Diminished Inotropic Response to Amrinone in Ventricular Myocytes from Myopathic Hamsters Is Linked to Depression of High-Gain Ca\(^{2+}\)-Induced Ca\(^{2+}\) Release

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ABSTRACT

This study investigates whether amrinone (100–1000 μM), a phosphodiesterase-III inhibitor, can alleviate depression of contractions in ventricular myocytes from prefailure cardiomyopathic (CM) hamsters (80–100 days). Cell shortening and ion currents were measured in voltage-clamped cells at 37°C. Normal myocytes exhibited low-gain Ca\(^{2+}\) -induced Ca\(^{2+}\) release (CICR) initiated by test steps from −40 mV and high-gain CICR initiated from more negative potentials. In normal myocytes, amrinone selectively increased contractions initiated by high-gain CICR (fractional shortening increased from 3.6 ± 0.5% to 5.3 ± 0.6%, 300 μM amrinone) but had no effect on low-gain CICR. Amrinone decreased L-type Ca\(^{2+}\) current (I\(_{\text{Ca-L}}\); −5.5 ± 0.8 mV, 0 to −3.7 ± 0.5 picoAmp/picoFarad, 300 μM amrinone). In contrast, in CM myocytes, high-gain CICR was virtually absent, and amrinone had no inotropic effect. Amrinone inhibited I\(_{\text{Ca-L}}\) less in CM than normal myocytes. Sarcoplasmic reticulum (SR) Ca\(^{2+}\) stores, assessed by caffeine, were significantly increased by amrinone in normal but not CM myocytes. Thus, the positive inotropic effect of amrinone in normal hamster myocytes was mediated by selective enhancement of high-gain CICR. This effect was not mediated by stimulation of I\(_{\text{Ca-L}}\) because I\(_{\text{Ca-L}}\) is inhibited by this drug in hamster. High-gain CICR, which is depressed in CM myocytes, cannot be restored by amrinone. However, minimal stimulation of adenyl cyclase with forskolin restored the positive inotropic effect of amrinone in CM cells. This positive inotropic effect of amrinone may reflect increased SR Ca\(^{2+}\) stores because increased stores accompanied the positive inotropic effect in normal myocytes but were absent in CM myocytes.

The cardiomyopathic (CM) golden Syrian hamster develops an inherited cardiomyopathy triggered by a large genomic deletion in the δ-sarcoglycan gene (Sakamoto et al., 1997). This gene mutation leads to a deficiency in the dystrophin-associated glycoprotein in the sarcolemma of cardiac and skeletal muscles (Robers et al., 1993) and leads to deleterious changes in contractile function of these muscles (for review, see Howlett et al., 1999). In CM hamster heart, focal necrosis is observed at 40 to 50 days of age and reaches a peak around 90 days of age (Jasmin and Proschek, 1982; Hunter et al., 1984). Necrotic changes are followed by progressive ventricular hypertrophy beginning at about 120 days of age, and heart failure develops between 200 and 300 days of age (Jasmin and Proschek, 1982; Hunter et al., 1984). Thus, the CM hamster exhibits predictable and progressive cardiomyopathy with hypertrophy and terminal heart failure.

Contractile defects can be observed in cardiac tissues from CM hamsters before development of hypertrophy and heart failure (Bobet et al., 1991; Howlett et al., 1991). This suggests that depression of contraction is an early event that may contribute to the development of heart failure. This defect can be observed in isolated single ventricular myocytes, which suggests that decreased contractile strength represents a defect in excitation-contraction (EC)-coupling (Howlett et al., 1999). In a previous study, we showed that the defect in contraction could be observed in voltage-clamped myocytes and therefore could not be attributed to changes in membrane or action potentials (Howlett et al., 1999). Furthermore, our studies showed that a defect in

**ABBREVIATIONS:** CM, cardiomyopathic; EC, excitation-contraction; CICR, Ca\(^{2+}\)-induced Ca\(^{2+}\) release; I\(_{\text{Ca-L}}\), L-type Ca\(^{2+}\) current; SR, sarcoplasmic reticulum; PKA, protein kinase A; PDE-III, phosphodiesterase III.
contraction could only be observed when contractions were initiated by voltage steps from negative membrane potentials approaching the resting potential but not when cells were activated by steps from −40 mV. Contractions activated by steps from −40 mV are believed to be initiated by Ca^{2+}-induced Ca^{2+} release (CICR) coupled to influx of Ca^{2+} by way of L-type Ca^{2+} current (ICa-L) (Bers, 2001). CICR initiated from depolarized potentials typically exhibits low gain (Ca^{2+} release/Ca^{2+} current). In contrast, we have presented evidence that contractions activated by steps from membrane potentials near the resting potential involve a high-gain mode of CICR in which very little current is required to initiate sarcoplasmic reticulum (SR) release of Ca^{2+} (Ferrier et al., 2004). Thus, the defect in contraction observed in myocytes from CM hamsters was caused by selective depression of high-gain CICR (Howlett et al., 1999).

The contribution of high-gain CICR to myocyte contraction is modulated by phosphorylation through the cAMP-dependent protein kinase A (PKA) and Ca^{2+}/calmodulin kinase pathways (Hobai et al., 1997; Ferrier et al., 1998; Zhu and Ferrier, 2000; Ferrier and Howlett, 2003). Modulation of high-gain CICR by PKA appears to be highly compartmentalized. This was shown in experiments on guinea pig ventricular myocytes where selective phosphodiesterase III (PDE-III) inhibition by amrinone augmented high-gain CICR with virtually no effect on low-gain CICR and little effect on ICa-L (Xiong et al., 2001). Because the defect in contraction in CM myocytes is primarily linked to depression of high-gain CICR, it is possible that stimulation of high-gain CICR with amrinone might restore strength of contraction in myocytes from these animals.

The objectives of this study were: 1) to determine whether amrinone selectively increases high-gain CICR in hamster myocytes, 2) to determine whether amrinone can reverse depression of contractile function that occurs in CM hamster myocytes prior to the onset of heart failure, and 3) to determine whether the inotropic effects of amrinone in myocytes from normal and/or CM hamsters are mediated by actions on ICa-L or SR Ca^{2+}.

Materials and Methods

All experiments were performed on isolated ventricular myocytes from 80- to 100-day-old male CM hamsters (CHF 146) and age-matched male normal hamsters (CHF 148), purchased from Canadian Hybrid Farms (Halls Harbor, NS, Canada). Experiments were conducted in accordance with the guidelines published by the Canadian Council on Animal Care, and this investigation was approved by the Dalhousie University Committee on Animal Care. Hamsters were injected intraperitoneally with heparin (3.3 IU/g) and sodium pentobarbital (80 mg/kg) to induce anesthesia. Following thoracotomy, the ascending aorta was cannulated in situ and perfused retrogradely (10 ml/min) with solution gassed with 100% O2. The solution had the following composition: 120 mM NaCl, 4 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 0.05 mM CaCl2, 10 mM HEPES, and 12 mM glucose (37°C, pH 7.4 with NaOH). Na+ current and transient outward current were inhibited with 200 μM lidocaine and 2 mM 4-aminopyridine, respectively. Most solutions were delivered from a reservoir at 3 ml/min and were changed by switching the inlet to the pump between different solutions. The solution exchange time was about 2 min, as determined by the response to changing extracellular K+ . To assess SR Ca2+ stores, caffeine (10 mM) was applied with a heated (37°C) rapid solution switcher for 4 s. This device allowed exchange of the extracellular solution surrounding the myocyte within approximately 300 ms. The rapid solution switcher was controlled by pCLAMP software (version 6.0.3; Axon Instruments Inc., Union City, CA) to ensure precise timing.

Recordings were made from quiescent, rod-shaped ventricular myocytes with clear striations and resting membrane potentials more negative than −85 mV. Discontinuous single-electrode voltage-clamp recordings (7–10 kHz) were made with an Axoclamp 2A amplifier (Axon Instruments Inc.) and high-resistance microelectrodes (18–25 MΩ, filled with 2.7 M KCl) to minimize cell dialysis. Switching frequency was adjusted to insure adequate settling time for accurate voltage control. A 2.7 M KCl-agar bridge was used as a bath ground to reduce liquid junction potential changes. Voltage-clamp protocols were generated, and currents and membrane potentials were recorded with pCLAMP 6.0 software (Axon Instruments Inc.). Myocyte images were monitored with a closed circuit television camera (model 1-GP-CD 60; Panasonic, Secaucus, NJ) and were displayed on a video monitor. Unloaded cell shortening was sampled at 120 Hz with a video edge detector (model VED 103; Crescent Electronics, Sandy, UT) coupled to the television camera. Cell length was measured by tracking both ends of each cell with the video edge detector. Contractions, current and voltage were digitized with a Labmaster A/D interface (TLI-125, Axon Instruments Inc.) and stored on a computer.

In all experiments, voltage-clamp test steps were preceded by trains of 200-ms conditioning pulses from a holding potential of −80 mV to 0 mV delivered at approximately 3 Hz to maintain consistent activation of the myocyte. Conditioning pulses were followed by a 500-ms-long postconditioning potential of −40 or −60 mV from which the test steps were made. Current, voltage, and contractions were measured with pClamp analysis software. Inward ICa-L was measured as the difference between the peak inward current and a reference point near the end of the voltage step (Li et al., 1995). Peak amplitudes of inward currents were normalized to cell capacitance (membrane area). Cell capacitance was estimated with pClamp software by integrating capacitive transients elicited by test steps from −60 to −50 mV, where little inward current is activated. Specific details of particular voltage-clamp protocols are provided in the appropriate results sections. Amplitudes of contraction were measured as the difference between a point immediately before onset of cell shortening and peak cell shortening. Fractional shortening was determined by normalizing contraction amplitudes to diastolic cell length.

The statistical significance of differences between means was tested with a Student’s t test or an analysis of variance. Current-voltage relationships and contraction-voltage relationships were analyzed with a two-way repeated measures analysis of variance. Post hoc comparisons were made with a Student-Newman-Keuls test.
statistical analyses were performed with SigmaStat (version 2.03, SPSS Inc., Chicago, IL). Data are presented as means ± S.E. The value of "n" represents the number of cells sampled. No more than two cells from one heart were included in each data set.

Amrinone, lidocaine, caffeine, forskolin, 4-aminopyridine, and HEPES were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). All other chemicals were purchased from BDH Inc. (Toronto, ON, Canada). Amrinone was dissolved in HCl in stock solution, and the pH of the final solution of amrinone was adjusted to 7.4 with NaOH.

**Results**

In a previous study, we demonstrated that contractions initiated from negative membrane potentials were selectively depressed in myocytes from CM hamsters (Howlett et al., 1999). Figure 1 compares contractions elicited in voltage-clamped myocytes from normal and CM hamsters. The voltage-clamp protocol is shown in Fig. 1A. Following the train of conditioning pulses, the cells were clamped to a postconditioning potential of −60 mV, followed by sequential steps to −40 and 0 mV. Figure 1B shows records of contractions and currents recorded from a myocyte from a normal heart. The test step to −40 mV initiated a phasic contraction with little accompanying inward current. The subsequent step to 0 mV elicited a second phasic contraction and activated I_{Ca,L}. In contrast, in the CM myocyte, the voltage step to −40 mV failed to elicit a contraction (Fig. 1C). However, the voltage-clamp step to 0 mV initiated a phasic contraction and I_{Ca,L}. Figure 1D shows mean amplitudes of contractions (fractional shortening) and current densities recorded from normal and CM myocytes. The amplitudes of contractions elicited by steps to −40 mV were significantly smaller in CM compared with normal myocytes. There was no difference in the amplitudes of the accompanying small inward currents. However, the step to 0 mV elicited contractions and I_{Ca,L} of comparable magnitudes in myocytes from normal and CM hearts. Thus, myocytes from CM hearts exhibited marked depression of contractions initiated by steps from −60 to −40 mV, with no significant differences compared with normal myocytes. Inward currents initiated by both steps were similar in amplitude in the two groups. * significantly different from normal (p < 0.05); n = 5 normal and 14 CM myocytes.
difference in contractions initiated by steps from −40 to 0 mV.

The next series of experiments used the same voltage-clamp protocol as in Fig. 1 to investigate the effects of amrinone on contractions and currents in myocytes from normal hamsters. Figure 2, A and B, respectively, show records of contractions and currents made before and after superfusion of a normal myocyte with 300 μM amrinone. In this example, amrinone increased the amplitude of the contraction initiated by the step from −60 to −40 mV but had little if any effect on the contraction elicited by the step from −40 to 0 mV. Interestingly, amrinone decreased the amplitude of ICa-L activated by the step to 0 mV. Figure 2C shows mean amplitudes of contractions and current densities in normal cells exposed to 0, 300, and 1000 μM amrinone. Amrinone caused a concentration-dependent increase in the amplitudes of contractions initiated by steps from −60 to −40 mV but did not alter the amplitudes of contractions initiated by steps from −40 to 0 mV. Mean inward current density initiated by the step to −40 mV was slightly but not significantly reduced in the presence of amrinone. However, amrinone caused large and significant decreases in ICa-L initiated by the step to 0 mV.

We next determined whether comparable effects could be demonstrated in CM myocytes superfused with amrinone. Figure 3, A and B, compare recordings of contractions and currents made from a CM myocyte in the absence and presence of 300 μM amrinone. Little or no contraction was initiated by the step from −60 to −40 mV in the absence of amrinone, and superfusion with amrinone had no observable effect on responses to either voltage-clamp step, except for a slight decrease in the amplitude of ICa-L. Interestingly, contractions initiated by the step from −60 to −40 mV in CM myocytes were unaffected by amrinone and remained small in control and treated myocytes (Fig. 3C). Thus, amrinone did not restore contractions initiated by steps from negative potentials in CM myocytes. Amrinone also had no significant effect on contractions initiated by the step from −40 to 0 mV.
in CM myocytes. However, amrinone did significantly reduce the amplitudes of $I_{Ca-L}$ in CM myocytes, although this effect appeared to be slightly smaller than in normal myocytes.

It is possible that the lack of inotropic effect of amrinone on CICR initiated by steps from $-40$ to $0$ mV in both normal and CM myocytes is related to the use of sequential activation steps. This might occur if the first step from $-60$ to $-40$ mV partially depletes SR stores. Therefore, we examined effects of amrinone on contractions and currents initiated by steps $-40$ to $0$ mV with a protocol that omitted the first step (Fig. 4, top). In this protocol, the last conditioning pulse was followed by return to a postconditioning potential of $-40$ mV. Figure 4A compares contractions and currents recorded from a normal myocyte in the absence and presence of 300 $\mu$M amrinone. Amrinone had no effect on the magnitude of contraction although it decreased inward current. Figure 4B presents mean data that show that amrinone had no inotropic effect in normal myocytes activated with this protocol. However, amrinone continued to exert an inhibitory effect on $I_{Ca-L}$ (Fig. 4C). Figure 4D shows that amrinone had no effect on contraction in a CM myocyte but decreased $I_{Ca-L}$. Mean data demonstrate that amrinone had no significant effect on contraction in CM myocytes but significantly decreased $I_{Ca-L}$ (Fig. 4, E and F). Thus, the lack of inotropic effect of amrinone on low-gain CICR was not related to the order of activation of contractions. In the next set of experiments, we examined the effects of amrinone when steps to $0$ mV were made from a postconditioning potential to $-60$ mV (Fig. 5, top). Figure 5A shows recordings of contractions and currents in a normal myocyte in the absence and presence of 300 $\mu$M amrinone. Amrinone increased the amplitude of contraction initiated by a step from $-60$ to $0$ mV and decreased inward current (Fig. 5A). Figure 5, B and C, show mean data for amplitudes of contraction and current densities recorded in normal myocytes. With this protocol, amrinone caused a significant increase in contraction in normal myocytes, despite continued inhibition of $I_{Ca-L}$ (Fig. 5, B and C). However, with the same voltage-clamp protocol, amrinone still did not exert a positive inotropic effect in CM myocytes, although inhibition of $I_{Ca-L}$ persisted (Fig. 5, D–F). These data suggest that amrinone does not restore contractions initiated by high-gain CICR in CM myocytes. The voltage-clamp protocol is shown at the top. A and B, representative recordings of contractions and currents elicited in a CM myocyte before and after exposure to 300 $\mu$M amrinone. Amrinone did not restore contraction for the step from $-60$ to $-40$ mV. C, mean data demonstrate that amrinone did not enhance contractions initiated by high-gain CICR triggered by steps to $-40$ mV. Amrinone also did not increase contractions initiated by the step to $0$ mV but did significantly decrease $I_{Ca-L}$, significantly different from control in the absence of drug ($p < 0.05$; $n = 6–10$ myocytes).
The inotropic effect of amrinone on normal cells is only available when test steps originate from more negative potentials. Furthermore, this inotropic effect is not available in myocytes from CM hearts.

It is possible that the presence or absence of effects of amrinone on contractions and currents could be related to shifts in the voltage dependence of these responses, rather than absolute changes in maximum amplitudes. To investigate this, we determined contraction-voltage and current-voltage relations over a wide range of test potentials. Also, we compared contraction-voltage and current-voltage relations initiated from postconditioning potentials of −40 and −60 mV. Mean relations determined in normal cells are shown in Fig. 6, and the voltage-clamp protocol is shown at the top.

Figure 6A shows that amrinone had no inotropic effect across the entire activation voltage range tested when the postconditioning potential was −40 mV and that there was no obvious shift in the voltage dependence of contraction. In contrast, the current-voltage relations demonstrated a prominent concentration-dependent inhibition of ICa,L (Fig. 6B). Inhibition of ICa,L occurred without a shift in voltage dependence. However, when the postconditioning potential was −60 mV (Fig. 6C), a marked positive inotropic effect of amrinone appeared over a wide range of test potentials. This effect was accompanied by inhibition of ICa,L, again with no apparent shift in voltage dependence (Fig. 6D). These data show that amrinone can exert a prominent positive inotropic effect over a wide range of test potentials in normal myocytes but only when steps are made...
from negative membrane potentials. Furthermore, the effects of amrinone cannot be attributed to shifts in voltage dependence of either contraction or current.

Figure 7 shows mean contraction-voltage and current-voltage relations determined in CM myocytes. The data demonstrate that, regardless of postconditioning potential, amrinone did not alter either the configuration or amplitudes of the contraction-voltage relations (Fig. 7, A and C). However, amrinone did cause a significant inhibition of ICa-L initiated by steps from either −40 or −60 mV. Inhibition of ICa-L was not accompanied by a shift in voltage dependence. Although inhibition of ICa-L was significant, the effect appeared smaller in CM compared with normal myocytes. To determine whether inhibition of ICa-L was greater in normal than in CM myocytes, we compared current-voltage relations from normal and CM myocytes in the absence and presence of the highest concentration of amrinone (Fig. 8). Figure 8, A and B, show mean current-voltage relations elicited by test steps from −40 and −60 mV for normal and CM myocytes in the absence of amrinone. Control current-voltage relations were not significantly different between normal and CM myocytes with either postconditioning potential. Figure 8, C and D, compare current-voltage relations in the presence of 1000 μM amrinone. With either postconditioning potential, ICa-L was significantly smaller in normal than CM myocytes.

To determine whether changes in SR Ca2+ stores contribute to the inotropic effects of amrinone, we estimated SR stores by rapid application of 10 mM caffeine. The protocol is...
shown at the top of Fig. 9. Caffeine application was substituted for the test step normally following the train of conditioning pulses. Caffeine induces release of SR Ca\(^{2+}\), which initiates an inward Na\(^{+}\)-Ca\(^{2+}\) exchange current. The integral of this current provides a measure of Ca\(^{2+}\) released from SR stores (Varro et al., 1993). Figure 9, A and B, respectively, show representative recordings of caffeine-induced contractions (top) and accompanying Na\(^{+}\)-Ca\(^{2+}\) exchange current (bottom) in a normal myocyte before and after exposure to amrinone. Mean amplitudes of contractures and currents are shown in Fig. 9C. The amplitudes of contractures were not different in the absence and presence of amrinone. However, amrinone significantly increased the integral of the Na\(^{+}\)-Ca\(^{2+}\) exchange current. This suggests that amrinone increases SR Ca\(^{2+}\) stores in normal hamster myocytes. Figure 10, A and B, show caffeine-induced contractures and currents recorded in a CM myocyte in the absence and presence of amrinone. Figure 10C shows that the mean amplitudes of contractures and integrals of Na\(^{+}\)-Ca\(^{2+}\) exchange currents were not significantly different in myocytes from CM hearts before and after exposure to amrinone. Thus, amrinone increased SR Ca\(^{2+}\) stores in normal but not CM myocytes.

Inhibition of PDE by amrinone would be expected to promote phosphorylation of protein targets only if there is adequate production of cAMP in the cell. Thus, the absence of a positive inotropic effect of amrinone may indicate that cAMP production is inadequate in CM myocytes. To investigate this, we used forskolin to stimulate adenylyl cyclase and thus stimulate cAMP production in myocytes from CM heart. We selected a low concentration forskolin (0.3 \(\mu\)M), which does not, by itself, cause a significant increase in contraction (Feldman et al., 1987). Then, we determined whether amrinone (300 \(\mu\)M) could increase contraction in CM myocytes in the presence of forskolin. The protocol is shown at the top of Fig. 11. Figure 11A shows representative traces of contraction (top) and current (bottom) recorded from a CM myocyte in response to a test step from -60 to 0 mV. Figure 11B demonstrates that 0.3 \(\mu\)M forskolin had little effect on magnitudes of either inward current or contraction activated by voltage steps from -60 to 0 mV. However, when 0.3 \(\mu\)M forskolin was added in combination with amrinone, contraction increased markedly in the CM myocyte (Fig. 11C). Mean contraction-voltage and current-voltage curves are shown in Fig. 11, D and E. These data show that a low concentration of forskolin had no significant effect on either contraction or current at any voltage examined. However, in the presence of amrinone (300 \(\mu\)M), the contraction and current increased significantly in the presence of forskolin.
of forskolin, amrinone significantly increased the amplitudes of contractions (Fig. 11D). Interestingly, amrinone plus forskolin had no significant effect on the magnitude of inward current (Fig. 11E). Thus, minimal stimulation of adenylyl cyclase with forskolin restored the positive inotropic effect of amrinone in myocytes from CM heart.

**Discussion**

The objectives of this study were: 1) to determine whether amrinone selectively increases high-gain CICR in hamster myocytes, 2) to determine whether amrinone can reverse depression of contractile function that occurs in myocytes from CM hamsters prior to the onset of heart failure, and 3) to determine whether the inotropic effects of amrinone in myocytes from normal and/or CM hamsters are mediated by actions on I\textsuperscript{ca-L} or SR Ca\textsuperscript{2+}. Our results demonstrated that amrinone caused a concentration-dependent increase in contractions initiated from negative membrane potentials in myocytes from normal hearts. This effect was selective for high-gain CICR because low-gain CICR initiated by steps from −40 mV was not affected by amrinone. Surprisingly, we found that amrinone did not exert a positive inotropic effect on either low- or high-gain CICR in myocytes from CM hearts, except in the presence of forskolin, which directly stimulates adenylyl cyclase. Furthermore, we found that amrinone caused a significant inhibition of I\textsuperscript{ca-L} in both normal and CM myocytes. Interestingly, amrinone caused a significant increase in caffeine-releasable SR Ca\textsuperscript{2+} in normal myocytes. Thus, the positive inotropic effect observed in normal myocytes cannot be attributed to stimulation of I\textsuperscript{ca-L} but may involve an increase in SR Ca\textsuperscript{2+} stores.

In the present study, we found that amrinone selectively increased contractions initiated by voltage steps from negative membrane potentials in normal hamster myocytes. We previously reported a similar selectivity for contractions initiated from membrane potentials near the resting potential in guinea pig ventricular myocytes (Xiong et al., 2001). Interestingly, earlier studies demonstrating the positive inotropic effects of amrinone in in vitro cardiac muscle were conducted in multicellular preparations, where contractions were activated by action potentials triggered from the resting potential (Rosenthal and Ferrier, 1982; Kondo et al., 1983; Malecot et al., 1985; Morner and Wohlfart, 1990). In contrast, our present study and our earlier study (Xiong et al., 2001) demonstrate that amrinone has virtually no effect on low-gain CICR initiated from more depolarized membrane poten-
Since we have shown that responses initiated from more negative potentials are initiated by high-gain CICR (Ferrier et al., 2004), our results indicate that amrinone selectively enhances high-gain CICR. Because the main defect in EC coupling in myocytes from CM hamsters can be attributed to a defect in high-gain CICR (Howlett et al., 1999), we hypothesized that amrinone would improve function in CM myocytes. Surprisingly, we found that amrinone was unable to restore high-gain CICR in CM cells. Thus, our results suggest that the lack of inotropic effect of amrinone in CM myocytes occurs because of a defect in CM cells that prevents activation of high-gain CICR.

In myocytes from guinea pig (Xiong et al., 2001) and normal hamster hearts, amrinone caused significant increases in caffeine-releasable SR Ca^{2+} stores. Increased SR Ca^{2+} stores could result from enhancement of SR Ca^{2+} uptake by amrinone. This is likely because a closely related PDE-III inhibitor, milrinone, has been shown to increase SR Ca^{2+} ATPase activity and SR Ca^{2+} uptake in homogenates and SR vesicles from canine ventricular muscle (Yano et al., 2000). Thus, it is possible that the key action for the positive inotropic effect of amrinone in normal myocytes is stimulation of SR Ca^{2+} uptake. However, in myocytes from CM hearts, SR Ca^{2+} stores were not increased by amrinone, which suggests that amrinone does not stimulate SR Ca^{2+} uptake in CM myocytes. The observation that both the inotropic effect and increase in SR Ca^{2+} stores were absent in CM myocytes further suggests that these two events are causally linked.

If amrinone produces its positive inotropic effect in normal myocytes by increasing SR Ca^{2+} stores, it is important to ask why amrinone did not increase CICR initiated by steps from -40 mV. One possibility is that the positive inotropic effect of increased SR stores is counteracted by the significant decrease in the amplitude of I_{Ca-L} observed in the present study. A decrease in I_{Ca-L} would be expected to cause a negative inotropic effect because SR Ca^{2+} release is proportional to the magnitude of I_{Ca-L} in conventional CICR (Bers, 2001). Thus, the combined effects of increased SR Ca^{2+} stores and decreased trigger for release may cancel one another out and result in no net inotropic effect on contractions initiated by conventional CICR. On the other hand, high-gain CICR shows a marked positive inotropic effect. This inotropic effect

![Diagram of I-V relations](attachment:image.png)
may persist because SR Ca\(^{2+}\) release triggered by high-gain CICR is not proportional to the amplitude of I\(_{\text{Ca-L}}\) (Ferrier and Howlett, 2003; Ferrier et al., 2004). Therefore, the positive inotropic effect of increased SR Ca\(^{2+}\) is not countered by the decrease in the magnitude of I\(_{\text{Ca-L}}\).

The mechanism by which amrinone inhibits I\(_{\text{Ca-L}}\) in hamster myocytes has not been established. It is possible that the effects of amrinone on I\(_{\text{Ca-L}}\) are mediated by inhibition of PDE-III. Inhibition of PDE-III increases both cAMP and cGMP (Movsesian, 2002). If elevation of cAMP predominates, an increase in I\(_{\text{Ca-L}}\) would be predicted (Mubagwa et al., 1993). However, if an increase in cGMP predominates in the vicinity of the L-type Ca\(^{2+}\) channel in hamster myocytes, a decrease in I\(_{\text{Ca-L}}\) may result. Elevation of cGMP can inhibit I\(_{\text{Ca-L}}\) through activation of protein kinase G, which can phosphorylate the L-channel at an inhibitory site (Mery et al., 1991; Sumii and Sperelakis, 1995). Alternatively, protein kinase G may activate protein phosphatases, which would dephosphorylate the PKA-phosphorylated site on the L-channel (Shen and Pappano, 2002). Inhibition of I\(_{\text{Ca-L}}\) was greater in normal myocytes compared with CM myocytes. The basis for this difference is unknown, but one could speculate that less inhibition of I\(_{\text{Ca-L}}\) might occur if the levels of PDE-III are less in CM myocytes compared with normal hamster myocytes (Yu et al., 1996). Interestingly, previous studies in guinea pig and ferret ventricular myocytes have shown that amrinone either has no effect on I\(_{\text{Ca-L}}\) or actually increases the magnitude of I\(_{\text{Ca-L}}\) (Kondo et al., 1983; Malecot et al.,

**Fig. 9.** Amrinone increases caffeine-releasable SR Ca\(^{2+}\) stores in normal hamster myocytes. Caffeine (10 mM) was applied for 4 s with a rapid solution switcher as shown in the protocol (top). A, caffeine-induced contracture (top) and associated inward current (bottom) recorded in a normal myocyte in the absence of amrinone. B, representative contracture and current from the same cell in the presence of 300 \(\mu\)M amrinone. C, mean amplitudes of caffeine-induced contractures and integrals of inward Na\(^{+}\)-Ca\(^{2+}\) exchange currents in the absence and presence of amrinone. Although amrinone did not affect mean amplitudes of contractures, it caused a significant increase in inward Na\(^{+}\)-Ca\(^{2+}\) exchange current. Denotes significantly different from control in the absence of drug \((p < 0.05)\); \(n = 5\) myocytes.

**Fig. 10.** Amrinone had no effect on caffeine-releasable SR Ca\(^{2+}\) stores in CM hamster myocytes. Caffeine (10 mM) was applied for 4 s with a rapid solution switcher as shown in the protocol (top). A, caffeine-induced contracture (top) and associated inward current (bottom) recorded in a CM myocyte in the absence of amrinone. B, representative contracture and current from the same cell in the presence of 300 \(\mu\)M amrinone. C, mean amplitudes of caffeine-induced contractures and integrals of inward Na\(^{+}\)-Ca\(^{2+}\) exchange currents in the absence and presence of amrinone. Amrinone did not affect mean amplitudes of contractures or the mean integrals of inward Na\(^{+}\)-Ca\(^{2+}\) exchange currents. \(n = 14\) myocytes.
Thus, effects of amrinone on $I_{\text{Ca-L}}$ appear to vary with species and experimental conditions and are not well correlated with inotropic effects.

A central finding in the present study is that amrinone can increase high-gain CICR in normal myocytes, but these effects are absent in CM myocytes where high-gain CICR is depressed (Howlett et al., 1999). The mechanisms underlying depression of high-gain CICR and the lack of a stimulatory effect of amrinone in myocytes from CM animals are not certain. However, cAMP content is significantly reduced in CM hamster hearts (Wikman-Coffelt et al., 1986). We have shown previously that high-gain CICR is promoted by the cAMP-PKA pathway (Ferrier et al., 1998; Ferrier and Howlett, 2003; Ferrier et al., 2004). Thus, lower levels of cAMP in CM myocytes might lead to weaker activation of high-gain CICR. Indeed, a low concentration of forskolin, which did not in itself increase contraction, restored the stimulatory effect of amrinone in CM myocytes. Thus, it is likely that the absence of high-gain CICR in CM myocytes reflects a reduction in basal cAMP production in CM myocytes. Interestingly, reduced synthesis of cAMP also has been shown to underlie reduced sensitivity and maximum effect of PDE-III inhibitors in trabeculae from hearts of patients in end-stage heart failure (Feldman et al., 1987; Bohm et al., 1988). Because CM hamsters eventually develop heart failure (Jasmin and Proschek, 1982; Hunter et al., 1984), our results suggest that a defect in the cAMP-PKA pathway is present before the onset of overt failure and therefore may contribute to development of failure.

There is evidence that an increase in myofilament $\text{Ca}^{2+}$ sensitivity may contribute to the positive inotropic effects of certain PDE inhibitors (Schmitz et al., 1989), although it is not clear whether this occurs with amrinone. The related PDE-III inhibitor, milrinone, either has no effect or actually decreases sensitivity to $\text{Ca}^{2+}$ (Gwathmey and Morgan, 1985; Alousi et al., 1988). Thus, it is unlikely that sensitization of myofilaments to $\text{Ca}^{2+}$ contributes to the positive inotropic effects of amrinone we observed in normal myocytes.
unlikely that changes in myofilament Ca\(^{2+}\) sensitivity are responsible for the lack of effect of amrinone in CM myocytes because our results suggest that reduced cAMP production is primarily responsible for the insensitivity of CM myocytes to PDE inhibitors. However, down-regulation of PDE, which occurs in heart failure (Smith et al., 1997), also might contribute to the reduced efficacy of PDE inhibitors in CM myocytes.

In summary, our results demonstrate that amrinone selectively enhances high-gain CICR in normal hamster myocytes but is not able to restore high-gain CICR, which is depressed in myocytes from CM hamsters, except in the presence of forskolin which stimulates adenyl cyclase. Furthermore, the positive inotropic effect of amrinone in normal myocytes cannot be attributed to effects on I\(_{\text{Ca-L}}\), which is inhibited in hamster myocytes by this drug. However, the positive inotropic effect may reflect increased SR Ca\(^{2+}\) stores because increased SR stores accompanied the positive inotropic effect in normal myocytes but were absent in CM myocytes. Interestingly, loss in sensitivity to PDE-III inhibitors has been observed in end-stage heart failure in humans (Feldman et al., 1988; Bohm et al., 1988). Because the loss of sensitivity to PDE-III inhibitors in CM hamster myocytes occurred prior to the onset of heart failure, our results suggest that this defect may contribute to the development of heart failure in these animals.

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References


