$\text{IPF} - 5$

A mechanism distinct from the L-type Ca current or Na–Ca exchange contributes to Ca entry in rat ventricular myocytes

P. Kupittayanant[1], A.W. Trafford, M.E. Díaz[2], D.A. Eisner[*]

Unit of Cardiac Physiology, University of Manchester, 3.18 Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, UK

Abstract

The aim of this paper was to characterize the pathways that allow Ca^{2+} ions to enter the cell at rest. Under control conditions depolarization produced an increase of intracellular Ca concentration ($[Ca^{2+}]_i$) that increased with depolarization up to about 0 mV and then declined. During prolonged depolarization the increase of $[Ca^{2+}]_i$ decayed. This increase of $[Ca^{2+}]_i$ was inhibited by nifedipine and the calculated rate of entry of Ca increased on depolarization and then declined with a similar timecourse to the inactivation of the L-type Ca current. We conclude that this component of change of $[Ca^{2+}]_i$ is due to the L-type Ca current. If intracellular Na was elevated then only part of the change of $[Ca^{2+}]_i$ was inhibited by nifedipine. The nifedipine-*insensitive* component increased monotonically with depolarization and showed no relaxation on prolonged depolarization. This component appears to result from Na–Ca exchange (NCX). When the L-type current and NCX were both inhibited (nifedipine and Na-free solution) then depolarization *decreased* and hyperpolarization *increased* $[Ca^{2+}]_i$. These changes of $[Ca^{2+}]_i$ were unaffected by modifiers of B-type Ca channels such as chlorpromazine and AlF_3 but were abolished by gadolinium ions. We conclude that, in addition to L-type Ca channels and NCX, there is another pathway for entry of Ca^{2+} into the ventricular myocyte but this is distinct from the previously reported B-type channel.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Calcium; Heart; Channel

1. Introduction

There is much information about the sarcolemmal Ca fluxes, which occur during systole. Depolarization allows calcium to enter via the L-type Ca current and there may also be influx on Na–Ca exchange (NCX). Ca will be pumped out of the cell largely on the sarcolemmal Na–Ca exchange with some contribution from extrusion on the sarcolemmal Ca-ATPase (PMCA). These sarcolemmal fluxes serve both to trigger Ca release from the SR and to maintain the loading of the SR with Ca (see Ref. [1] for review).

In contrast to this knowledge of systolic fluxes, comparatively little is known as to what happens at rest. The resting Ca concentration of a cardiac cell is of the order of 100 nM so, given that both NCX and PMCA will be producing some Ca efflux, there must presumably be Ca entry into the cell. The source of this Ca entry is not clearly established and there are at least three possibilities: (1) Ca entry via L-type Ca channels. Although the L-type channel only appears to activate on depolarization to potentials positive to −60 mV, it is possible that infrequent openings at more negative potentials may contribute. (2) Ca entry on NCX may contribute but, since the reversal potential of the exchange is generally thought to be more positive than the resting potential, it is likely that such an entry will be less than the efflux produced by the exchange. (3) There may be another Ca entry pathway. For example, a background ("B-type") Ca entry has been reported

[*] Corresponding author. Tel.: +44 161 275 2702; fax: +44 161 275 2703.
  E-mail address: eisner@man.ac.uk (D.A. Eisner).
  [1] Present address: School of Animal Production Technology, Suranaree University of Technology, University Avenue, Naknon Ratchasima 30000, Thailand.
  [2] Present address: Veterinary Biomedical Sciences, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH, United Kingdom.

[2]. These channels are activated by chlorpromazine [3] and have recently been suggested to result from channel activity of the PMCA [4,5]. In addition to these channels, in the heart there are stretch-dependent [6,7] and possibly store-operated channels [8]. Much recent work in a variety of tissues has also examined the role of the transient receptor potential (TRP) family of channels [9,10] and it is also possible that these may contribute to background Ca influx.

Given this background, the aim of the present study was to investigate the effects of membrane potential on $[Ca^{2+}]_i$ while inhibiting selectively one or both of the L-type channel and NCX. In order to see the effects of sarcolemmal fluxes, Ca handling by the sarcoplasmic reticulum was inhibited. The results show a significant contribution from a third pathway but are not consistent with a role for B-type Ca channels.

2. Methods

Rat ventricular myocytes were isolated using a collagenase and protease technique as previously described [11]. Rats were killed by stunning and cervical dislocation. All experiments and animal care were in accordance with the provisions of the Animals (Scientific Procedures) Act 1986. The experimental methods have been described previously [12]. Briefly, cells were loaded with the acetoxymethyl (AM) ester of the fluorescent indicator fluo-3 and voltage clamped using the perforated patch technique. To overcome access resistance problems associated with this method, the switch clamp facility of the Axoclamp 2B (Axon Instruments Inc., USA) amplifier was used.

2.1. Solutions

The control superfusate contained (in mM): NaCl, 134; KCl, 4; CaCl$_2$, 1; MgCl$_2$, 1.2; HEPES, 10; Glucose, 11. In all experiments the sarcoplasmic reticulum was disabled with thapsigargin (1 μM) and ryanodine (1 μM). In some experiments nifedipine, 10 μM was used in order to block L-type Ca channel. Na-free solutions were produced by equimolar replacement of Na by Li. The pipette solution contained (in mM): KCH$_3$O$_3$S, 125; KCl, 20; HEPES, 10; MgCl$_2$, 5; K$_2$EGTA, 0.1. NaCl was added to this solution at the desired concentration. Amphotericin-B was dissolved in DMSO (60 mg ml^{-1}) and added to the pipette solution to a final concentration of 240 μg ml^{-1} immediately before use.

All experiments were carried out at room temperature (23 °C).

2.2. Calculation of Ca fluxes

In order to estimate the fluxes of Ca across the surface membrane, changes of $[Ca^{2+}]_i$ were converted to total Ca $[Ca_T]$ using the Ca buffering properties of the cell measured during application of caffeine [13]. The records of $[Ca_T]$ were then smoothed and differentiated using a 29-point recursive

linear regression around the point of interest. Data are presented as mean ± S.E.M.

3. Results

The experiment of Fig. 1A shows the effects of prolonged changes of membrane potential on $[Ca^{2+}]_i$. In this, and all the other experiments in this paper, the sarcoplasmic reticulum was disabled with thapsigargin (1 μM) and ryanodine (1 μM). Depolarization produced a rise of $[Ca^{2+}]_i$ to a peak which was followed by a slow decay to a steady level. The amplitude of these responses increased from −20 to 0 mV and then decreased with further depolarization. The addition of nifedipine (10 μM) removed all these changes of $[Ca^{2+}]_i$ on depolarization. The dependence of peak $[Ca^{2+}]_i$ on voltage is shown in Fig. 1B.

The fact that nifedipine abolishes the changes of $[Ca^{2+}]_i$ produced by depolarization suggests that the L-type Ca current alone is responsible for the increase of $[Ca^{2+}]_i$ on depolarization. We have therefore compared the current with the calculated rate of increase of total Ca concentration. Panel (a) of Fig. 2 shows the timecourse of $[Ca^{2+}]_i$ during a depolarization from −40 to 0 mV. The complete inhibition by nifedipine is obvious. Panel (b) shows changes of *total* Ca (Ca_T) calculated from the assumed buffering properties (see Section 2). The control data show that the increase of $[Ca_T]$ is at first rapid and then slow. This is emphasised by panel (c) which plots $d[Ca_T]/dt$. In panel (d), $d[Ca_T]/dt$ is inverted and superimposed on the membrane current record. To a first approximation the calculated rate of rise of $[Ca_T]$ has the same timecourse as that of the L-type Ca current.

The experiments described above were performed with 10 mM Na in the patch pipette. In subsequent experiments
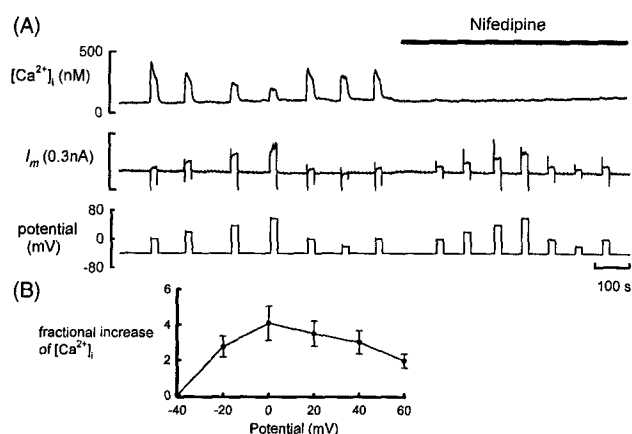

Fig. 1. The effects of prolonged steps of membrane potential on $[Ca^{2+}]_i$. (A) Original data. Traces show: top, $[Ca^{2+}]_i$; middle, current; bottom, membrane potential. Nifedipine (10 μM) was applied for the period shown. The pipette contained 10 mM Na. In this, and all the other experiments in this paper, the sarcoplasmic reticulum was disabled with thapsigargin (1 μM) and ryanodine (1 μM). (B) Mean data showing the peak fractional increase of $[Ca^{2+}]_i$ as a function of membrane potential (14 cells).
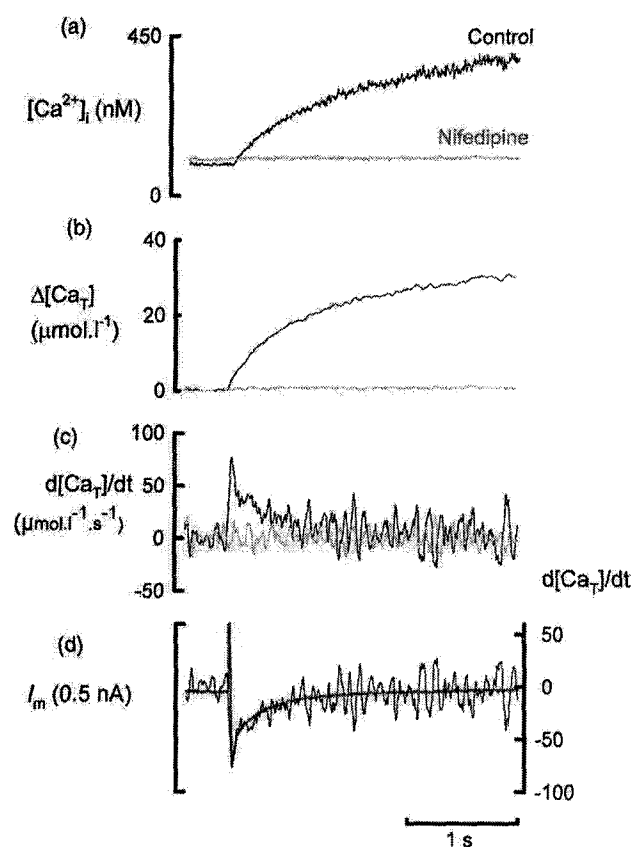
Fig. 2. Comparison of the nifedipine-sensitive Ca entry with that of the L-type Ca current. Data were obtained from the initial 20 s of a depolarizing pulse from −40 to 0 mV. Data are shown for pulses obtained in both control and nifedipine (10 μM). Panels show: (a) $[Ca^{2+}]_i$; (b) calculated change of total Ca ($\Delta[Ca_T]$); (c) $d[Ca_T]/dt$; (d) membrane current (red) superimposed on the inverted $d[Ca_T]/dt$. For (a)–(c) the black trace is the control and the green is nifedipine. For (d) only the control is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Fig. 3. The effects of membrane potential on $[Ca^{2+}]_i$ under conditions of elevated $[Na^+]_i$. Traces show: top, $[Ca^{2+}]_i$; middle, current; bottom, membrane potential. Nifedipine (10 μM) was applied for the period shown. The pipette contained 30 mM Na.

we have examined the effect of changing this Na concentration. In Fig. 3 there was 30 mM Na in the pipette. Under these conditions the $[Ca^{2+}]_i$ response was fairly constant in amplitude for depolarizations to potentials from −20 to +50 mV. Furthermore, the secondary decay of $[Ca^{2+}]_i$, although still present was less marked than was the case for experiments such as Fig. 1A. A more striking difference was seen when nifedipine was added when an increase of $[Ca^{2+}]_i$ with depolarization persisted. This increase of $[Ca^{2+}]_i$ increased monotonically with the degree of depolarization and never showed a secondary decrease during the depolarization.

We have analyzed the rising phase of these responses in more detail in Fig. 4A. The calculated $[Ca_T]$ (b) shows a biphasic rise in control and a much slower and linear rise in nifedipine (well fit by a straight line). The nifedipine-sensitive increase (red) rises exponentially to a plateau. Panel (c) shows the calculated rate of rise of $[Ca_T]$. The nifedipine sensitive component peaks and then decays to zero. In contrast, the
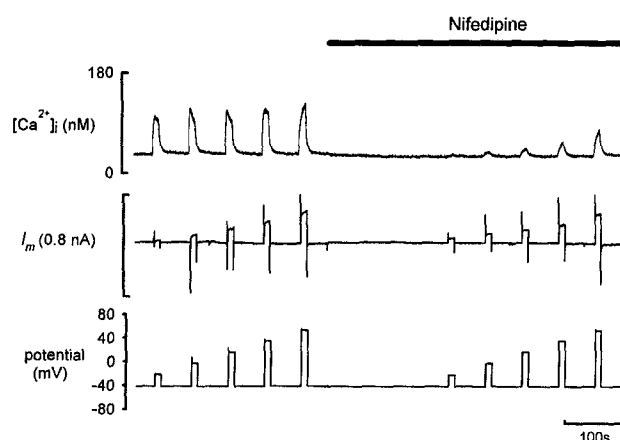
value in nifedipine (calculated from the linear regression as the raw data are too noisy) shows a constant small positive $d[Ca_T]/dt$. As was the case for Fig. 2, panel (d) shows that the nifedipine-sensitive entry has a similar timecourse to that of the L-type Ca current.

The data of Fig. 4B show the voltage dependence of the nifedipine-sensitive and insensitive components of Ca entry and also that of the peak L-type Ca current. It is clear that the voltage dependence of the nifedipine-sensitive Ca entry is similar to that of the L-type Ca current whereas the nifedipine-insensitive component increases monotonically with depolarization.

The experiments described above suggest that NCX can play a role depending on the transmembrane Na gradient. In subsequent experiments, therefore, we have removed the role of NCX by superfusing the cells with a Na-free solution and having no Na in the pipette. A typical result is shown in Fig. 5A. Initially, increasing depolarization increases the magnitude of the rise of $[Ca^{2+}]_i$ but with further depolarization a maximum is obtained and depolarization to potentials above +50 mV produces only a small response. This bell-shaped dependence on voltage is to be expected for Ca entry via the L-type current. When nifedipine was added, the increase of $[Ca^{2+}]_i$ on depolarization disappeared and was replaced by a decrease. This decrease of $[Ca^{2+}]_i$ on depolarization in nifedipine is also seen in Fig. 5A. In fact, even in the absence of nifedipine sufficient depolarization can decrease $[Ca^{2+}]_i$ (see the 5th and 6th pulses in Fig. 5B). In addition Fig. 5B also shows that, in these Na-free solutions, in both the presence and absence of nifedipine, hyperpolarization *increases* $[Ca^{2+}]_i$. Mean data from five cells are shown in Fig. 6. It is clear that there are two components. (1) A nifedipine-sensitive increase of $[Ca^{2+}]_i$ observed between about −40 and +60 mV and (2) a nifedipine-insensitive change of $[Ca^{2+}]_i$ such that hyperpolarization increases and depolarization decreases $[Ca^{2+}]_i$.
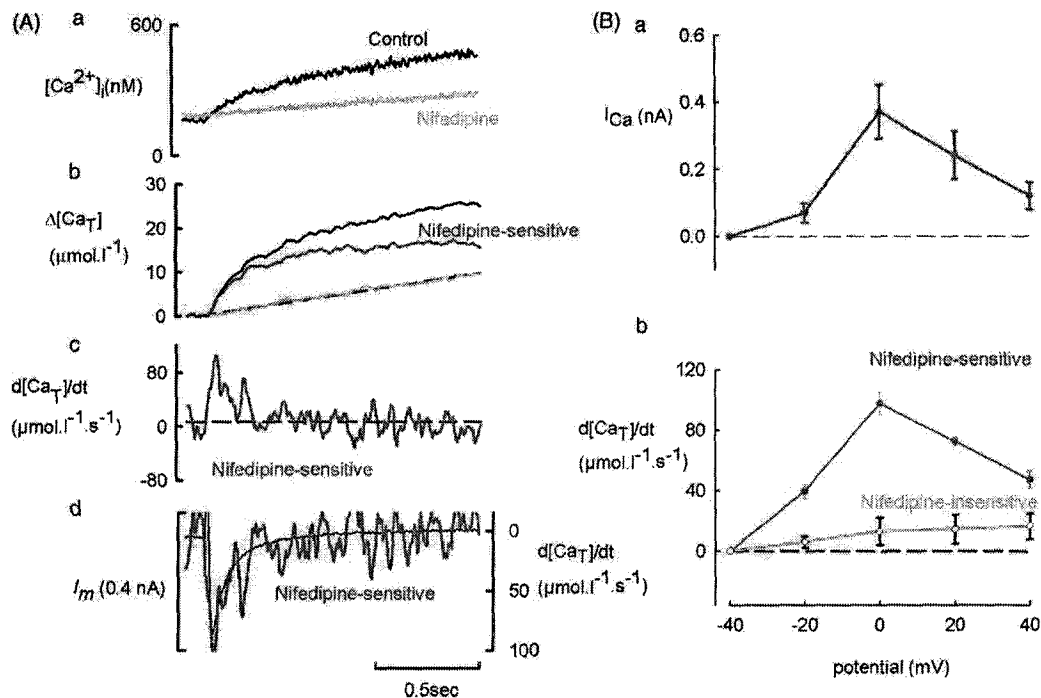
Fig. 4. Comparison of the nifedipine-sensitive Ca entry with the L-type Ca current. (A) Original data obtained from the initial 15 s of a depolarizing pulse from −40 to 0 mV. Data are shown for pulses obtained in both control (black) and nifedipine (10 μM, green). Panels show: (a) $[Ca^{2+}]_i$; (b) $[Ca_T]$, total Ca, note that the nifedipine data (green) have been fitted to a straight line (blue); (c) $d[Ca_T]/dt$. The nifedipine-sensitive (red) data in (b) and (c) have been calculated from the raw data while the nifedipine trace is from the regression line of (b); (d) membrane current superimposed on the inverted nifedipine-sensitive $d[Ca_T]/dt$. (B) Mean data (five cells); (a) shows the amplitude of the L-type Ca current and (b) the maximum rate of rise of $[Ca_T]$ (separated into both nifedipine-sensitive and insensitive components). The data were obtained from pulses to the potentials indicated from a holding potential of −50 mV. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

3.1. Characterization of I_{Ca} and NCX-independent route of Ca entry

The previous data suggest that there is a Ca influx pathway in addition to the L-type current and NCX. In subsequent work we have attempted to investigate the origin of this pathway with various pharmacological interventions.

B-type channels have been reported to be activated by chlorpromazine (CPZ) [3]. We have therefore investigated the effect of 50 μM CPZ. We found that, in contrast to the increase expected from an activator, CPZ decreased the effects of membrane potential on $[Ca^{2+}]_i$ (Table 1). AlF$_3$ has been reported to block B-type channels [4] but, again, we found (Table 1) no significant effect on the voltage dependent changes of $[Ca^{2+}]_i$.

Another possible candidate is the T-type channel. This can be inhibited by low concentrations of Ni [14]. However, as shown in Table 1, even at a concentration of 250 μM, Ni did not inhibit Ca entry. We also found that inhibitors of store-activated channels such as clotrimazole (100 μM), SKF-96365 (10 μM), and 1-APB (100 μM) [15–17] had no effect on the changes of $[Ca^{2+}]_i$.

The only substance we found to have an inhibitory action at reasonably low concentrations was gadolinium. As shown in Fig. 7, 10 μM gadolinium completely inhib-

ited the changes of $[Ca^{2+}]_i$ produced by changes of membrane potential. Significant inhibition was also observed at 1 μM (Table 1). Gadolinium is known to block stretch activated channels [18] and we have therefore also investigated the effects of other substances which block stretch activated channels. However, we found (Table 1) that streptomycin had no effect on the Ca influx produced by hyperpolarization.

Table 1
The effects of various substances on the increase of $[Ca^{2+}]_i$ produced by hyperpolarization from −40 to potentials in the range −100 to −120 mV

Substance	n	Peak $[Ca^{2+}]_i$/control
AlF$_3$ (100 μM)	4	0.81 ± 0.1
Chlorpromazine (50 μM)	4	0.8 ± 0.03
Nickel (0.25 mM)	4	0.77 ± 0.12
Clotrimazole (100 μM)	4	1 ± 0.0
2-APB (100 μM)	4	1.4 ± 0.2
SKF-96365 (10 μM)	4	0.98 ± 0.02
Gadolinium (1 μM)	4	0.29 ± 0.02
Gadolinium (10 μM)	7	0.19 ± 0.03
Gadolinium (250 μM)	4	0 ± 0.0
Streptomycin (80 μM)	4	0.81 ± 0.1

Data show the amplitude of the rise of $[Ca^{2+}]_i$ as a fraction of that in absence of the substance.
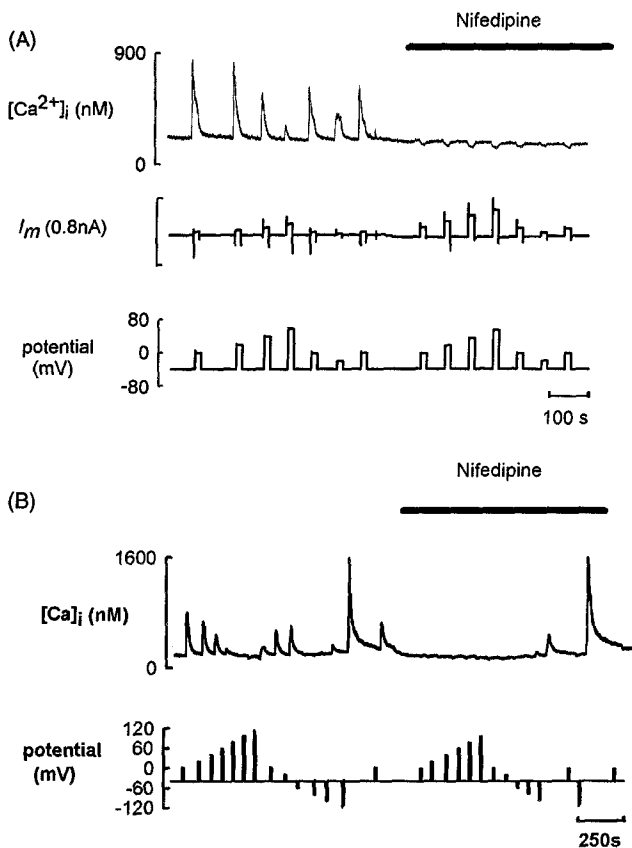
(A)

Fig. 5. The effects of membrane potential on $[Ca^{2+}]_i$ in Na-free conditions. In both parts traces show: top, $[Ca^{2+}]_i$; bottom membrane potential. In A membrane current is also shown. Nifedipine (10 μM) was applied for the period shown. (A) and (B) show examples from two different cells.
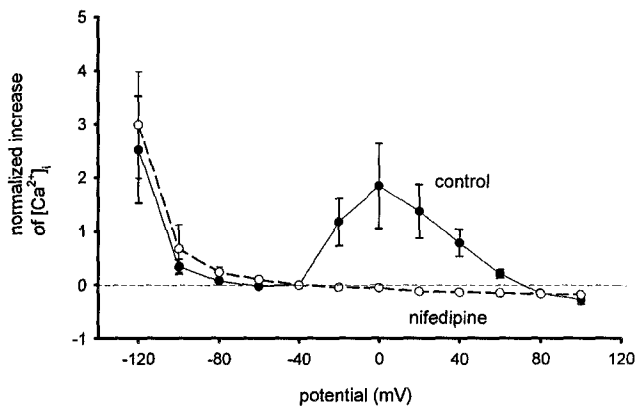

Fig. 6. Voltage-dependence of $[Ca^{2+}]_i$ in Na-free solutions. Symbols show: (●) control; (○) nifedipine.

4. Discussion

In this paper we have investigated the effects of steady state changes of membrane potential on $[Ca^{2+}]_i$ under a variety of conditions. The results show three different routes for Ca entry: (1) via the L-type Ca current; (2) via Na–Ca exchange and (3) via a pathway which is increased by hyperpolarization.
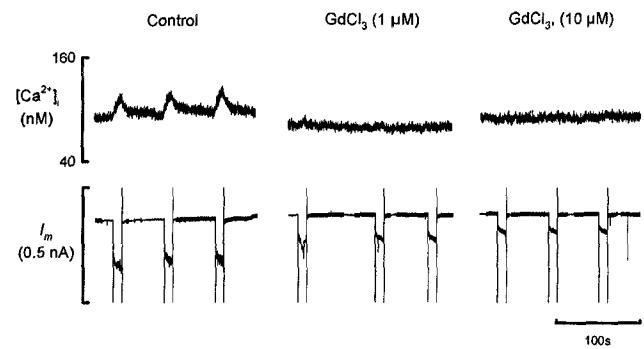

Fig. 7. Gadolinium ions inhibit the increase of $[Ca^{2+}]_i$ produced by hyperpolarization. Traces show: top, $[Ca^{2+}]_i$; bottom, membrane current. The pulses were from a holding potential of −40 to −120 mV. Solutions were Na-free and contained nifedipine (10 μM).

It is not surprising that there are both nifedipine-sensitive and insensitive pathways of Ca entry. Considerable previous work has examined the source of the Ca influx on depolarization that activates Ca release from the SR. The results show that depolarization produces Ca entry via two routes to release Ca from the SR: the L-type Ca current and NCX [19,20]. In the present work we have inhibited Ca release from the SR and measured the Ca influx directly. This is arguably more direct than using SR release as an index of entry. In the absence of inhibitors, depolarization produces an initial increase of $[Ca^{2+}]_i$ which is either maintained or decays. Under conditions when Ca entry on NCX is not prominent (control or Na-free) the response is most transient and, conversely, when Ca entry on NCX is augmented (elevated intracellular Na concentration) the decay is absent. This suggests that the decaying component is due to Ca entry on the L-type current. This is plausible since, in contrast to the NCX, Ca entry on the L-type current is known to inactivate on depolarization. Quantitative support for this hypothesis is provided by examining the effects of nifedipine. Under conditions where both NCX and the L-type current contribute to Ca entry, net Ca entry (as assessed by $d[Ca_T]/dt$) has an initial peak which decays to a steady level (Fig. 2). The initial peak is abolished by nifedipine. Furthermore, within the limits of the noise, the timecourse of decay of the initial peak of Ca entry occurs over a similar period of time as the inactivation of the L-type Ca current (see Figs. 2 and 4).

4.1. Ca entry in the absence of L-type Ca current or NCX

When experiments were performed in Na-free solutions to inhibit Na–Ca exchange and the presence of nifedipine to inhibit the L-type Ca current, depolarization decreased and hyperpolarization increased $[Ca^{2+}]_i$. This increase of Ca on hyperpolarization is what would be expected from a channel, which is always open and therefore voltage affects only the driving force for Ca. A similar voltage-dependence of $[Ca^{2+}]_i$ has been seen in Purkinje fibres exposed to Na-free solutions containing high Ca concentrations [21]. Previous

work measuring single channel currents (but not $[Ca^{2+}]_i$) has shown the existence of background (B-type) channels [2,4,22]. Such channels would have the properties expected to account for the voltage-dependence of $[Ca^{2+}]_i$ in the present work. However, these channels have previously been shown to be activated by chlorpromazine [3] and to be blocked by AlF_3 [4] and no effect of these compounds was found in the present work. We therefore conclude that the hyperpolarization activated Ca entry is unlikely to be through the B-type Ca channel.

We have examined the effects of blockers of other putative Ca entry pathways. We find no effect of blockers of store-operated Ca channels such as SKF-96365 or cotrimazole. Indeed the only agent we find to inhibit the Ca entry is gadolinium. A concentration of $10\,\mu M$ reduced the rise of $[Ca^{2+}]_i$ to 19% of control and $250\,\mu M$ abolished it. Previous work has shown that gadolinium inhibits stretch operated Ca channels in cardiac muscle [6] thus raising the possibility that the change of $[Ca^{2+}]_i$ is due to Ca entry through these channels. A study on chick skeletal myotubes found that hyperpolarization increased $[Ca^{2+}]_i$ and that this was also inhibited by gadolinium leading the authors to suggest a role for stretch activated channels [23]. We attempted to investigate this further by examining the effects of streptomycin (another inhibitor of stretch activated channels) but found no effect. Some members of the transient receptor potential channels family, which are expressed in cardiac myocytes, are also stretch-activated and inhibited by Gd (for review see Ref. [9]). This is particularly relevant as some TRP channels are known to be activated during Ca store depletion, e.g. SR being disabled by thapsigargin and ryanodine. However, as discussed above, SKF-96365, which is known to block some TRP channels isoforms [24,25] did not affect changes in $[Ca^{2+}]_i$. Unfortunately, the lack of functional characterisation of TRP channels in cardiac tissue makes it impossible for us to conclude whether they are the possible pathway for Ca influx during hyperpolarization recorded in our experimental conditions.

In conclusion, our data provide clear evidence for the existence of a mechanism for Ca entry that is gadolinium-sensitive and distinct from NCX and the L-type Ca current although its molecular identity is as yet unknown.

Acknowledgements

References

[1] D.M. Bers, Excitation–Contraction Coupling and Cardiac Contractile Force, Kluwer Academic Publishers, Dordrecht/Boston/London, 2001.

[2] A. Coulombe, I.A. Lefèvre, I. Baró, E. Coraboeuf, Barium- and calcium-permeable channels open at negative membrane potentials in rat ventricular myocytes, J. Membr. Biol. 111 (1989) 57–67.

[3] T. Lefevre, E. Coraboeuf, A. Ghazi, A. Coulombe, Divalent cation channels activated by phenothiazines in membrane of rat ventricular myocytes, J. Membr. Biol. 147 (1995) 147–158.

[4] S. Antoine, C. Pinet, A. Coulombe, Are B-type Ca^{2+} channels of cardiac myocytes akin to the passive ion channel in the plasma membrane Ca^{2+} pump? J. Membr. Biol. 179 (2001) 37–50.

[5] C. Pinet, S. Antoine, A.G. Filoteo, J.T. Penniston, A. Coulombe, Reincorporated plasma membrane Ca^{2+}-ATPase can mediate B-type Ca^{2+} channels observed in native membrane of human red blood cells, J. Membr. Biol. 187 (2002) 185–201.

[6] H. Hu, F. Sachs, Stretch-activated ion channels in the heart, J. Mol. Cell. Cardiol. 29 (1997) 1511–1523.

[7] N. Sasaki, T. Mitsuiye, A. Noma, Effects of mechanical stretch on membrane currents of single ventricular myocytes of guinea-pig heart, Jpn. J. Physiol. 42 (1992) 957–970.

[8] A. Uehara, M. Yasukochi, I. Imanaga, M. Nishi, H. Takeshima, Store-operated Ca^{2+} entry uncoupled with ryanodine receptor and junctional membrane complex in heart muscle cells, Cell Calcium 31 (2002) 89–96.

[9] D.E. Clapham, TRP channels as cellular sensors, Nature 426 (2003) 517–524.

[10] L.W. Runnels, L. Yue, D.E. Clapham, The TRPM7 channel is inactivated by PIP(2) hydrolysis, Nat. Cell Biol. 4 (2002) 329–336.

[11] D.A. Eisner, C.G. Nichols, S.C. O'Neill, G.L. Smith, M. Valdeolmillos, The effects of metabolic inhibition on intracellular calcium and pH in isolated rat ventricular cells, J. Physiol. (Lond) 411 (1989) 393–418.

[12] A.W. Trafford, M.E. Díaz, D.A. Eisner, Coordinated control of cell Ca^{2+} loading and triggered release from the sarcoplasmic reticulum underlies the rapid inotropic response to increased L-type Ca^{2+} current, Circ. Res. 88 (2001) 195–201.

[13] A.W. Trafford, M.E. Díaz, D.A. Eisner, A novel, rapid and reversible method to measure Ca buffering and timecourse of total sarcoplasmic reticulum Ca content in cardiac ventricular myocytes, Pflügers Arch. 437 (1999) 501–503.

[14] J.H. Lee, J.C. Gomora, L.L. Cribbs, E. Perez-Reyes, Nickel block of three cloned T-type calcium channels: low concentrations selectively block alpha1H, Biophys. J. 77 (1999) 3034–3042.

[15] M. Hecker, A.T. Bara, J. Bauersachs, R. Busse, Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals, J. Physiol. (Lond) 481 (1994) 407–414.

[16] M. Prakriya, R.S. Lewis, Potentiation and inhibition of Ca^{2+} release-activated Ca^{2+} channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP3 receptors, J. Physiol. (Lond) 536 (2001) 3–19.

[17] J.M. Diver, S.O. Sage, J.A. Rosado, The inositol trisphosphate receptor antagonist 2-aminoethoxydiphenylborate (2-APB) blocks Ca^{2+} entry channels in human platelets: cautions for its use in studying Ca^{2+} influx, Cell Calcium 30 (2001) 323–329.

[18] G.R. Li, C.M. Baumgarten, Modulation of cardiac Na^+ current by gadolinium, a blocker of stretch-induced arrhythmias, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H272–H279.

[19] S.E. Litwin, J. Li, J.H. Bridge, Na–Ca exchange and the trigger for sarcoplasmic reticulum Ca release: studies in adult rabbit ventricular myocytes, Biophys. J. 75 (1998) 359–371.

[20] K.R. Sipido, M. Maes, F. Van de Werf, Low efficiency of Ca^{2+} entry through the Na^+-Ca^{2+} exchanger as trigger for Ca^{2+} release from the sarcoplasmic reticulum, Circ. Res. 81 (1997) 1034–1044.

[21] M.B. Cannell, D.A. Eisner, W.J. Lederer, M. Valdeolmillos, Effects of membrane potential on intracellular calcium concentration in sheep Purkinje fibres in sodium-free solutions, J. Physiol. (Lond) 381 (1986) 193–203.

[22] T. Lefevre, A. Coulombe, E. Coraboeuf, Tonically active (background) calcium channels unmasked by phenothiazines in rat ventricular myocytes, Biophys. J. 66 (1994) a321.

[23] J.Y. Park, D. Lee, J.U. Maeng, D.S. Koh, K. Kim, Hyperpolarization, but not depolarization, increases intracellular Ca^{2+} level in cultured chick myoblasts, Biochem. Biophys. Res. Commun. 290 (2002) 1176–1182.

[24] A. Riccio, C. Mattei, R.E. Kelsell, et al., Cloning and functional expression of human short TRP7, a candidate protein for store-operated Ca^{2+} influx, J. Biol. Chem. 277 (2002) 12302–12309.

[25] C.R. Halaszovich, C. Zitt, E. Jungling, A. Luckhoff, Inhibition of TRP3 channels by lanthanides. Block from the cytosolic side of the plasma membrane, J. Biol. Chem. 275 (2000) 37423–37428.