Functional Cross-Talk Between Aldosterone and Angiotensin-(1-7) in Ventricular Myocytes

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Abstract—High serum levels of aldosterone have been linked to the development of cardiac disease. In contrast, angiotensin (Ang)-(1-7) was extensively shown to possess cardioprotective effects, including the attenuation of cardiac dysfunction induced by excessive mineralocorticoid activation in vivo, suggesting possible interactions between these 2 molecules. Here, we investigated whether there is cross-talk between aldosterone and Ang-(1-7) and its functional consequences for calcium (Ca^{2+}) signaling in ventricular myocytes. Short-term effects of aldosterone on Ca^{2+} transient were assessed in Fluo-4/AM-loaded myocytes. Confocal images showed that Ang-(1-7) had no effect on Ca^{2+} transient parameters, whereas aldosterone increased the magnitude of the Ca^{2+} transient. Quite unexpectedly, addition of Ang-(1-7) to aldosterone-treated myocytes further enhanced the amplitude of the Ca^{2+} transient suggesting a synergistic effect of these molecules. Aldosterone action on Ca^{2+} transient amplitude was mediated by protein kinase A, and was related to an increase in Ca^{2+} current (I_{Ca}) density. Both changes were not altered by Ang-(1-7). When cardiomyocytes were exposed to aldosterone, increased Ca^{2+} spark rate was measured. Ang-(1-7) prevented this change. In addition, a NO synthase inhibitor restored the effect of aldosterone on Ca^{2+} spark rate in Ang-(1-7)-treated myocytes and attenuated the synergistic effect of these 2 molecules on Ca^{2+} transient. These results indicate that NO plays an important role in this cross-talk. Our results bring new perspectives in the understanding of how 2 prominent molecules with supposedly antagonist cardiac actions cross-talk to synergistically amplify Ca^{2+} signals in cardiomyocytes. **(*Hypertension*. 2013;61:425-430.)** ● **Online Data Supplement**

Key Words: calcium ■ myocytes ■ electrophysiology ■ angiotensin

Progression of chronic heart failure is mediated largely via persistent activation of different neuroendocrine systems. Both experimental and clinical studies have linked aldosterone excess to the development and progression of several different cardiovascular disease processes,[1,2] and aldosterone levels are often elevated in patients with heart failure.[2–4] In addition, the presence of both mineralocorticoid (MR)[5] and glucocorticoid receptors (GR)[6] has been reported in the heart, supporting a direct effect of aldosterone on cardiomyocyte function in normal condition and during pathological remodeling.

There is accumulating evidence that modulation of calcium (Ca^{2+}) influx plays a central role in the action of aldosterone in cardiomyocytes. Consistently, aldosterone upregulates L-type Ca^{2+} current (I_{Ca}),[7] enhances cardiomyocyte shortening,[8] and increases ryanodine receptor (RyR) activity leading to abnormal Ca^{2+} release in ventricular myocytes.[9]

Previously, it has been shown in vivo that angiotensin (Ang)-(1-7) infusion protects the heart against deoxycorticosterone acetate–mediated cardiac dysfunction.[10] Similar findings were observed in a transgenic rat with increased circulating levels of Ang-(1-7).[11] Taken together, these data suggest an important interaction between Ang-(1-7) and aldosterone signaling in the heart that culminates with the activation of protective mechanisms. How these 2 signaling pathways interact and the impact of this interplay for Ca^{2+} handling in ventricular myocytes is still not known. To begin to investigate whether

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cardioprotective effects of Ang-(1-7) against aldosterone excess represent a consequence of antagonistic outcomes or result from more complex interactions, we assessed short-term effects of aldosterone and Ang-(1-7) on freshly isolated ventricular myocytes, under conditions where both MR and GR were activated. Here, our goal was 2-fold: (1) to investigate whether there is a signaling cross-talk between aldosterone and Ang-(1-7) in ventricular myocytes and (2) to identify its functional consequences and underlying mechanisms.

For the detailed Methods Section, please see the online-only Data Supplement.

Results

In ventricular myocytes the global $[Ca^{2+}]_i$ transient is central to cardiac function; it underlies contraction and contributes to the regulation of electric activity.[12] To directly investigate the short-term effects of aldosterone on Ca^{2+} transient parameters, ventricular myocytes were loaded with the Ca^{2+} sensitive fluorescent dye Fluo-4/AM and visualized by confocal microscopy. Here, we used a supraphysiological concentration of aldosterone (1 μmol/L) to fully activate both MR and GR in a redox-independent manner, as previously shown.[13] Treatment of cardiomyocytes with aldosterone led to a significant increase in Ca^{2+} transient amplitude when compared with untreated cells, an effect that was partially abolished by preincubation with the MR antagonist spironolactone (10 μmol/L; Figure S1A in the online-only Data Supplement). Incubation of cardiomyocytes with mifepristone (10 μmol/L), a GR antagonist, also partially prevented aldosterone from inducing an increase in Ca^{2+} transient amplitude (Figure S1A). Finally, combining spironolactone and mifepristone completely prevented the increase in peak Ca^{2+} in aldosterone-treated myocytes (Figure S1B). Taken together, these data validated the use of supraphysiological concentrations of aldosterone for activating MR and GR, as previously shown by others.[14]

We next investigated the effects of Ang-(1-7) on global Ca^{2+} levels under conditions of excessive aldosterone signaling. Top panels of Figure 1A display representative line-scanning images recorded from ventricular cells. Below the line scanning is a graphical representation of each selected cell. As we have previously shown,[15] Ang-(1-7) (100 nmol/L) alone had no effect on Ca^{2+} transient amplitude (Figure 1B). Quite unexpectedly, addition of Ang-(1-7) to aldosterone-treated myocytes further enhanced the magnitude of the Ca^{2+} transient above the levels observed in cells exposed to aldosterone alone (Figure 1A and 1B). Thus, this finding suggests an important synergistic effect between aldosterone and Ang-(1-7) in ventricular myocytes, pointing to an important role of Ang-(1-7) in the modulation of intracellular Ca^{2+} under conditions of increased aldosterone signaling. To investigate the role of receptor Mas on Ang-(1-7) effects on Ca^{2+} transient in aldosterone-treated cardiomyocytes, we isolated cardiac cells from wild-type and *Mas*$^{-/-}$ mice. Confirming our findings using rat ventricular myocytes, aldosterone significantly enhanced Ca^{2+} transient levels in myocytes from wild-type mice, an effect that was exacerbated in the presence of Ang-(1-7; Figure S2A and S2C). In contrast, *Mas*$^{-/-}$ myocytes lack the synergistic effect of Ang-(1-7) on Ca^{2+} transient amplitude in aldosterone-treated cells (Figure S2B and S2D), suggesting an important role of receptor Mas on Ang-(1-7) actions in the presence of aldosterone.

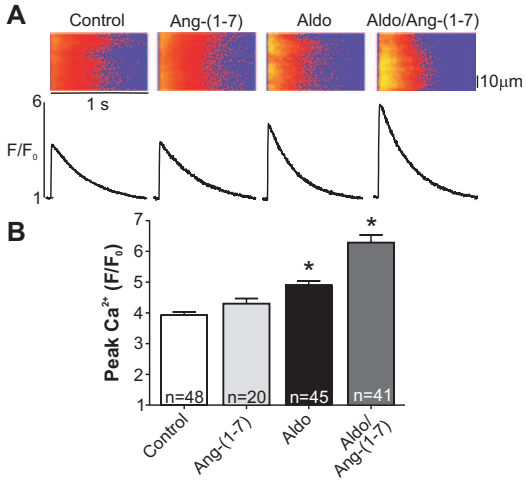

Figure 1. Cross-talk between aldosterone (Aldo) and angiotensin (Ang)-(1-7) enhances Ca^{2+} transient in ventricular myocytes. **A**, **Top**, representative images of Ca^{2+} transients recorded from control, Ang-(1-7) (100 nmol/L), aldosterone (1 μmol/L), or a combination of aldosterone plus Ang-(1-7). **Bottom**, Ca^{2+} transient line-scan profile. **B**, Average-bar graph showing that the cross-talk between aldosterone and Ang-(1-7) signaling pathways further enhances Ca^{2+} transient amplitude in aldosterone-treated cardiomyocytes. **P*<0.05 when compared with the other groups. n=number of cells.

To investigate the cellular basis of this phenomenon, we assessed $I_{Ca,L}$ by electrophysiological techniques. As shown in Figure S3, Ang-(1-7) alone had no effect on $I_{Ca,L}$ current density. In contrast, aldosterone treatment significantly enhanced $I_{Ca,L}$ in cardiomyocytes when compared with untreated controls (at 0 mV: 8.1±0.4 pA/pF in 26 control versus 9.6±0.3 pA/pF in 30 aldosterone-treated cardiomyocytes; *P*<0.05; Figure S4A and S4B), as previously shown by others.[7] Importantly, this aldosterone-mediated effect was unchanged in the presence of Ang-(1-7). The importance of these data is 2-fold. First, increased $I_{Ca,L}$ current density can explain the effects of aldosterone on Ca^{2+} transient. Second, synergistic effects of aldosterone and Ang-(1-7) were not observed at the $I_{Ca,L}$ level, indicating that other factors would be possibly contributing to the observed effects of these 2 molecules on Ca^{2+} transient magnitude. Therefore, we next investigated the subcellular mechanisms underlying Ang-(1-7) enhancement of Ca^{2+} transient magnitude in conditions of aldosterone excess. Alterations in sarcoplasmic reticulum (SR) Ca^{2+} content contribute to enhance Ca^{2+} release in ventricular myocytes. To investigate this possible contribution, we recorded SR Ca^{2+} load in caffeine-dumped ventricular cells. Figure 2A shows that SR Ca^{2+} content was not significantly different between control and aldosterone-treated myocytes. However, it should be noted that there was a tendency for lower SR Ca^{2+} content in aldosterone-treated myocytes. Importantly, Ang-(1-7) induced a significant increase in SR Ca^{2+} content (by ≈34%) in aldosterone-treated myocytes.

SR Ca^{2+} content reflects the balance between Ca^{2+} uptake via SR Ca^{2+} pump (SERCA) and Ca^{2+} efflux via RyR. Therefore, to assess RyR activity, Ca^{2+} sparks were examined in Fluo-4/AM loaded myocytes. Representative images of resting Ca^{2+} spark rate are shown in Figure 2B. Ang-(1-7) alone had no effect on

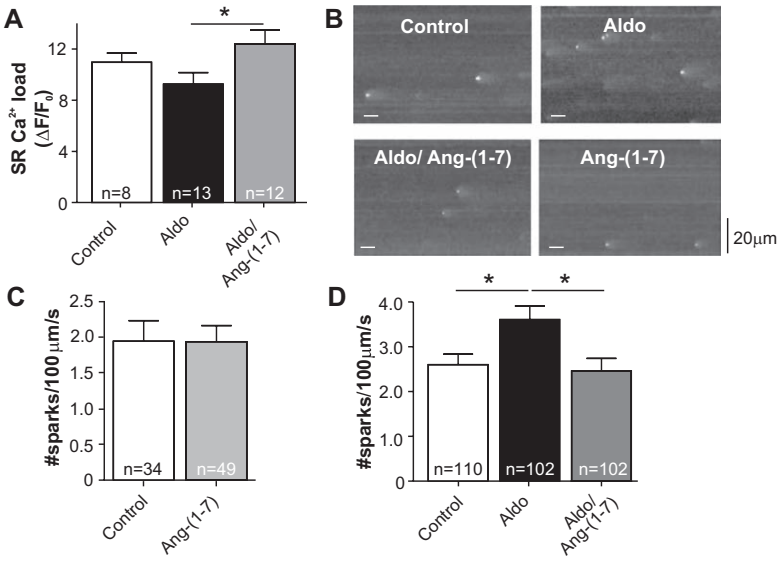

Figure 2. Angiotensin (Ang)-(1-7) increases sarcoplasmic reticulum (SR) Ca^{2+} content in aldosterone-treated myocytes. **A**, Ang-(1-7) significantly increases SR Ca^{2+} load in aldosterone (Aldo)-treated myocytes. SR Ca^{2+} content was not different between control and aldosterone-treated myocytes. **B**, Representative Ca^{2+} sparks recorded at rest. Bar, 100 ms. **C**, Ang-(1-7) alone does not modulate Ca^{2+} spark rate. **D**, Ang-(1-7) significantly decreases Ca^{2+} spark rate in aldosterone-treated myocytes. n=number of cells. **P*<0.05.

Ca^{2+} spark rate (Figure 2C), whereas aldosterone significantly increased Ca^{2+} spark rate (Figure 2D). We now show for the first time that addition of Ang-(1-7) markedly reduced Ca^{2+} spark rate in aldosterone-treated myocytes (Figure 2D). Thus, by reducing SR Ca^{2+} spark frequency in aldosterone-treated myocytes, Ang-(1-7) contributes to increased SR Ca^{2+} content, consequently enhancing Ca^{2+} transient magnitude in these cells. Therefore, the increase in SR Ca^{2+} load observed when aldosterone-treated myocytes were exposed to Ang-(1-7) can account fully for the further enhancement of the Ca^{2+} transient magnitude found in this group when compared with aldosterone-treated cardiomyocytes.

Our next goal was then to investigate the molecular determinants of changes in Ca^{2+} signaling observed in response to aldosterone and Ang-(1-7). As previously shown by Christ et al,[16] treatment of vascular smooth muscle cells with aldosterone increased cyclic adenosine 3′,5′-monophosphate levels. To gain further insight into the signaling pathway by which aldosterone modulates global Ca^{2+} transient in cardiomyocytes, we investigated whether protein kinase A (PKA), a known cyclic adenosine 3′,5′-monophosphate target, is a downstream mediator of aldosterone effects. We found that PKi, a PKA inhibitor, abolished the effect of aldosterone on peak Ca^{2+} transient amplitude (Figure 3A). Similar results were observed when aldosterone-treated cells were incubated with H89, another PKA inhibitor (data not shown). Thus, we conclude that aldosterone alters global Ca^{2+} transient, through activation of PKA. Importantly, protein kinase inhibitor significantly reduced peak Ca^{2+} transient amplitude in cells treated with aldosterone and Ang-(1-7) (Figure 3B) indicating that PKA activation plays a major role in this cross-talk.

PKA targets phospholamban (PLN) a protein that regulates SERCA activity by increasing phosphorylation of Ser.[16] Dephosphorylated PLN inhibits SERCA, whereas phosphorylation of PLN reverses this inhibition, thus increasing the rate of Ca^{2+} uptake by the SR. Therefore, we next assessed PLN Ser^{16} phosphorylation levels by Western blot techniques. Figure 3C shows that aldosterone significantly increases PLN Ser^{16} phosphorylation. A similar effect was observed in cardiomyocytes exposed to aldosterone and Ang-(1-7). Thus these data suggest that SERCA activity is increased in cells treated with aldosterone, whether or not it is combined with Ang-(1-7). Interestingly, the increase in SERCA activity in aldosterone-treated myocytes leads to no change in SR Ca^{2+} content, indicating that the full effect of PKA activation on SR Ca^{2+} load was mitigated by increased Ca^{2+} spark rate in these cells. On the other hand, PKA activation and reduced Ca^{2+} spark activity explain the increased SR Ca^{2+} content found in aldosterone/Ang-(1-7) treated myocytes.

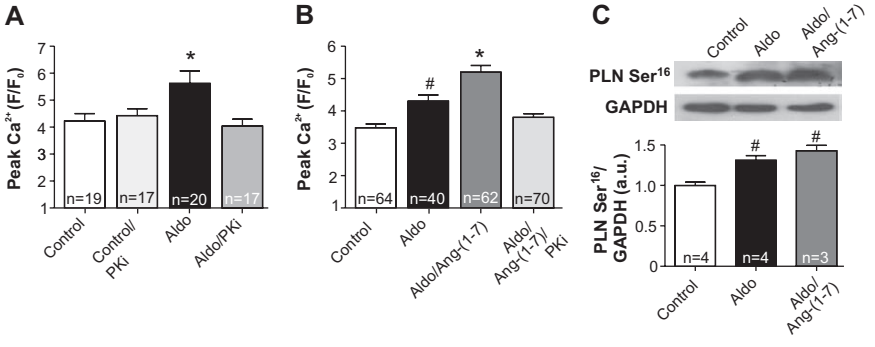

Figure 3. Protein kinase A is a downstream mediator of the synergistic effects of aldosterone and angiotensin (Ang)-(1-7) on Ca^{2+} transients. **A**, Cardiomyocytes were preincubated with 10 μmol/L protein kinase inhibitor (PKi) and then treated with 1 μmol/L aldosterone (Aldo) in the continuous presence of PKi for 30 minutes. PKi treatment completely abolished the effect of aldosterone on peak Ca^{2+} transient. **B**, PKi significantly prevented the increase in Ca^{2+} transient magnitude induced by aldosterone and Ang-(1-7). **C**, Phospholamban (PLN) phosphorylation levels at Ser^{16} are increased in cardiomyocytes exposed to aldosterone with or without Ang-(1-7). **P*<0.05 when compared with the other groups. #*P*<0.05 when compared with control. **A** and **B**, n= number of cells. **C,** n= number of cardiomyocyte samples from each experimental group.

To establish the mechanisms involved in Ang-(1-7) effects on Ca^{2+} spark rate in cells treated with aldosterone was our next goal. NO is a well known modulator of Ca^{2+} handling proteins, including the RyR.[17] Indeed, we have previously shown that Ang-(1-7) leads to NO production in cardiomyocytes.[15] Therefore, we next assessed whether NO contributes to the observed effect of Ang-(1-7) on Ca^{2+} signaling under conditions of aldosterone excess. We assessed NO levels by using the fluorescent indicator diaminofluorescein-FM diacetate. Consistent with our previous findings,[15,18] Ang-(1-7)-treated cardiomyocytes (100 nmol/L) showed an increase in NO generation (Figure 4A and 4B). We now show that Ang-(1-7) effects on NO were not affected by aldosterone, which alone had no effect on NO levels in cardiomyocytes. Figure 4B shows that the Ang-(1-7)-induced NO increase was completely abolished in cells preincubated with NO synthase (NOS) inhibitor N^G-nitro-Larginine methyl ester (L-NAME, 10 μmol/L). Cardiomyocytes constitutively express both NOS1 and NOS3. We have previously shown that Ang-(1-7) increases NOS3 phosphorylation at the Ser^{1177} activation site in myocytes.[15] To further investigate which NOS isoform is activated by Ang-(1-7) in aldosterone-treated myocytes, we assessed NOS3 and NOS1 phosphorylation levels at Ser^{1177} (activation site) and Ser^{852} (inhibitory site), respectively. As shown in Figure S5A, treatment with aldosterone alone did not affect NOS3 phosphorylation. In contrast, Ang-(1-7) stimulation of aldosterone-treated myocytes led to a significant increase in NOS3 Ser^{1177} phosphorylaton levels confirming and extending our previous findings that NOS3 is activated by Ang-(1-7) even in the presence of aldosterone. A lack of aldosterone effects on NOS1 phosphorylation levels was also observed (Figure S5B). Furthermore, there was a significant decrease in NOS1 phosphorylation at the inhibitory site (Ser^{852}) in cardiomyocytes treated with a combination of aldosterone and Ang-(1-7). To confirm that NOS1 and NOS3 protein levels were unaltered during our experimental conditions, we measured total NOS protein levels. Figure S5C and S5D shows no significant changes in NOS1/NOS3 levels during our experiments. Importantly, Ang-(1-7) alone led to reduced NOS1 phosphorylation levels at Ser^{852} showing for the first time that this peptide modulates NOS 1 activity (Figure S6A and S6B). Reduced NOS1 phosphorylation at Ser^{852} increases enzyme activity, suggesting an increase in NO production under this condition. To investigate whether NO generation contributes to Ang-(1-7) effects on Ca^{2+} handling in aldosterone-treated myocytes, we measured Ca^{2+} transients in cells incubated with L-NAME. Although L-NAME alone had no effect on Ca^{2+} transient amplitude (Figure 5A), it significantly reduced the magnitude of Ca^{2+} transient in cells treated with aldosterone and Ang-(1-7) (Figure 5B). Thus, these data suggest that NO participates in the cross-talk between Ang-(1-7) and aldosterone contributing to the significant increase in Ca^{2+} transient amplitude observed under this condition. Because PKA is activated in cardiomyocytes treated with a combination of aldosterone and Ang-(1-7), it is expected that L-NAME would not completely abolish the increase in Ca^{2+} transient amplitude observed in this group. NO can interact with proteins involved in Ca^{2+} handling, including the RyR.[17] Therefore, a possibility is that Ang-(1-7)-derived NO stabilizes the RyR reducing the channel open probability (P_o) leading to an increase in SR Ca^{2+} load. If so, we would expect an increase in Ca^{2+} spark rate because of increased RyR activity in aldosterone/Ang-(1-7)-treated myocytes when NO synthesis is blocked. We next tested this hypothesis by recording Ca^{2+} sparks in the presence of L-NAME. Figure 5C shows that addition of L-NAME to aldosterone and Ang-(1-7) treated-cardiomyocytes significantly increased Ca^{2+} spark rate, whereas L-NAME alone did not influence Ca^{2+} spark rate. Taken together, these data support a direct role of Ang-(1-7)-derived NO as a modulator of Ca^{2+} handling in the presence of aldosterone and implicates NOS1 and NOS3 as sources of NO production.

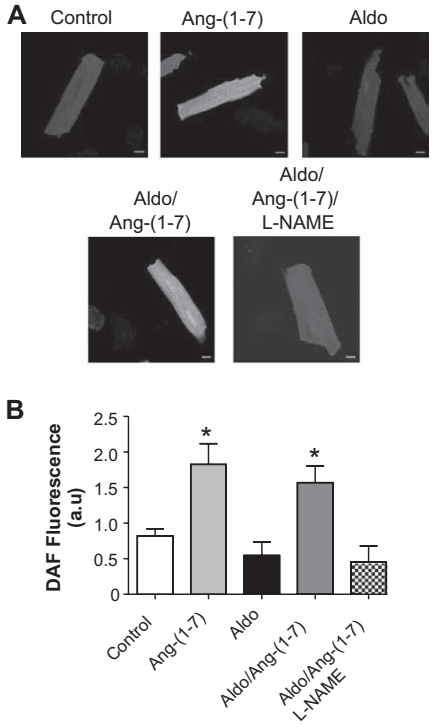

Figure 4. Angiotensin (Ang)-(1-7) increases NO in aldosterone (Aldo)-treated myocytes. **A**, Sample images of diaminofluorescein (DAF) fluorescence in ventricular myocytes. Bar, 10 μm. **B**, Averaged-bar graph represents NO generation in ventricular myocytes after acute Ang-(1-7) treatment (100 nmol/L; 30 minutes). The significant increase in NO levels after Ang-(1-7) incubation was not affected by aldosterone. In L-NAME pretreated cells, the Ang-(1-7)–dependent NO raise was abolished. Aldosterone alone had no effect on DAF fluorescence. n=20 to 50 cardiomyocytes per group. *P<0.05 when compared with control, aldosterone, and aldo/Ang-(1-7)/L-NAME treated myocytes.

Table S1 summarizes the important cellular changes found in Aldo/Ang-(1-7) treated myocytes.

Discussion

Our results bring new perspectives into the understanding of how 2 prominent molecules with supposedly antagonistic cardiac actions cross-talk to functionally amplify Ca^{2+} signals in cardiomyocytes. High serum levels of aldosterone have been identified as an independent predictor of all-cause mortality risk in patients with chronic heart failure of any cause and severity.[19] In contrast, Ang-(1-7) was repeatedly shown to possess cardioprotective effects, preventing cardiac remodeling in vitro[18,20] and in vivo[18,21–24] and attenuating cardiac dysfunction and fibrosis induced by excessive MR activation.[10,11] Notably, acute Ang-(1-7) treatment synergistically

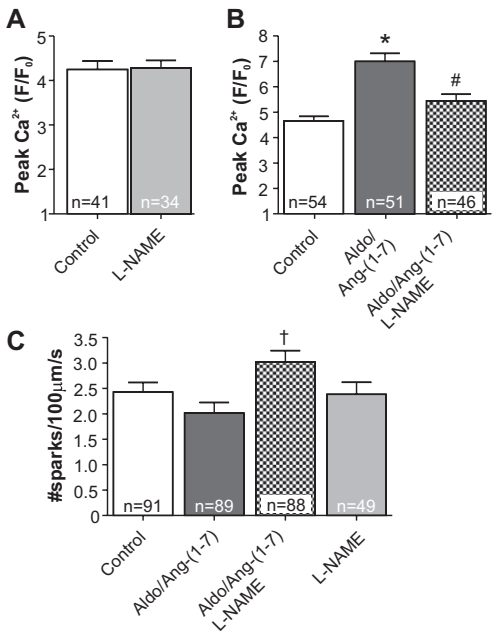

Figure 5. NO mediates Angiotensin (Ang)-(1-7) effects on Ca^{2+} signaling in aldosterone-treated cardiomyocytes. **A**, Treatment of myocytes with L-NAME alone had no effect on Ca^{2+} transient amplitude. **B**, L-NAME significantly reduced the magnitude of the Ca^{2+} transient in cardiomyocytes treated with aldosterone and Ang-(1-7). **C**, L-NAME restored the effect of aldosterone (Aldo) on Ca^{2+} spark rate in aldosterone/Ang-(1-7) treated myocytes. n=number of cardiomyocytes. **P*<0.05 when compared with other experimental groups. #*P*<0.05 when compared with control. †*P*<0.05 when compared with control and Aldo/Ang-(1-7)-treated cardiomyocytes.

enhanced aldosterone mediated effects on Ca^{2+} handling. This observation was initially unexpected because previous studies showed antagonistic effects exerted by these 2 molecules in the heart.[10,11] We now show that Ang-(1-7) functionally cross-talks with aldosterone, via parallel and converging transduction pathways that include PKA activation and NO production, culminating with enhanced Ca^{2+} release in ventricular myocytes. These findings have important implications for our understanding of how 2 important signaling pathways interact in cardiac cells. One possibility is that this synergistic effect might result in amplification of Ang-(1-7) cardioprotection, preventing the progressive deterioration in myocardial contractility associated with increased aldosterone levels.

Short-term aldosterone incubation induces moderated PKA activation, which leads to increased Ca^{2+} transient, possibly through enhanced I_{Ca}. Consistent with our data, another study has shown that aldosterone increases cyclic adenosine 3′,5′-monophosphate levels in vascular smooth muscle cells.[16] Moreover, SR Ca^{2+} content is not altered in aldosterone-treated cardiomyocytes despite increased PLN Ser^{16} phosphorylation. Therefore we conclude that the full effect of PKA activation on SR Ca^{2+} load was mitigated by increased Ca^{2+} spark rate in aldosterone-treated cells.

Interestingly, PKA remains activated in cells treated with a combination of aldosterone and Ang-(1-7), as seen by the significant reduction in Ca^{2+} transient amplitude observed in this group, when treated with protein kinase inhibitor. In addition to increased PKA activation, Ang-(1-7) also leads to NO generation in aldosterone-treated myocytes, with NO exerting a crucial role on Ang-(1-7) synergistic effects on Ca^{2+} transient. NO is known to regulate the L-type Ca^{2+} channel[25] and the RyR,[26] working as both positive or negative inotropic agent. Because Ang-(1-7) alone increases NO levels without changing Ca^{2+} transient amplitude, it is conceivable that NO effects on Ca^{2+} transient under conditions of Ang-(1-7)/aldosterone cross-talk depend on other factors, such as the degree of PKA activation. Accordingly, Ziolo et al[17] have shown that NO effects on RyR activity and Ca^{2+} transients can be modulated by the state of PKA activation. PKA activation leads to the phosphorylation of several proteins involved in excitation-contaction coupling, including the RyR. In this context, PKA-phosphorylated RyR presented increased P_o, that is seen as an increase in resting Ca^{2+} spark frequency, which was reversed by addition of a NO donor.[17] Our Ca^{2+} spark results support this assumption because cells treated with aldosterone/Ang-(1-7) showed a significant reduction in Ca^{2+} spark rate when compared with aldosterone-treated myocytes, an effect that was abolished by addition of L-NAME. Taken together, these findings suggest that RyR open probability is significantly reduced in aldosterone-treated myocytes when exposed to Ang-(1-7). Thus, our findings reveal a previously unidentified role for Ang-(1-7) as a modulator of diastolic Ca^{2+} sparks in the heart under conditions of elevated aldosterone levels. In fact, Gomez et al[9] have shown that aldosterone directly affects RyR activity, by dissociating FKBP12.6, leading to abnormal Ca^{2+} sparks in ventricular myocytes. Aberrant Ca^{2+} release during diastole has been linked to the development of cardiac arrhythmia in heart failure models.[27] A potentially important implication of our findings is that Ang-(1-7), by reducing the Ca^{2+} spark rate in the presence of aldosterone, may protect the heart against life threatening arrhythmias. Further investigation will be necessary to understand whether Ang-(1-7) prevents aldosterone-induced arrhythmias.

NOS3 phosphorylation of Ser^{1177} was increased by Ang-(1-7) in aldosterone-treated myocytes. This supports our observation that Ang-(1-7) increases NOS3 phosphorylation in cardiomyocytes.[15] We have now shown that NOS1 is activated by Ang-(1-7) in ventricular myocytes, and this effect is not altered by aldosterone. In addition, we have previously shown that transgenic rats with a chronic increase in circulating levels of Ang-(1-7) have increased NOS1 expression levels in cardiomyocytes,[18] suggesting an important association between the Ang-(1-7) and NOS1 signaling pathways in cardiomyocytes. Therefore, it is possible that NOS1-derived NO plays a role in Ang-(1-7) effects on cardiomyocytes. However the specific contributions of each NOS isoform to Ang-(1-7) effects on Ca^{2+} handling still need to be determined.

Perspectives

Our finding of cross-talk between Ang-(1-7) and aldosterone signaling under conditions where both receptors are activated in cardiomyocytes uncovered a previously unrecognized signaling pathway that includes synergistic actions at the Ca^{2+} transient level, giving further insight into how these 2 signaling pathways interact in cardiac cells. As such, the NO-dependent action of Ang-(1-7) associated with its ability to keep aldosterone-induced PKA activation may confer to this peptide the capacity to maintain contractile function in conditions of

aldosterone excess. In addition, abnormal Ca^{2+} release during diastole has been linked to the development of cardiac arrhythmia in heart failure models. A potential implication of our findings is that Ang-(1-7) by reducing diastolic Ca^{2+} spark rate in the presence of aldosterone may protect the heart against life threatening arrhythmias. In light of Ang-(1-7) therapeutic potential it will also be extremely important to investigate whether these signaling pathways may account in vivo for Ang-(1-7) protective actions in models of hyperaldosteronism.

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Disclosures

None.

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Novelty and Significance

What Is New?

- We demonstrate that Aldosterone is capable of cross-talk with Ang-(1-7) to amplify Ca^{2+} release in ventricular myocytes.

What Is Relevant?

- We provide a better understanding of Ang-(1-7) actions under conditions of increased aldosterone levels.

Summary

Our data uncover a previously unrecognized signaling that helps to explain the ability of Ang-(1-7) to protect the heart against increased aldosterone signaling.