Na\(^+/\)Ca\(^{2+}\) exchange current \((I_{Na/Ca})\) and sarcoplasmic reticulum Ca\(^{2+}\) release in catecholamine-induced cardiac hypertrophy

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Abstract

Objective: Catecholamines that accompany acute physiological stress are also involved in mediating the development of hypertrophy and failure. However, the cellular mechanisms involved in catecholamine-induced cardiac hypertrophy, particularly Ca\(^{2+}\) handling, are largely unknown. We therefore investigated the effects of cardiac hypertrophy, produced by isoprenaline, on \(I_{Na/Ca}\) and sarcoplasmic reticulum (SR) function in isolated myocytes.

Methods: \(I_{Na/Ca}\) was studied in myocytes from Wistar rats, using descending (+80 to –110 mV) voltage ramps under steady state conditions. Myocytes were also loaded with fura-2 and either field stimulated or voltage clamped to assess \([Ca^{2+}]_i\) and SR Ca\(^{2+}\) content.

Results: Ca\(^{2+}\)-dependent, steady state \(I_{Na/Ca}\) density was increased in hypertrophied myocytes \((P<0.05)\). Ca\(^{2+}\) release from the SR was also increased, whereas resting \([Ca^{2+}]_i\) and the rate of decline of \([Ca^{2+}]_i\) to control levels were unchanged. SR Ca\(^{2+}\) content, estimated by using 10.0 mmol/l caffeine, was also significantly increased in hypertrophied myocytes, but only when myocytes were held and stimulated from their normal resting potential \((-80 \text{ mV})\) but not from \(-40 \text{ mV}\). However, the rate of decline of caffeine-induced Ca\(^{2+}\) transients or \(I_{Na/Ca}\) was not significantly different between control and hypertrophied myocytes. Ca\(^{2+}\)-dependence of \(I_{Na/Ca}\) was examined by comparing the slope of the descending phase of the hysteresis plots of \(I_{Na/Ca}\) vs. \([Ca^{2+}]_i\). was also similar in the two groups of cells.

Conclusion: Data show that SR Ca\(^{2+}\) release and SR Ca\(^{2+}\) content were increased in hypertrophied myocytes, despite an increase in the steady state \(I_{Na/Ca}\) density. The observation that increased SR function occurred only when myocytes were stimulated from \(-80 \text{ mV}\) suggests that Na\(^+\) influx may play a role in altering Ca\(^{2+}\) homeostasis in hypertrophied cardiac muscle, possibly through increased reverse Na\(^+/\)Ca\(^{2+}\) exchange, particularly at low stimulation frequencies.

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1. Introduction

Myocardial contraction is dependent on a transient rise in \([Ca^{2+}]_i\). During normal excitation–contraction coupling (ECC), Ca\(^{2+}\) enters the cell through the L-type Ca\(^{2+}\) channels [1] and to a lesser extent via reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange [2]. Trans-sarcolemmal Ca\(^{2+}\) entry activates the ryanodine receptors (RyR) on the sarcoplasmic reticulum (SR) to release larger quantities of Ca\(^{2+}\) by a process known as calcium-induced calcium release (CICR). However, to maintain a steady state, Ca\(^{2+}\) entering the cell must be matched by extrusion from the cytosol on a beat-to-beat basis. The principal pathways for Ca\(^{2+}\) removal are the forward-mode Na\(^+/\)Ca\(^{2+}\) exchange (Na\(^+\) influx/Ca\(^{2+}\) efflux) and also the sarcolemmal Ca\(^{2+}\)-ATPase. The quantitative contribution of the sarcolemmal Ca\(^{2+}\)-ATPase is, however, thought to be less than 5% thereby making Na\(^+/\)Ca\(^{2+}\) exchange the main route for Ca\(^{2+}\) extrusion in normal cardiac muscle [3,4].

Previous studies have shown that expression of mRNA and the resulting protein responsible for Na\(^+/\)Ca\(^{2+}\) exchange are increased in several animal models of experimental hypertrophy and/or failure [5–13]. However, the functional consequences of increased expression of the Na\(^+/\)Ca\(^{2+}\) exchange protein remain unclear. Some studies have reported blunting of the positive force–frequency relationship to correlate with increased Na\(^+/\)Ca\(^{2+}\) exchange protein and suggested that over-expression of Na\(^+/\)Ca\(^{2+}\) exchange...
may result in enhanced depletion of Ca\(^{2+}\) from the SR and thereby decrease SR Ca\(^{2+}\) release, and hence contractility \([10,11]\). In contrast, some studies have shown the opposite; increased Na\(^{+}/Ca\(^{2+}\) exchange has been associated with increased [Ca\(^{2+}\)\]i and/or contraction \([12,13]\). Differences have also been reported in transgenic mice over-expressing the exchanger \([14,15]\). The reasons for the observed differences in the functional consequences of remodelling the Na\(^{+}/Ca\(^{2+}\) exchange protein during hypertrophy and/or failure remain unclear (see Ref. [16] for review), but may also be complicated by the extent to which other Ca\(^{2+}\) handling proteins, such as L-type Ca\(^{2+}\) channels, sarcolemmal and SR Ca\(^{2+}\)-ATPases, RyR and phospholamban, are also altered. In addition, factors associated with the nature and severity of the hypertrophic insult may also dictate the extent to which these key proteins are up or down regulated.

In particular, the animal species used, the procedure employed to induce hypertrophy as well as the stage and severity of the disease are all likely to impact the functional response \([17]\). The relative importance of each of these parameters therefore needs to be established for each of the specific model(s) of hypertrophy and/or failure that are studied. In the present study, we used a rat model of hypertrophy where cardiac enlargement was produced by injection of isoprenaline, to determine whether the previously reported increase in the expression and translation of mRNA encoding the Na\(^{+}/Ca\(^{2+}\) exchange protein \([7]\) results in increased \(I_{NaCa}\) and whether any such upregulation altered SR function that could account for the known changes in contractility \([18]\).

2. Materials and methods

2.1. Model of hypertrophy, cell isolation and measurement of [Ca\(^{2+}\)\]i, and cell shortening

Catecholamine-induced hypertrophy, which has a distinctly different aetiology from hypertrophy produced by pressure-overload, was produced in male Wistar rats (180–200 g body weight) following injection of isoprenaline hydrochloride (5.0 mg/kg, i.p.) once daily for 7 days. Age and weight-matched cohorts received the same volume of 0.9% saline and served as control. On day 8, animals were stunned by a blow to the head and killed by cervical dislocation in accordance with Home Office Guidelines on the Operation of the Animals (Schedule 1, Scientific Procedures Act 1986), which conform to the NIH Guide for the Care and Use of Laboratory Animals. Myocytes were isolated following collagenase (Type I; Worthington, Lakewood, USA) and protease (Type XIV; Sigma, UK) digestion of the heart and [Ca\(^{2+}\)\]i was determined with fura-2 (R) using the Optoscan monochromator unit (Cairn Research, Faversham, Kent, UK) \([19]\). R was converted to [Ca\(^{2+}\)\]i following in vivo calibration of fura-2 in myocytes permeabilised with the Ca\(^{2+}\) ionophore 4-bromo-A-23187 \([20]\).

Cell shortening was measured at room temperature using a photodiode array edge detection system \([21]\).

2.2. Membrane currents

Myocytes were voltage clamped using the Axopatch 200B patch clamp amplifier linked to a Digidata 1200B A/D interface. Currents were acquired using Clampex 8.1 and analysed with Clampfit 8.1 (all Axon Instruments, Foster, CA). Micropipettes were fabricated from filamented borosilicate glass (GC150TF; Harvard, Kent, UK) to give tip resistances of 1 MΩ (perforated patch clamp) and 2–5 MΩ (ruptured patch clamp) when filled with standard KCl or CsCl filling solutions (see below).

Caffeine-induced membrane currents were measured by the perforated patch clamp method using 240–480 µg/ml amphotericin B (Sigma) as the pore-forming agent. Caffeine (10.0 mmol/l) was applied rapidly by TTL-triggered switching of solenoids, using a computer-controlled solution switcher capable of achieving complete solution exchange in 200–300 ms at 35 ± 1 °C. Conventional whole cell patch clamp was used to determine the steady state \(I_{NaCa}\). Descending voltage ramps were applied from +80 to −120 mV at a rate of 100 mV/s (holding potential −40 mV, stimulation frequency 0.1 Hz). Currents recorded were subsequently plotted against voltage and normalised for membrane capacitance to generate current–voltage (I–V) curves. Membrane capacitance was determined using 10 mV hyperpolarising pulses from holding potential of −40 mV or the analogue controls on the Axopatch 200B amplifier. All [Ca\(^{2+}\)]i and current measurements were undertaken at 35 ± 1 °C.

2.3. Solutions and drugs

The physiological salt solution used for isolation and storage of myocytes contained (mmol/l): NaCl, 130.0; KCl, 5.4; MgCl\(_2\)-6H\(_2\)O, 1.5; NaH\(_2\)PO\(_4\), 0.4; creatine, 10.0; taurine, 20.0; glucose, 10.0; and HEPES, 10.0; titrated to pH 7.25 with NaOH. This was supplemented with EGTA or Ca\(^{2+}\) at different stages of the isolation procedure, as described previously \([19]\).

The external Tyrode solution for field stimulation and perforated patch clamp experiments contained (mmol/l): NaCl, 140.0; KCl, 5.4; MgCl\(_2\)-6H\(_2\)O, 1.0; CaCl\(_2\), 1.0; glucose, 10.0; HEPES, 10.0; adjusted to pH 7.35 with NaOH. For the experiments measuring exchange current, 10.0 µmol/l DIDS (4,4′diisothiocyanatostilbene-2,2′-disulphonic acid disodium) was added to the external solution to inhibit Cl− current and 20 mmol/l CsCl was added to inhibit background potassium currents \([20]\). The internal pipette solution for perforated patch clamp was (mmol/l): K-glutamate, 120.0; KCl, 20.0; NaCl, 10.0; HEPES, 10.0 adjusted to pH 7.2 with KOH.

The external solution for conventional whole cell patch clamp method contained (mmol/l): NaCl, 140.0; MgCl\(_2\)-6H\(_2\)O, 1.0; CsCl, 5.4; CaCl\(_2\), 1.0 or 0.2; glucose, 10.0;
HEPES, 10.0 adjusted to pH 7.35 with NaOH. This solution also contained inhibitors of other current systems including (μmol/l): nifedipine, 15.0 (L-type Ca\textsuperscript{2+} channels); DIDS, 10.0 (Cl\textsuperscript{-} current) and strophanthidin, 50.0 (sodium pump). A total of 1.0 mmol/l BaCl\textsubscript{2} was also added to inhibit any non-selective cationic currents. The internal pipette solution composition for conventional patch clamp was (mmol/l): CsCl, 120.0; TEACl, 20.0; Na\textsubscript{2}ATP, 5.0; CaCl\textsubscript{2}, 3.5; MgCl\textsubscript{2}6H\textsubscript{2}O, 5.0; EGTA, 5.0; Heps, 10.0 adjusted to pH 7.25 with CsOH. Free [Ca\textsuperscript{2+}] in this solution was 0.3 μmol/l, as calculated with the Sliders programme provided free of charge by Dr. Chris Patton, Stanford University. Isoprenaline hydrochloride (Saventrine I.V., Pharmax, UK) ampoules and saline (NaCl 0.9% w/v; B. Braun Medical, UK) were used for injections. All other drugs were purchased from either Sigma or BDH, UK.

2.4. Data analysis

Data are shown as mean ± S.E.M. Comparison between means was made using Student’s t-test for unpaired data.

3. Results

3.1. Catecholamine-induced cardiac hypertrophy

Administration of catecholamines has previously been used to produce cardiac hypertrophy in the rat [18]. These studies showed that heart/body weight ratio, an index of hypertrophy was increased by up to 36%. In the present study, heart/body weight ratio increased by 30%, as described recently [22]. In addition, we also measured membrane capacitance as a more direct index of cell size and found that whole cell capacitance was 150.6 ± 6.0 pF (n = 37) in control cells and 175.3 ± 8.3 pF (n = 55) in hypertrophied myocytes, representing an increase of 16.4% (P<0.05), which is consistent with mild hypertrophy.

3.2. Effects of cardiac hypertrophy on the steady state \textit{I}_{Na/Ca} density

Measurements of \textit{I}_{Na/Ca} have previously been carried out by using (ascending or descending) voltage ramps

![Fig. 1. Effects of catecholamine-induced hypertrophy on steady state \textit{I}_{Na/Ca} density in cardiac myocytes isolated from rat left ventricle. (A) Descending voltage ramps were used to determine the current–voltage (\textit{I}–\textit{V}) relationship before and after switching external [Ca\textsuperscript{2+}] from 1.0 to 0.2 mmol/l to generate the driving force for Ca\textsuperscript{2+} extrusion via the exchanger. (B) \textit{I}–\textit{V} relationship in the presence of 1.0 (i) or 0.2 (ii) mmol/l external Ca\textsuperscript{2+} and then after 5.0 mmol/l Ni\textsuperscript{2+} plus 0.2 mmol/l Ca\textsuperscript{2+} (iii). (C) \textit{I}–\textit{V} curves isolated by subtraction of those obtained in the presence of Ni\textsuperscript{2+} from those obtained in the presence of either 1.0 (i) or 0.2 (ii) mmol/l Ca\textsuperscript{2+}. (D) Currents elicited by the decrease in external [Ca\textsuperscript{2+}] from 1.0 to 0.2 mmol/l in myocytes from control and hypertrophied hearts. (E) Mean (± S.E.M.) Ca\textsuperscript{2+}-dependent \textit{I}_{Na/Ca} at −110 mV in control (n = 11) and hypertrophied (n = 8) myocytes (P<0.05) from at least three separate animals in each group.](image)

![Fig. 2. Effects of cardiac hypertrophy on Ca\textsuperscript{2+} release from the SR in isolated left ventricular myocytes. (A) Representative time-averages of 30 individual Ca\textsuperscript{2+} transients. (B) Mean (± S.E.M.) resting (hatched bar) and peak [Ca\textsuperscript{2+}] (open bar) in control and hypertrophied myocytes. (C) The time constant (\textit{t}) for the rate of decline of [Ca\textsuperscript{2+}] to resting levels. n = 24 in control and 18 in hypertrophied myocytes.](image)
(e.g. Fig. 1A), where Ni\(^{2+}\) (an inhibitor of Na\(^+/Ca^{2+}\) exchange) was used to isolate \(I_{\text{Na/Ca}}\) (e.g. Ref. [23]). However, Ni\(^{2+}\) has the potential to inhibit other current types. The use of Ni\(^{2+}\) alone could therefore potentially confound measurements of \(I_{\text{Na/Ca}}\). We therefore used changes in extracellular [Ca\(^{2+}\)] to generate the driving force for Ca\(^{2+}\) extrusion via the exchanger and hence generate an inward current carried by the exchanger [24].

Fig. 1B shows superimposed \(I–V\) curves to demonstrate that 5.0 mmol/l Ni\(^{2+}\) inhibited the current elicited by the decrease in external [Ca\(^{2+}\)], confirming the current to be sensitive to changes in external [Ca\(^{2+}\)], and also to inhibition by Ni\(^{2+}\), features that are well-known characteristics of \(I_{\text{Na/Ca}}\). Also consistent with the expected properties of the \(I_{\text{Na/Ca}}\) was the observation that alterations in the external [Ca\(^{2+}\)] produced a shift in the \(I–V\) curve so that the reversal potential was more positive when external [Ca\(^{2+}\)] was 0.2 mmol/l. The expected reversal potentials at the two Ca\(^{2+}\) concentrations were −5.0 mV at 1.0 mmol/l and +36 mV at 0.2 mmol/l, a shift of 41 mV. The expected reversal potentials at the two Ca\(^{2+}\) concentrations were −20 and +15 mV at 1.0 and 0.2 mmol/l, respectively, exhibiting a shift of 35 mV (Fig. 1C). The small discrepancies between the calculated and observed values could result from the fluctuations in the ramp currents that can occur when measuring such small currents and also from the possibility that dialysis of the pipette solution constituents with the interior of the cell was incomplete. A discrepancy of only a 5-mV shift in the reversal potentials between the observed and the calculated values at the two Ca\(^{2+}\) concentrations is therefore within reasonable error limits. Consequently, the difference currents recorded at the two external [Ca\(^{2+}\)] were taken as the steady state \(I_{\text{Na/Ca}}\). Fig. 1D and E shows that such currents under steady state conditions are generally low in normal (control) rat cardiac myocytes. However, in hypertrophied myocytes, \(I_{\text{Na/Ca}}\) density (quantified at −110 mV) was increased (\(P<0.05\)).

3.3. Effects of catecholamine-induced cardiac hypertrophy on SR function

Fig. 2A shows time-averaged Ca\(^{2+}\) transients, in myocytes field stimulated at 0.5 Hz, to demonstrate that systolic [Ca\(^{2+}\)] was increased (\(P<0.05\)) in hypertrophied myocytes whereas resting [Ca\(^{2+}\)] remained unchanged (B). The time constant (\(\tau\)) for the decline of the Ca\(^{2+}\) transient to resting values, fitted with a single exponential, was also not significantly altered (Fig. 2C).

![Graphs](https://via.placeholder.com/150)

Fig. 3. Effects of hypertrophy on the Ca\(^{2+}\) content of the SR in rat left ventricular myocytes. Myocytes were field stimulated at 0.5 Hz by a train of 30 conditioning pulses and then rapidly exposed to 10.0 mmol/l caffeine. (A–B) Original fura-2 fluorescence tracings from control and hypertrophied myocytes in response to two successive exposures to caffeine. (C) Mean (± S.E.M.) resting (hatched bars) and peak (open bars) values of [Ca\(^{2+}\)] obtained in response to caffeine. (D) Integral of the caffeine-induced Ca\(^{2+}\) transient. (E) Time constant (\(\tau\)) for the decline of the caffeine response. \(n=41–44\) for each group.
Given that Ca\(^{2+}\) influx via \(I_{\text{Ca}}\) has previously been shown to be unchanged in this model of hypertrophy [25], one possible mechanism for the observed increase in SR Ca\(^{2+}\) release could be increased SR Ca\(^{2+}\) content, possibly by increasing the gain function of CICR [26]. Application of caffeine causes Ca\(^{2+}\) contained in the SR to be liberated and then extruded across the cell membrane, mainly by the exchanger so that measurement of caffeine-induced Ca\(^{2+}\) transients or membrane currents can be used to estimate the Ca\(^{2+}\) load of the SR. Field stimulation of myocytes with a train of 30 conditioning pulses (at 0.5 Hz) was paused for 5 seconds before 10.0 mmol/l caffeine was applied, using an automated solution switcher (Fig. 3A–B). Peak amplitude of the caffeine-induced Ca\(^{2+}\) transient (Fig. 3C) and also the integral of the response (Fig. 3D) were increased (\(P<0.05\)) in hypertrophied myocytes, confirming that Ca\(^{2+}\) content of the SR was indeed increased. However, the time-constant for the decline of the caffeine-induced Ca\(^{2+}\) transients was not significantly different between the two groups of myocytes (\(t=1180 \pm 48\) ms in control, \(n=42\) and 1209 ± 68 ms in hypertrophy, \(n=43\); Fig. 3E).

In addition to the use of fura-2 (Fig. 3), we also determined caffeine-induced inward \(I_{\text{Na/Ca}}\) as another means of determining the SR Ca\(^{2+}\) content. Fig. 4A shows recordings of membrane currents obtained using the perforated patch clamp technique (to minimise internal dialysis). In myocytes held and stimulated from −80 mV, peak amplitude (Fig. 4B) and the integral of \(I_{\text{Na/Ca}}\) (Fig. 4D) elicited by caffeine were increased in hypertrophied myocytes (\(P<0.05\)). However, the time constant for decline of the \(I_{\text{Na/Ca}}\) to resting values was

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**Fig. 4.** Effects of changes in the holding potential on Ca\(^{2+}\) content of the SR in control and hypertrophied myocytes. (A) Original records of caffeine-induced \(I_{\text{Na/Ca}}\) to assess the Ca\(^{2+}\) content of the SR in control and hypertrophied myocytes held at different holding potentials. (B and C) Peak amplitudes of \(I_{\text{Na/Ca}}\) elicited by caffeine after stimulation from, either −80 or −40 mV, respectively. (D and E) Integrals of \(I_{\text{Na/Ca}}\) elicited by caffeine after stimulation from −80 or −40 mV, respectively. Control myocytes (open bars), hypertrophied myocytes (solid bars), mean ± S.E.M. from at least three hearts in each group.
Fig. 5. Simultaneous measurement of $I_{Na/Ca}$ and $[Ca^{2+}]_i$ in control and hypertrophied myocytes: comparison of the $Ca^{2+}$-dependence of $I_{Na/Ca}$. (A) $Ca^{2+}$ transient (top trace) and $I_{Na/Ca}$ (lower trace) elicited by 10.0 mmol/l caffeine. (B–E) Caffeine-induced $[Ca^{2+}]_i$ and the accompanying $I_{Na/Ca}$ re-plotted both in control (B and D) and hypertrophied (C and E) myocytes clamped to holding potentials of $-40$ or $-80$ mV. (F and G) Mean (± S.E.M.) slope of the relationship between $I_{Na/Ca}$ and $[Ca^{2+}]_i$ during the descending phase of the caffeine-induced $Ca^{2+}$ transient to compare $Ca^{2+}$-dependance of $I_{Na/Ca}$ in control (open bars) and hypertrophied (filled bars) myocytes.
only slightly but not significantly accelerated in hypertrophied myocytes (1022 ± 225 ms, n = 5 in control and 883 ± 91, n = 7 in hypertrophied myocytes). In contrast, when the same myocytes were stimulated from a holding potential of −40 mV, peak amplitude (Fig. 4C) and the integral (Fig. 4E) of ISNa/Ca were no longer significantly different between control and hypertrophied myocytes (see Section 4). The τ for the decline of ISCaNa was 1031 ± 98 ms in control and 900 ± 105 ms (n = 3, ns).

A recent study [9] has shown that, in a canine model of hypertrophy, ISNa/Ca was increased only in myocytes where [Ca2+]i was permitted to change but not when it was buffered. These data were used to suggest that [Ca2+]i may alter the physiological regulation of the Na+/Ca2+ exchange mechanism during hypertrophy (e.g. by alterations in the 3:1 stoichiometry). Increased ISNa/Ca for given [Ca2+]i, has also been reported for a rabbit model of heart failure [11]. However, Fig. 1 shows that, in the present study, ISNa/Ca density was increased under conditions where [Ca2+]i was buffered. However, in view of this earlier study [9], we also measured both ISNa/Ca and [Ca2+]i simultaneously. Fig. 5A shows recordings of [Ca2+]i, and the accompanying membrane current elicited by 10 mmol/l caffeine. These data were re-plotted in Fig. 5B–E to compare the relationship between ISNa/Ca and [Ca2+]i in control and hypertrophied myocytes. The arrows in Fig. 5B–E indicate the increase in ISNa/Ca as [Ca2+]i approaches the peak value. It is clear from the hysteresis loops in these figures that ISNa/Ca observed at any given [Ca2+]i, is greater as the Ca2+ transient approaches the peak value (i.e. the ascending part of the loop) compared to the ISNa/Ca during the decline of the Ca2+ transient. The most favoured explanation for the observed hysteresis is that subsarcolemmal [Ca2+]i, sensed by the exchanger during the ascending phase of the loop is probably higher than that actually reported by fura-2, which represents global [Ca2+]i across the whole cell [27]. However, during the descending phase of the hysteresis loop, ISNa/Ca and [Ca2+]i are closely related so that there is a linear relationship between the two parameters. Consequently, it is possible to examine the Ca2+-dependence of ISNa/Ca during the descending phase of the hysteresis loop. Fig. 5F–G shows that the slope of Ca2+-dependence of ISNa/Ca was not significantly different between control and hypertrophied myocytes (n = 3), whether the myocytes were stimulated from a holding potential of −40 or −80 mV. The Ca2+-dependence of ISNa/Ca was therefore not affected by the levels of [Ca2+]i, which were higher at −80 mV, particularly in hypertrophied myocytes.

These data could be interpreted to suggest that increased expression of the Na+/Ca2+ exchange protein [7] and the concomitant increase in steady state ISNa/Ca (Fig. 1 above) may not necessarily result in depletion of the SR during hypertrophy. The normal Na+/Ca2+ exchange protein density may not therefore be rate limiting in rat cardiac myocytes. To determine whether this might be the case, we compared the rate of decline of caffeine-induced Ca2+ transients in normal control myocytes at two different Ca2+ loads of the SR. β-Adrenergic receptor stimulation was used to increase SR Ca2+ content as described previously [19]. Fig. 6A shows that Ca2+ content of the SR is markedly increased in the presence of 10.0 mmol/l isoprenaline. However, when the Ca2+ transients were normalised to the same amplitude (Fig. 6B), the time course of decline of [Ca2+]i, to resting levels was unchanged in the presence of isoprenaline, both in control (n = 7; Fig. 6C) and hypertrophied myocytes (n = 9; Fig. 6D).

A previous [13] study showed that in hypertrophied canine ventricular myocytes, where ISNa/Ca and SR Ca2+ release were also increased, the positive shortening-frequency relationship was blunted. We therefore tested whether this was also the case in the rat model used in the present study. Fig. 7 shows that the positive inotropic effect of increasing stimulation frequency was observed in control but not in hypertrophied myocytes, whereas the rate of relaxation, estimated by measuring the time taken for cell

![Fig. 6. Effect of β-adrenergic receptor stimulation on the rate of decline of caffeine-induced SR Ca2+ release in normal and hypertrophied rat ventricular myocytes. (A) Caffeine-induced Ca2+ transients in the absence and presence of 10.0 mmol/l isoprenaline. (B) Normalised caffeine-induced Ca2+ transients superimposed to compare the time course of decline of [Ca2+]i. (C) Mean (± S.E.M.) time constants, fitted with a single exponential, for the decline of caffeine-induced Ca2+ transients in control (C) and hypertrophied (D) myocytes in the absence (open bars) and presence (hatched bars) of isoprenaline.](image)
to relax to 50% of the maximum \(T_{1/2}\) was similar in the two groups of cells (ns; not shown).

4. Discussion

The main finding of the present study was that steady state \(I_{Na/Ca}\) density was increased in myocytes from hypertrophied hearts. However, increased \(I_{Na/Ca}\) did not cause depletion of \([Ca^{2+}]_i\) from the SR. Instead, the amplitude of Ca\(^{2+}\) transients was significantly increased. Ca\(^{2+}\) content of the SR was also increased in myocytes from hypertrophied hearts but only when myocytes were stimulated from their normal resting potential (≈ −80 mV) and not when the membrane was clamped to a holding potential of −40 mV. \([Ca^{2+}]_i\) dependence of \(I_{Na/Ca}\), determined during the response to 10.0 mmol/l caffeine, was similar in control and hypertrophied myocytes, implying that operation of the Na\(^+\)/Ca\(^{2+}\) exchange mechanism was unaffected by hypertrophy. Consistent with an overloaded SR, increasing the stimulation frequency abolished the positive force–frequency relationship in hypertrophied myocytes. The following discussion considers some of the possible mechanisms that could account for the observed results and evaluates the broader significance of these data to myocardial dysfunction and the genesis of arrhythmias.

4.1. Mechanisms responsible for alterations in Ca\(^{2+}\) handling during catecholamine-induced hypertrophy

A previous study [7] has reported that mRNA and the levels of the Na\(^+\)/Ca\(^{2+}\) exchange protein were both increased in rats after 3 and 7 days of isoprenaline administration and could therefore potentially account for the observed increase in the steady state \(I_{Na/Ca}\) (Fig. 1). However, the consequences of upregulating the exchanger, particularly with respect to Ca\(^{2+}\) handling by the SR, are unclear. A previous study has shown that \(I_{Ca}\) density and the activation/inactivation kinetics of the L-type Ca\(^{2+}\) channels were unaltered in this model of hypertrophy [25] and are therefore unlikely to contribute to the observed increase in SR Ca\(^{2+}\) release. However, SR Ca\(^{2+}\) content is also an important determinant of SR Ca\(^{2+}\) release and could therefore potentiate SR Ca\(^{2+}\) release by increasing the gain function of CICR and thus explain why SR Ca\(^{2+}\) release is increased, despite there being no change in the density or the kinetics of \(I_{Ca}\).

The primary mechanism(s) responsible for increasing SR load are unknown but may be related to alterations in \([Na^+]_i\). A recent study [22] has shown that \([Na^+]_i\) is increased in this model of hypertrophy, either through inhibition of the Na\(^+\)–K\(^+\)-ATPase (Na\(^+\)-pump) that normally extrudes Na\(^+\) or by increased Na\(^+\) influx via other mechanisms (e.g. \(I_{Na}\) or reverse Na\(^+\)/Ca\(^{2+}\) exchange). \(I_{Na}\) has, indeed, been shown to be increased in a guinea-pig model of hypertrophy [5] and \([Na^+]_i\) has also been shown to be elevated in yet another model of hypertrophy in the guinea-pig [28]. Given that SR Ca\(^{2+}\) release has previously been shown to correlate with Na\(^+\)/Ca\(^{2+}\) exchange and \([Na^+]_i\) in normal myocytes [29], it seems likely that elevated \([Na^+]_i\) could increase the electrochemical gradient for reverse Na\(^+\)/Ca\(^{2+}\) exchange, particularly if the density of the Na\(^+\)/Ca\(^{2+}\) exchange protein is also increased. Consequently, increased Ca\(^{2+}\) entry could occur via reverse exchange at the beginning of the beat [30] and enhance the trigger function of Ca\(^{2+}\) for CICR as well as loading the SR with more Ca\(^{2+}\). The observation that increased SR load did not occur when myocytes were held and stimulated from a holding potential of −40 mV (i.e. when \(I_{Na}\) is absent) implies that alterations in Na\(^+\) influx may also be involved in increasing Ca\(^{2+}\) content of the SR.
possibly by increasing the driving force for reverse exchange. That the SR Ca\(^{2+}\) content is increased, at the low frequencies used in the present study, is also consistent with an overloaded SR and the possibility that [Na\(^+\)] may also be elevated.

4.2. Upregulation of Na\(^+\)/Ca\(^{2+}\)exchange and the time course of Ca\(^{2+}\) extrusion

Although the preceding section may explain how an increase in the expression of the Na\(^+\)/Ca\(^{2+}\) exchange protein and the concomitant increase in the \(I_{\text{Na/Ca}}\) might be compatible with increased SR function in hypertrophied myocytes, it does not explain why the time course of the decline of [Ca\(^{2+}\)] during a twitch or in response to caffeine is not accelerated (Figs. 2–5) if the density of the exchanger is increased. One possible explanation is that the basal levels of exchanger are not rate limiting so that the relationship between the rate of Ca\(^{2+}\) efflux and the levels of the Na\(^+\)/Ca\(^{2+}\) exchanger are not rate limiting so that the relationship data in Fig. 6 show that, even when the SR Ca\(^{2+}\) content was increased, at the low frequencies used in the present study, is also consistent with an overloaded SR and the possibility that [Na\(^+\)] may also be elevated.

4.3. Role of \(I_{\text{Na/Ca}}\) in prolongation of the action potential and arrhythmogenesis during hypertrophy and heart failure

Although increased \(I_{\text{Na/Ca}}\) density is widely believed to generate the transient inward current (\(I_d\)) that could potentially contribute to prolongation of the action potential and generation of delayed after depolarisations (DADs) in the hypertrophied or failing myocardium, data from the present study suggest that in catecholamine-induced hypertrophy, this is unlikely to result directly from the increased expression of the exchanger protein alone but rather as a secondary consequence to the increase in [Ca\(^{2+}\)], and other electrophysiological changes such as down regulation of the transient outward current (\(I_{\text{to}}\)) [33]. Increased SR function, in turn, may result from an increased reverse \(I_{\text{Na/Ca}}\) that would be expected if [Na\(^+\)] was elevated (above). Increased reverse Na\(^+\)/Ca\(^{2+}\) exchange has also been suggested to be responsible for enhanced SR function in other models of hypertrophy [12,13]. The intricate inter-dependence of Na\(^+\) and Ca\(^{2+}\) under physiological conditions means that identifying the primary defect during hypertrophy still remains to be established in this model. The effects of hypertrophy on the mechanisms responsible for Na\(^+\) homeostasis are therefore particularly important targets for future study.

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References


Terracciano CMN, De Souza AI, Philipson KD, MacLeod KT. Na⁺–Ca²⁺ exchange and sarcoplasmic reticular Ca²⁺ regulation in ventricular myocytes from transgenic mice overexpressing the Na⁺–Ca²⁺ exchanger. J Physiol 1998;512:651–67.


Trafford AW, Diaz ME, Eisner DA. Ca-activated chloride current and Na–Ca exchange have different time courses during sarcoplasmic reticulum Ca release in ferret ventricular myocytes. Pflugers Arch 1998;435:743–5.


