Prevention of Hypertrophy by Overexpression of Kv4.2 in Cultured Neonatal Cardiomyocytes

Carsten Zobel, MD; Zameneh Kassiri, PhD; The-Tin T. Nguyen, MsC; Yang Meng, PhD; Peter H. Backx, DVM, PhD

Background—Prolonged action potentials (APs) and decreased transient outward K⁺ currents (Iₒ) are consistent findings in hypertrophic myocardium. However, the connection of these changes with cardiac hypertrophy is unknown. The present study investigated the effects of changes in Iₒ and the associated alterations in AP on myocyte hypertrophy induced by phenylephrine.

Methods and Results—Chronic incubation of cultured neonatal ventricular rat myocytes (NVRMs) with phenylephrine (PE) reduced Iₒ density and prolonged AP duration, leading to a 2-fold increase in the net Ca²⁺ influx per beat and a 1.4-fold increase in Ca²⁺-transient amplitude. PE treatment of chronically paced (2-Hz) NVRM also induced increases in cell size, protein/DNA ratio, atrial natriuretic factor mRNA expression, as well as βαR myosin mRNA ratio. These hypertrophic changes were associated with a 2.4-fold increase in activation of nuclear factor of activated T-cells (NFAT), indicating increased activity of the Ca²⁺-dependent phosphatase calcineurin. Overexpression of Kv4.2 channels using adenovirus prevented the AP duration prolongation as well as the increases in Ca²⁺ influx and Ca²⁺-transient amplitude induced by PE. Kv4.2 overexpression also prohibited the PE-induced increases in cell size, protein/DNA ratio, atrial natriuretic factor expression, βαR myosin mRNA ratio, and NFAT activation.

Conclusions—Our results demonstrate that PE-mediated hypertrophy in NRVM seems to require Iₒ reductions and AP prolongation associated with increased Ca²⁺ influx and Ca²⁺ transients as well as calcineurin activation. The clinical implications of these studies and the possible involvement of other signaling pathways are discussed. (Circulation. 2002;106:2385-2391.)

Key Words: action potentials ■ myocytes ■ ion channels ■ hypertrophy ■ calcium

Cardiac hypertrophy is a crucial compensatory mechanism for normalizing wall stress in many forms of heart disease. Nevertheless, chronic activation of hypertrophic pathways is believed to contribute to heart disease progression toward heart failure. A consistent finding in cardiac hypertrophy is electrical remodelling and, in particular, action potential (AP) prolongation.1–3 Reductions in Iₒ density toward heart failure. A consistent finding in cardiac pathways is believed to contribute to heart disease progression. Nevertheless, chronic activation of hypertrophic pathways is believed to contribute to heart disease progression toward heart failure. A consistent finding in cardiac hypertrophy is electrical remodelling and, in particular, action potential (AP) prolongation.1–3 Reductions in Iₒ density have been linked to altered AP profiles as well as slowed early repolarization4 and occur early in cardiac disease, suggesting a possible role for Iₒ changes in hypertrophic process.5 On a molecular level, cardiac Iₒ is generated by Kv4.2, Kv4.3, and Kv1.4 genes, with their relative contribution varying between species and different regions of the heart.6 However, only reductions in Kv4.2 and Kv4.3 expression have been linked consistently to the diminished Iₒ densities observed in cardiac hypertrophy.7

AP prolongation after reduced Iₒ densities can lead to increases in Ca²⁺ influx through L-type Ca²⁺ channels, resulting in elevated intracellular Ca²⁺ levels.8,9 Because Ca²⁺ is an essential cofactor for hypertrophic signaling,9 it is conceivable that reductions in Iₒ density play a significant role in mediating cardiac hypertrophy. Indeed, we have recently demonstrated that reductions of Iₒ encoded by Kv4.2/3 cause hypertrophy in cultured neonatal ventricular rat myocytes (NVRMs).10 The α₁-adrenoceptor agonist phenylephrine (PE) has been commonly used to induce hypertrophy in NVRM.11 Furthermore, exposure of NVRM to PE results in a reduction of Iₒ density and AP prolongation12 as well as reduced expression levels of Kv4.213 and Kv4.3.14 The implications of these changes in Iₒ on the induction of hypertrophy through PE are not known. Therefore, the present study was designed to investigate whether prevention of the reductions in Iₒ could inhibit the hypertrophic effects of chronic PE treatment.

Methods

Construction of Recombinant Adenoviruses

Recombinant adenoviruses were generated15 to overexpress either the green fluorescence protein (GFP) alone (AdGFP) or in combi-
nation with the Kv4.2 K+ channels (AdGFPKv4.2). Both viruses were plaque purified, and virus titers were determined using the 50% tissue culture infectious dose (TCID50) method: AdGFP 6.3×10^6/mL and AdGFPKv4.2 3.95×10^6/mL.

**Neonatal Rat Ventricular Myocyte Isolation**

NVRMs were isolated and cultured as described previously. For patch-clamp recording experiments, 1.5×10^6 myocytes were plated on laminin-coated coverslips in 35-mm culture dishes. To assess the hypertrophic effects of PE treatment, 9.6×10^6 myocytes were split on laminin-coated coverslips in 35-mm culture dishes. After 24 hours in culture, the medium was replaced by serum-free medium and virus infections were performed (AdGFP 5 TCID50/myocyte or 2 TCID50/myocyte and AdGFPKv4.2 5 TCID50/myocyte). Typically, 80% to 90% of the myocytes showed expression of GFP 24 hours after infection. PE in a concentration of 10 μmol/L was added when required. To stimulate and synchronize myocyte contraction, alternating rectangular-wave electrical pulses (50 V) of 5-ms duration at 2 Hz were applied via platinum wires that were submersed at opposite ends of each well in the 4-well culture dishes, as described previously.

**Electrophysiological Recordings**

Electrophysiological recordings were performed 48 hours after serum withdrawal using borosilicate glass micropipettes (3 to 4 MOhm). Myocytes were perfused with drug-free bath solution for at least 15 minutes before measurements were performed. Whole-cell currents were filtered at 2 kHz (Axon 200A amplifier; Axon Instruments, Inc.). I Ca was measured at 21°C in an extracellular solution containing (in mmol/L) NaCl 140, KCl 2, MgCl2 1, CaCl2 0.5, HEPES 10, and glucose 10, pH 7.4. The intracellular solution contained (in mmol/L) KCl 140, MgCl2 1, EGTA 10, HEPES 10, and MgATP 5, pH 7.25. APs were acquired at 32°C in the same extracellular solution as above with the exception of CaCl2. The pipette solution for the AP studies contained (in mmol/L) KCl 140, MgCl2 1, HEPES 5, and Na2ATP 5, pH 7.25. Ca2+ currents were recorded with a bath solution containing (in mmol/L) TMA-OH 130, aspartic acid 130, HEPES 10, glucose 10, CaOH2 1.8, and MgOH2 1, pH 7.4. The pipette solution for I Ca measurements contained (in mmol/L) CsOH 150, aspartic acid 120, EGTA 10, HEPES 10, TEA-Cl 10, and Mg2+ATP 5, pH 7.3. In voltage-clamp studies, cells were stimulated with either step depolarizations or AP clamps using typical APs recorded under current clamp conditions. Junction potentials were −4.9 mV for the ΔI Ca measurements (uncorrected) and −11.1 mV for the ΔI Ca measurements (corrected).

Ca2+ Transients

Ca2+ transients were measured essentially as described earlier using Indo-1 at 30°C in a heating chamber. Autofluorescence was measured before myocytes were loaded with Indo-1AM dye, and 405/485-nm ratios were calculated after autofluorescence subtraction following stimulation of the cardiomyocytes at 1 Hz with a Grass S44 stimulator.

**Confocal Images**

Confocal images of NRVM cultured in 4-well dishes were obtained using a Bio-Rad MRC 600 laser-scanning confocal microscope with a 50× water immersion objective lens.

**Measurement of Protein-DNA Ratio**

Protein-DNA ratios were measured as described previously.

**RNAse Protection Assay**

After 48 hours of chronic stimulation, total RNA was isolated using the RNeasy Mini Kit (Qiagen). Antisense riboprobes for α/β myosin mRNAs and atrial natriuretic factor (ANF) (both probes were a gift from Dr. T.G. Parker, University Health Network, Toronto, Canada) were labeled with biotin-16-uridine-5'-triphosphate (Boehringer Mannheim) by in vitro transcription with T7 (α/β myosin) or SP6 (ANF, GAPDH) RNA polymerase of appropriate RNA synthesis vectors. The probes were hybridized with total RNA isolated as described above, and RNAase-resistant hybrids were recovered using the RPA III Kit (Ambion), analyzed on 8 mol/L urea 6% polyacrylamide sequencing gels, and visualized with the BrightStar Biodetect Kit (Ambion).

**Luciferase Assay to Measure Calcineurin Activity**

To assess calcineurin activity, a luciferase assay was applied as described previously.

**Statistics**

All data are expressed as mean±SEM. Statistical significance was determined using the unpaired or paired t test while comparing 2 groups and ANOVA to compare multiple groups.

**Results**

**Relationship Between AP Duration, I Ca, and Ca2+ Transients**

Figure 1 shows typical I Ca traces, APs, and Ca2+ transients from cultured neonatal myocytes before and 5 minutes after application of 10 μmol/L PE. Both I Ca density and AP duration50 (APD50) were slightly (but significantly) altered, whereas APD90 was unchanged by PE application. By contrast, chronic exposure to PE reduced I Ca density by ~70%
while increasing APD_{50} by ~10-fold and APD_{90} by ~4-fold (Figure 2). Despite the large differences in the effects of PE on I_{Ca} and AP between acute and chronic applications, the effects of PE on Ca^{2+}-transient amplitudes were quite similar (Figures 1 and 2), suggesting that the acute effects of PE on APD and Ca^{2+} handling are distinct from its chronic effects, as suggested previously.12

As expected from previous studies,7,18 the integrated Ca^{2+} influx per beat increased ~2-fold in PE-treated myocytes stimulated with their own prolonged AP compared with nontreated myocytes stimulated with their corresponding typical short, control AP (Figure 3), despite reductions \((P<0.01)\) in the I_{Ca} peak (Table). These differences in net Ca^{2+} influx seem to result from variations in AP duration, because the net Ca^{2+} influx in PE-treated myocytes became indistinguishable from untreated myocytes after depolarization with typical control APs (Figures 3B and 3E, Table). Moreover, application of typical long APs obtained from PE-treated myocytes to AdGFP-infected control cells produced I_{Ca} profiles and integrated Ca^{2+} influxes matching closely those recorded from PE-treated cells stimulated with similar long APs (Figures 3A and 3D, Table). As expected,7,18,19 increased Ca^{2+} entry via I_{Ca} in PE-treated myocytes was associated with elevated \((P<0.05)\) Ca^{2+} transient amplitudes compared with untreated NVRM (Figures 1 and 2).

Overexpression of Kv4.2 abbreviated AP durations and prevented the increases in Ca^{2+} influx and Ca^{2+} transient amplitude resulting from PE treatment (Figures 2 and 3C, Table). These effects of Kv4.2 overexpression did not seem to be related to alterations in the density and biophysical properties of I_{Ca} itself, because I_{Ca} in PE-treated NRVMs was not different between AdKv4.2-infected versus AdKv4.2-uninfected myocytes when stimulated with similar prolonged AP (Figures 3D and 3F and Table). Thus, although PE might affect the expression and activity of many Ca^{2+}-handling proteins in NVRM, the alterations in I_{Ca} and AP profile seem to be necessary for the increases in I_{Ca} and Ca^{2+} transient amplitude induced by chronic PE treatment.

Effects of APD on Myocyte Hypertrophy

Next, the effects of Kv4.2 overexpression on hypertrophy induced by PE were investigated. Because beating rates can influence hypertrophic signaling as well as gene expression in cultured NRVM,16 and because PE treatment20 or overexpression of Kv4.2 can conceivably affect spontaneous beating rates, NVRMs were chronically paced for 48 hours at 2 Hz.

![Figure 2](image-url) Chronic effects of PE treatment on APs, I_{Ca} density, and Ca^{2+} transients. Left, Representative original traces acquired under the indicated conditions. Corresponding panels on the right depict the averaged data. APs were elicited by a brief (3-ms) suprathreshold pulse applied at a frequency of 2 Hz. I_{Ca} was elicited by 500-ms voltage steps to +60 mV from holding potential of −80 mV and quantified by subtracting the current at the end of the voltage pulse from the peak current. Ca^{2+} transients were measured as change in Indo-1 fluorescence ratio (405/485 nm) in electrically paced myocytes. *P<0.05 vs AdGFP.

![Figure 3](image-url) Ca^{2+} currents recorded in response to APs illustrated in the insets. Ca^{2+} currents are shown from a control AdGFP-infected myocyte stimulated with a typical AP recorded from AdGFP-infected myocytes (A) or a typical AP obtained from AdGFP-infected myocytes chronically exposed to PE (D). Typical Ca^{2+} currents are also illustrated for AdGFP-infected myocytes treated with PE (B) in response to a typical prolonged AP recorded from similar myocytes or a typical short control AP (E), as well as for AdKv4.2-infected myocytes treated with PE and stimulated with typical APs recorded from the same cells (C) or from typical long APs (F).
Properties of AP-Induced Ca\(^{2+}\) Currents

<table>
<thead>
<tr>
<th>Culture Condition</th>
<th>Applied Action</th>
<th>(I_{\text{Ca,peak}}) (pA/pF)</th>
<th>(Q_{\text{DNA}}) (fC/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdGFP (n=9)</td>
<td>AdGFP</td>
<td>−12.06±1.56</td>
<td>198.30±24.73†††</td>
</tr>
<tr>
<td></td>
<td>AdGFP+PE</td>
<td>−6.04±0.78††††‡‡</td>
<td>383.58±59.50†††</td>
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<tr>
<td>AdGFP+PE (n=11)</td>
<td>AdGFP</td>
<td>−11.83±1.32</td>
<td>218.90±25.48‡‡</td>
</tr>
<tr>
<td></td>
<td>AdGFP+PE</td>
<td>−6.48±1.09††††‡‡</td>
<td>425.66±59.84†††</td>
</tr>
<tr>
<td>PE+Adk4.2 (n=8)</td>
<td>PE+Adk4.2</td>
<td>−14.07±1.14</td>
<td>116.23±9.46††</td>
</tr>
<tr>
<td>AdGFP+PE</td>
<td>−4.80±0.47‡‡</td>
<td>378.28±25.17††††</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs AdGFP-infected myocytes exposed to a typical control AP.
†P<0.05 vs AdGFP-infected myocytes incubated with PE and exposed to a typical control AP.
‡P<0.05 vs Adk4.2-infected myocytes treated with PE and stimulated with a short AP (Adk4.2+PE).

The addition of PE for 48 hours to AdGFP-infected myocytes increased cell size (Figure 4). These alterations in cell size were accompanied by increases (\(P<0.05\)) in the protein/DNA ratio, enhanced expression of the ANF, and increases in the \(\beta\alpha\) myosin heavy chain (MHC) ratio compared with non-treated AdGFP-infected myocytes (Figures 5A and 6), demonstrating that PE is capable of enhancing hypertrophy in chronically paced cells. More important, Kv4.2 overexpression prevented both the increase in cell size induced by PE (Figure 4) and the increases in the protein/DNA ratio (Figure 5A), which were not related to differences in the amount of DNA levels (Figure 5B). Overexpression of Kv4.2 in PE-treated myocytes also prevented the increase in the \(\beta\alpha\) MHC ratio (\(P>0.05\)) and attenuated the increase in ANF mRNA levels (Figure 6). Collectively, these results establish that there is a strong link between AP prolongation and hypertrophy induced by PE.

Consistent with previous studies showing that the calcium-dependent phosphatase calcineurin is involved in cardiac hypertrophy\(^{21}\) and in PE-induced hypertrophy of cultured NRVMs,\(^{22}\) the level of activation of NFAT, a calcineurin-activated transcription factor, was increased (\(P<0.05\)) by PE, whereas overexpression of Kv4.2 prevented this increase of NFAT activity (activity normalized to AdGFP-infected myocytes: AdGFP+PE 242.3±34.5% versus AdKv4.2+PE 110.5±28.47%).

**Discussion**

The present study investigated the contribution of alterations in \(I_{\text{Ca}}\) density and corresponding changes in AP profile on the development of myocyte hypertrophy induced by PE. To separate the contribution of changes in APD on the hypertrophic effects of PE treatment and the blockade of this hypertrophy by Kv4.2 overexpression from possible secondary effects of these interventions on intrinsic beating rates of NRVM (which can indirectly affect hypertrophy\(^{16}\)), our studies were performed using cultured NRVMs electrically paced at 2 Hz. As observed previously in nonpaced NRVMs, PE treatment of paced NRVM increased cell sizes, protein/DNA ratios, and ANF mRNA levels as well as the \(\beta\alpha\) MHC mRNA ratio. Unlike in nonpaced and spontaneously beating NRVM,\(^{11,23}\) the relative increase in ANF mRNA expression induced by PE was remarkably modest in paced myocytes.\(^{17,24}\) This observation is probably related to the previous finding that rapid electrical pacing itself elevates ANF mRNA expression to levels comparable with those induced by PE treatment of spontaneously beating NRVMs, albeit through the activation of different signaling pathways.\(^{24}\)

Acute exposure of NRVMs to PE resulted in relatively minor reductions in \(I_{\text{Ca}}\) and APD prolongation, as reported previously.\(^{25}\) By contrast, treatment of paced NRVM for 48 hours with PE caused marked reductions of \(I_{\text{Ca}}\) and associated APD prolongation that seemed to originate from reduced functional expression of Kv4.2/3, as reported previously.\(^{12,14}\) These effects in chronically treated myocytes were largely unrelated to acute effects of PE, because our electrical recordings were made in the absence of PE.

The ability of Kv4.2 overexpression to prevent hypertrophy in our studies establishes that \(I_{\text{Ca}}\) reductions and APD prolongation are prerequisites for PE-mediated hypertrophy in NRVM. However, these observations do not establish that \(I_{\text{Ca}}\) reductions by PE are the primary cause of the observed hypertrophy. The issue of whether reductions in \(I_{\text{Ca}}\) can induce hypertrophy is controversial and seems to be model-dependent. In transgenic mice, elimination of Kv4.x-based \(I_{\text{Ca}}\) and AP prolongation may\(^{29,30}\) or may not\(^{39,41}\) lead to notable structural alterations of the heart, possibly depending on the specific dominant-negative strategy used. On the other hand, Kv4.x-based \(I_{\text{Ca}}\) reduction does cause hypertrophy in NRVMs\(^{10}\) requiring AP prolongation. The reasons for these inconsistencies are unclear. It is conceivable that adenoviral infections or excessive overexpression of exogenous dominant-negative proteins used to reduce \(I_{\text{Ca}}\)\(^{10,29,30}\) could stimulate hypertrophic signaling pathways that could be amplified or modulated by the associated reductions in \(I_{\text{Ca}}\) and APD prolongation, as occurs in NRVM treated with PE. Modulation of hypertrophy by \(I_{\text{Ca}}\) reductions would explain the observations that the amount of hypertrophy induced by PE is considerably greater in nonpaced, than in paced, NRVMs\(^{10}\) (C. Zobel, unpublished data, 2001) and that hypertrophy is enhanced in Kv4.2W362FxKv1.4\(^{−/−}\) mice subjected to pressure overload.\(^{42}\) Alternatively, \(I_{\text{Ca}}\) reductions might be sufficient for inducing hypertrophy in NRVMs and the developing heart owing to an increased propensity for growth in young myocytes. Regardless, more studies are clearly warranted to test further the modulatory effects of \(I_{\text{Ca}}\) on myocyte hypertrophy.

APD prolongation induced by chronic PE treatment was accompanied by a 2-fold increase in total Ca\(^{2+}\) influx per beat.
and a 1.4-fold increase in \( \text{Ca}^{2+} \) transient amplitude compared with untreated NVRM. The increases in \( \text{Ca}^{2+} \) influx seen with PE are clearly and directly linked to AP prolongation and not to changes in the number of active \( \text{Ca}^{2+} \) channels, because the application of a control AP to PE-treated NVRM resulted in \( \text{Ca}^{2+} \) currents indistinguishable from control cells and vice versa. The absence of measurable changes in \( I_{\text{Ca}} \) was somewhat unexpected, because chronic \( \alpha_1 \)-adrenoceptor stimulation with PE has previously been shown to reduce \( \text{Ca}^{2+} \) current densities by 50% in cultured NVRM. On the other hand, another study showed that chronic \( \alpha_1 \)-adrenoceptor stimulation increased L-type \( \text{Ca}^{2+} \) current densities. The reasons for these discrepancies are unclear and will require additional studies.

Chronic PE treatment was associated with hypertrophy and a 2.4-fold activation of calcineurin (whose \( \text{Ca}^{2+} \)/calmodulin-dependent phosphatase activity was measured using NFAT activity), both of which were prevented by AP abbreviation. These results confirm an important role for calcineurin in PE-mediated hypertrophy of NVRM, as reported previously, but additionally suggest that AP prolongation and elevated \( \text{Ca}^{2+} \) are required for calcineurin activation after PE treatment. The mechanism for activation of calcineurin in our study could be linked to local increases of \( [\text{Ca}^{2+}]_i \), particularly in the vicinity of \( \text{Ca}^{2+} \) channels, as a result of observed increases in \( \text{Ca}^{2+} \) entry after AP prolongation, because calcineurin has been shown to colocalize with L-type \( \text{Ca}^{2+} \) channels via the A-kinase anchoring protein AKAP79. Moreover, increased \( I_{\text{Ca}} \) has been shown to induce hypertrophy in NVRM treated with \( \text{Ca}^{2+} \) channel agonists as well as in transgenic mice with cardiac overexpression of either L-type \( \text{Ca}^{2+} \) channels or dominant-negative Kv4.2 channels that eliminate fast \( I_{\text{to}} \). This mechanism is also consistent with the observation that treatment with blockers of \( I_{\text{to}} \) can prevent the hypertrophy induced by AP prolongation.

Alternatively, the activation of calcineurin might also originate from global elevations in peak systolic \( [\text{Ca}^{2+}]_i \); although this cannot be ruled out, phospholamban knockout mice show marked increases in \( \text{Ca}^{2+} \) transient amplitudes without developing cardiac hypertrophy.

Of course, other hypertrophic signaling pathways and mechanisms, in addition to calcineurin, might also contribute to the PE-induced hypertrophy. For example, PE has been shown to activate protein kinase (PKC) \( \epsilon \) and \( \delta \) in cultured NVRM as well as members of the mitogen-activated protein kinase (MAPK) family, which are \( \text{Ca}^{2+} \)-dependent path-

**Figure 5.** The protein/DNA ratios (A) and the amount of DNA (B) induced by PE. Myocytes were plated as described in the Methods section and electrically paced for 48 hours. Data are mean±SEM for 9 to 11 myocyte isolations with 2 to 3 averaged individual experiments. *P<0.05 vs AdGFP.

**Figure 6.** Expression of hypertrophic marker genes is reduced by overexpression of Kv4.2. RNAase protection experiments performed for ANF (A) as well as for \( \alpha \) and \( \beta \) myosin heavy chain (MHC) mRNA (B), as described in the Methods section. Lane 1, probes; Lane 2, AdGFP; Lane 3, AdGFP+PE; Lane 4, AdKv4.2+PE. C, Histogram comparing the abundance of ANF mRNA and the ratio of \( \beta \) versus \( \alpha \) MHC mRNA normalized to beating myocytes infected with AdGFP (n=5 \( \beta /\alpha \) MHC, n=3 ANF). *P<0.05 vs AdGFP.
ways that might also be modulated by changes in AP profile and Ca\(^{2+}\) cycling. However, neither overexpression of constitutively active PKCe nor PKC\(\delta\) induces hypertrophy in NRVM,\(^{37}\) whereas hypertrophy induced in cultured NRVM by PE\(^{22}\) and other stimuli\(^{38}\) require the activation of the calcineurin pathway. Alternatively, it is also possible that elevated Ca\(^{2+}\) transients after PE treatment will increase mechanical activity, which could stimulate cell growth\(^{32}\) independent of Ca\(^{2+}\)-dependent hypertrophic signaling pathways.

Regardless of the connection between elevated Ca\(^{2+}\) as a result of APD prolongation with hypertrophy and calcineurin activation after PE treatment, it is clear that overexpression of Kv4.2 channels and associated increased \(I_{\text{Na}}\) current can prevent these PE-induced changes. This suggests that the reductions in \(I_{\text{Na}}\) currents generated by Kv4.x channels\(^{12-14}\) are necessary ingredients in the hypertrophic response induced by PE. Consequently, it is certainly possible that overexpression of other K\(^+\) channel genes besides Kv4.2-based \(I_{\text{Na}}\) could also inhibit PE-induced hypertrophy in NRVMs. However, the predominant effect of chronic PE treatment on membrane currents is the reduction of \(I_{\text{Na}}\) with little change in other K\(^+\) currents. In addition, because of its rapid gating kinetics compared with many other K\(^+\) channels, \(I_{\text{Na}}\) more strongly influences early repolarization and, thereby, the amount of Ca\(^{2+}\) entry through \(I_{\text{Ca}}\) per beat and Ca\(^{2+}\) transient amplitudes.\(^{7,8,18,46}\)

It is possible that Kv4.2 overexpression inhibits growth in NRVMs due to nonspecific effects of the expression of this K\(^+\) channel, independent of effects on AP profile. Although this possibility is difficult to rule out, it seems unlikely, because overexpression of the full-length dominant-negative Kv4.2 mutant (Kv4.2W363F), which apparently is processed in a manner similar to the wild-type Kv4.2 protein,\(^{39}\) induces hypertrophy in NRVMs.\(^{10}\) In non-PE-treated myocytes, cell growth was also significantly inhibited (\(\approx 15\%)\) by overexpression of Kv4.2 and APD abbreviation compared with GFP-infected myocytes (data not shown), which might, at first glance, suggest a nonspecific effect of AdKv4.2 infection. However, this mild inhibitory effect on hypertrophy is consistent with our previous studies showing that \(I_{\text{Na}}\) reductions alone can induce hypertrophy in NRVMs,\(^{10}\) which is anticipated from the link between APD duration, Ca\(^{2+}\) cycling, and calcineurin activation. This interpretation is also consistent with previous studies (and our unpublished data) establishing that pacing itself affects myocyte growth in the cultured NRVMs.\(^{17,24}\)

The relevance of our NRVM results to hypertrophy in intact adult myocardium and human disease remains unclear for several reasons. In rodents, \(I_{\text{Na}}\) represents the major repolarizing current, while making a relatively smaller contribution to repolarization in larger species and humans,\(^{43,44}\) leading to very different consequences of changes in \(I_{\text{Ca}}\) amplitude.\(^{45}\) For example, reduced \(I_{\text{Na}}\) in rodents leads to enhanced excitation-contraction coupling and contraction,\(^{7,8}\) whereas the opposite occurs in larger mammals,\(^{33-45}\) which can be traced to variability in the effects of \(I_{\text{Na}}\) on \(I_{\text{Ca}}\)\(^{46}\). In addition, as already mentioned, the responsiveness of cultured NRVMs to various hypertrophic stimuli and reduction in \(I_{\text{Na}}\) may differ markedly from that observed in intact myocardium or adult myocytes.

In summary, our results suggest that AP prolongation as a result of \(I_{\text{Na}}\) reduction plays a crucial role in cardiac hypertrophy of NRVM after chronic \(\alpha_1\)-adrenergic receptor activation, possibly by modulating calcineurin activity in response to altered Ca\(^{2+}\) cycling.

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**References**


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