atrial stroke volume (D) in perfused beating rabbit atria (0.8, 1, 1.3, 1.6, 2.0 Hz) ($n = 10$). Relationships between ANP secretion and atrial stroke volume (E), ECF translocation and atrial stroke volume (F), and ANP secretion and ECF translocation (G) were examined. Values are means ± SE.
perfusate in terms of the ECF translocation \((P < 0.05\) at 1.6 Hz) concomitantly with a decrease in atrial stroke volume (all \(P < 0.05\) at 1.0–2.0 Hz). Changes in translocation of the ECF were not significant (Fig. 7B). Mibebradil shifted relationships between ANP secretion or ECF translocation and atrial stroke volume leftward (both \(P < 0.01\); Fig. 7, E and F). Mibebradil shifted the relationship between ANP secretion and ECF translocation downward and rightward, which indicated an inhibition of myocytic release of ANP \((P < 0.001\), Fig. 7G).

**DISCUSSION**

The present study shows for the first time that changes in Ca\(^{2+}\) influx via L- and T-type channels are oppositely involved in the regulation of myocytic release of ANP.

Activation of L-type Ca\(^{2+}\) channel inhibits myocytic release of ANP. Whereas the activation by BAY K of L-type Ca\(^{2+}\) channel inhibited atrial myocytic release of ANP into the paracellular space, blockade by diltiazem returned the inhibition to control levels. As shown in Fig. 2, slight increases in the secretion of ANP and translocation of the ECF by BAY K at low atrial rate are related to the coincident increase in atrial stroke volume. Because the translocation of the ECF and secretion of ANP are positively related with changes in atrial stroke volume and the secretion of ANP is a function of the ECF translocation (Refs. 5 and 20, and present study), an apparent slight increase in ANP secretion by BAY K should be the result of washout of released ANP with convective ECF translocation from the paracellular space. However, despite the increase in ANP secretion, the concentration of ANP in
perfusate in terms of the ECF translocation was rather decreased by BAY K. The concentration of ANP in perfusate in terms of the ECF translocation reflects the concentration of extracellular ANP of the atrium and, therefore, the rate of myocytic release of ANP into the surrounding paracellular space (4, 5). The fact that an apparent increase by BAY K of ANP secretion could be a result of washout was tested by an alternative protocol. As shown in Fig. 5, BAY K first increased and then decreased ANP secretion at a low and fixed pacing frequency. The findings indicate that the slight increase by BAY K of ANP secretion is closely related with the translocation of the ECF, i.e., washout of the ECF with ANP released in the extracellular space of the atrium. After a steady point, a slight increase by BAY K of ANP secretion was followed by an inhibition of myocytic release of ANP.

Although atrial dynamics were accentuated by BAY K, the positive relationships between ANP secretion and atrial stroke volume or ECF translocation were shifted downward and rightward. The latter again indicates that BAY K inhibits atrial myocytic release of ANP. At higher atrial rate, BAY K suppressed translocation of the ECF as well as myocytic release of ANP. The slight but not significant suppression by BAY K of translocation of the ECF may be related to the shrinkage of the extracellular space by an increase in atrial workload, as suggested in our previous study (4). The specificity of the channel activation for the inhibition of ANP release by BAY K was further confirmed by diltiazem, a L-type Ca$^{2+}$ channel blocker. The effects of BAY K on atrial dynamics and secretion of ANP were reversed by simultaneous administration of diltiazem and restored control levels. Taken together, these data indicate that Ca$^{2+}$ influx via L-type channel inhibits myocytic release of ANP.

**Tonic inhibition by Ca$^{2+}$ influx via L-type channel in the mechanically stimulated ANP release.** In the present study, it was found that mechanically stimulated myocytic release of ANP is regulated by a tonic inhibition, possibly via increase in intracellular Ca$^{2+}$, especially at a higher atrial rate. Our finding that diltiazem increased myocytic release of ANP (Fig. 6) and that the concentration of paracellular ANP of the atrium waned at higher atrial rate support the notion.

An apparent increase in ANP secretion in response to increasing pacing frequency was related to the concomitant increase in ECF translocation, the second step of ANP secretion, as shown in control values of Figs. 2, 3, 6, and 7; A and G. Even in this occasion, however, myocytic ANP release, i.e., the concentration of ANP in perfusate in terms of the ECF translocation, the first step of ANP secretion, was rather inhibited by increasing atrial rate. The ratio increments in the concentration of ANP in response to pacing frequency were significantly different between low (1 Hz) and higher (2.0 Hz) atrial rates (1.01 ± 0.04 vs. 0.73 ± 0.06, P < 0.01, n = 9, Fig. 3C; 1.07 ± 0.05 vs. 0.85 ± 0.04, P < 0.01, n = 10, Fig. 6C; and 1.00 ± 0.05 vs. 0.79 ± 0.04, P < 0.01, n = 9, Fig. 7C). Although myocytic ANP release was slightly but significantly suppressed at a higher atrial rate, an increase in translocation of the ECF by an accentuation in atrial dynamics could overcome the inhibition in release and thus increased an apparent secretion of ANP. Because the increase in atrial rate may be accompanied by net cellular Ca$^{2+}$ uptake (21, 22, 36), the mechanism responsible for the waning of the secretion and the concentration of paracellular ANP at higher atrial rate may be partly related to an increase in intracellular Ca$^{2+}$ of atrial myocytes. It was observed (S. H. Kim, K. W. Ku, and W. X. Xu, unpublished data) that an increase in pacing frequency from 0.8 to 2.0 Hz resulted in an increase in intracellular Ca$^{2+}$ transients in isolated single atrial myocytes from rabbits.

The effect of diltiazem on ANP secretion may be related with a decrease in the intracellular concentration of Ca$^{2+}$. Figure 6 shows how diltiazem increased secretion of ANP and the concentration of ANP in perfusate in terms of the ECF translocation at higher atrial rate. At a low atrial rate, decreases in the secretion of ANP and translocation of the ECF were also observed. Even in this occasion, the concentration of ANP rather increased. Therefore, it is interpreted that the decrease in ANP secretion is related with a coincidental decrease in ECF translocation. Although the ANP secretion was apparently decreased, myocytic release of ANP into the paracellular space was increased by diltiazem, which in turn resulted in an increase in the concentration of ANP in perfusate in terms of the ECF translocation.

The present data showing an inhibition of myocytic release of ANP by activation of L-type Ca$^{2+}$ channel with BAY K in beating atria are consistent with previous results in perfused rat hearts (30) and isolated beating (10) and nonbeating (11, 17) rat atria. However, the present data are not consistent with the data reported in perfused rat hearts (29, 31), isolated beating rat atria (33, 34), and contracting cultured atrial myocytes (22), thus showing an increase in ANP secretion by BAY K and its blockade by specific inhibitor. The reason for the discrepancy is not clear, though the difference in methodology may be accounted for the discrepancy. Alternatively, species difference in the regulation of intracellular Ca$^{2+}$ homeostasis (36) may be related with the discrepancy, because many previous studies have been done mainly in rats. However, this is unlikely because, in our previous studies, an inhibition by Ca$^{2+}$ of the myocytic release of ANP has been observed in the perfused atria from rabbits (6) and rats (17). Ruskoaho et al. (30) also observed an inhibition by Ca$^{2+}$ of ANP secretion in perfused rat hearts.

The present results show the dual effects of BAY K on apparent ANP secretion and ECF translocation, i.e., an increase at low and decrease at higher atrial rate (Fig. 2). This is in relation to a previous report; Ruskoaho et al. (28) suggested that the effect of intracellular Ca$^{2+}$ on ANP secretion might be different by the rate of atrial contraction. In accordance with the two-step sequential mechanism for the regulation of ANP secretion (4, 5, 20), the suggestion could be interpreted...
as follows. At a low atrial rate, translocation of the ECF with the washout of ANP is increased by an increase in atrial dynamics. This is related to an apparent increase in ANP secretion with an inhibition of myocytic ANP release. At a higher atrial rate, both inhibition of myocytic ANP release by an increase in intracellular concentration of Ca$^{2+}$ and a decrease in translocation of the ECF by shrinkage of extracellular space are involved. This is related to an apparent inhibition in ANP secretion with an inhibition of myocytic ANP release (Fig. 2).

Both the increase in ANP secretion by L-type Ca$^{2+}$ channel inhibition and waning of ANP secretion at a higher atrial rate suggest that tonic inhibition of myocytic ANP release by an increase in Ca$^{2+}$ influx via L-type Ca$^{2+}$ channel activation is contained in the mechanically stimulated ANP secretion in beating atria. These indicate that, although translocation of the ECF with released ANP is ultimately coupled to the Ca$^{2+}$-dependent myocytic contraction, myocytic release of ANP is inhibited by an augmentation of Ca$^{2+}$ influx via L-type Ca$^{2+}$ channel. Ca$^{2+}$ influx via T-type channel may be related to an increase in ANP secretion. The present data suggest for the first time that an increase in Ca$^{2+}$ influx via T-type channel may increase ANP secretion. This is in relation to the speculation by Bonvallet and Rouquier (3), that T-type Ca$^{2+}$ channel may have a role in ANP secretion. Although the L-type Ca$^{2+}$ channel may also be partially blocked by mibefradil at a similar dose as used in the present experiments (24), the finding that mibefradil inhibits the ANP secretion that is opposite to the effect of the L-type Ca$^{2+}$ channel inhibition with diltiazem or nifedipine suggests that Ca$^{2+}$ influx via T-type channel increases ANP secretion. This finding is homologous to the previous report (9) that T-type Ca$^{2+}$ current plays a role in mediating the secretion of aldosterone in adrenal glomerulosa cells. The amplitude of inhibition of ANP secretion by mibefradil was not as large as that of increase by diltiazem with a similar concentration in terms of decrease in atrial stroke volume. For changes in atrial dynamics and ECF translocation, the second step of ANP secretion, the effects of both mibefradil and diltiazem were similar. This finding, therefore, suggests that the role of Ca$^{2+}$ influx via T-type channel in the regulation of ANP release may not be large in physiological condition. This notion is related with the finding (2, 39) that T-type Ca$^{2+}$ current is only a small fraction to the total Ca$^{2+}$ current in atrial cells. However, the role of T-type Ca$^{2+}$ channel in the regulation of ANP secretion may be more important in pathophysiological conditions, such as cardiac hypertrophy. Previously, it was shown that the T-type Ca$^{2+}$ channel activity increased in the cardiomyocytes from the atrium-bearing growth hormone-secreting tumor (38) or hypertrophied ventricle (26, 35). Therefore, it is interesting to note that ANP secretion is activated in the cardiac hypertrophy (8, 19, 32). The negative inotropic effect of mibefradil shown in the present experiment was similar to that shown in previous data obtained from guinea pig left atria (27).

In conclusion, the present data indicate that Ca$^{2+}$ influx via L- and T-type Ca$^{2+}$ channels elicits opposite effects in the regulation of ANP secretion: Ca$^{2+}$ influx via L-type channel inhibits, but Ca$^{2+}$ influx via T-type channel increases, atrial myocytic ANP release. The present data obtained by dissecting the functional pathway of the ANP secretion may explain, at least in part, diversity of reported effects of Ca$^{2+}$ or Ca$^{2+}$ channel modulators in the regulation of ANP secretion.

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