Cell Calcium 53 (2013) 120–124

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Calcium content of the endoplasmic reticulum of snail neurones releasable by caffeine

Roger C. Thomas*

Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3EG, UK

ARTICLE INFO

Article history:
Received 13 July 2012
Received in revised form 29 October 2012
Accepted 30 October 2012
Available online 29 November 2012

Keywords:
Caffeine
Neurone
Microelectrode
Ryanodine receptor

ABSTRACT

I have used Ca^{2+}- and pH-sensitive microelectrodes in and on large neurones in isolated ganglia of *Helix aspersa* to measure changes in intracellular Ca^{2+} (as V_{Ca}) and surface pH caused by caffeine (20 mM) application, and compared these changes with those caused by iontophoretic injection of Ca^{2+}. Mitochondrial uptake was blocked by pressure-injection of Ru360. In 8 experiments the mean increase in V_{Ca} in response to caffeine was 27.3 ± 2.8 mV (SEM) equivalent to a peak tenfold increase in ionised Ca^{2+}. Iontophoretic injection of Ca^{2+} into the same 8 cells showed that the caffeine responses were equivalent to those caused by an average injection charge of 235 ± 41 nC. Surface pH changes produced by the PMCA pumping out Ca^{2+} released by caffeine averaged $0.025 \pm .005$ pH units ($n = 7$), which was equivalent to surface pH changes induced by an average Ca^{2+} injection charge of 300 ± 86 nC. The average cell volume was 4.2 nl. Assuming that the injection transport index was 0.48 as previously measured, these V_{Ca} and pH changes suggest that the total Ca^{2+} content released by caffeine was about 0.175 mmol/l cell volume.

1. Introduction

The endoplasmic reticulum (ER) of animal cells plays a key role in both protein synthesis and Ca^{2+} signalling. In neurones the ER acts as a highly dynamic intracellular Ca^{2+} store, and is deeply involved in neuronal signalling (for review see [1]). It has been described as a "neurone within the neurone" [2] and there is much interest in the role of the ER in neuronal plasticity [3–5]. In spite of this the total Ca^{2+} intraluminal content has not been measured for any neurone. The free ionised Ca^{2+} in the ER of various animal cells has been estimated at between 20 and 2000 μM, while the total concentration (ionised and bound) may range from 1 to 5 mM [6]. Ca^{2+} is pumped into the ER by the sarco-endoplasmic reticulum ATPase, or SERCA, while Ca^{2+} can be released through channels opened by either inositol tris phosphate ($InsP_3$) or Ca^{2+} itself [1,7]. The latter release mechanism, calcium-induced calcium release or CICR, is via channels blocked by ryanodine and thus known as ryanodine receptors (RyRs). The RyRs can be fully activated at normal cytoplasmic and intraluminal [Ca^{2+}] by high levels of caffeine. Caffeine lowers the threshold for Ca^{2+} activation of the channels to below the normal cytoplasmic Ca^{2+} level.

In many cells caffeine effectively empties the ER, although in others two different pools of Ca^{2+} have been proposed, the other being released by $InsP_3$. Snail neurones however seem insensitive to $InsP_3$ injection (Thomas, unpublished). The total Ca^{2+} released from other cells has been measured only in cardiac myocytes [8], human T cells [9] and frog sympathetic neurones [10].

2. Methods

2.1. General

Experiments were done on large (180–250 μm diameter) neurones in isolated sub-oesophageal ganglia of the common snail, *Helix aspersa*. Cells were voltage-clamped to −50 mV using two microelectrodes. Caffeine was applied to empty the ER, which caused a transient rise in intracellular Ca^{2+} measured as V_{Ca}, (the potential recorded by a Ca^{2+}-sensitive microelectrode (CaSM) after the subtraction of the membrane potential). This rise was compared with those caused by brief iontophoretic injections of Ca^{2+} ions. In other experiments the changes in surface pH (ΔpH_s) resulting from the subsequent extrusion of the Ca^{2+} released by caffeine were measured using pH-sensitive microelectrodes with exposed glass tips which were pressed against the surface of the chosen cell. These pH changes were compared with those caused by the extrusion of injected Ca^{2+}.

2.2. Preparation

An aestivating snail was killed by rapid removal of the circumoesophageal ring of ganglia, and the large cells on the dorsal side of the suboesophageal ganglia exposed as previously described [11,12].

* Correspondence address: Physiological Laboratory, Department of PDN, University of Cambridge, Downing Site, Cambridge CB2 3EG, UK. Tel.: +44 01223 333869; fax: +44 01223 333840.
E-mail address: rct26@cam.ac.uk

0143-4160/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ceca.2012.10.007

All experiments were carried out at room temperature, 18–23 °C, starting at least 1 h after the dissection.

2.3. Solutions

The normal snail Ringer solution contained (mM): 80 NaCl, 4 KCl, 7 $CaCl_2$, 5 $MgCl_2$, and 20 Hepes, titrated with NaOH to pH 7.5. For surface pH measurements the Hepes concentration was 5 mM with additional NaCl to maintain tonicity. Caffeine was dissolved in normal or low-buffer solution with no adjustment of tonicity, since caffeine crosses the cell membrane much faster than water [13].

Ru360 was made up at 500 nM in 0.1 M KCl with 0.1% Fast green FCF to make it visible when injected. Aliquots of 0.1 ml of the solution were kept frozen until use.

2.4. Microelectrodes

Conventional micropipettes were pulled from 1.2 mm filamented borosilicate glass tubing and backfilled with 1 M CsCl for passing clamp current or recording membrane potential. Microelectrodes for iontophoretic injection were filled with 0.1 M $CaCl_2$ with tips broken by touching a pin in the bath to give resistances of 5–10 MΩ.

CaSMs were made from quartz glass and Hinke-style pHSMs from borosilicate and pH-sensitive glass [14,15].

2.5. Microelectrode placement

As shown in Fig. 1, the CaSM, which was inserted first, was pushed deep into the cell and left with the tip close to the far side. The membrane potential (E_m) and clamp microelectrodes were inserted next and left with their tips near the top of the cell. The clamp was activated and set to a holding potential of −50 mV. That all electrodes were in the same cell was tested by a brief 30 mV hyperpolarisation.

Finally, the Ru360 and Ca^{2+} injection electrodes were inserted, with the tip of the latter moved to near the cell centre. In some experiments intracellular pH electrodes were placed with the tip near the cell centre, in others a surface pH electrode was pressed firmly against the side of the cell, as shown in Fig. 1.

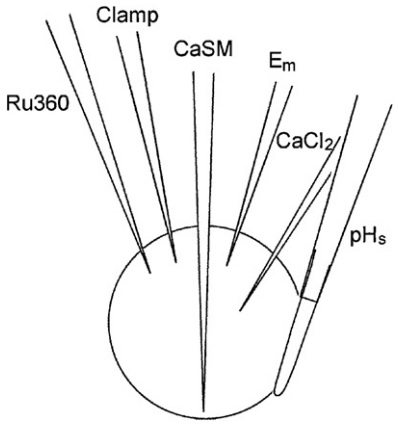

Fig. 1. Microelectrode positioning showing relative depths in the cell. From the left, Ru360 pressure-injection microelectrode, voltage clamp electrode, Ca^{2+}-sensitive microelectrode, membrane potential electrode, Ca^{2+} iontophoretic injection electrode and surface pH (pH_s) Hinke-style glass microelectrode. The last was pushed sideways against the cell to maximise contact.

2.6. Data collection and analysis

Potentials from the conventional microelectrodes, CaSMs and intracellular pHSMs were recorded as voltages referred to membrane potential, with the voltages from the pHSMs converted to pH before display. Potentials from the surface pHSMs were referred to the bath potential. Results were discarded if on withdrawal of an electrode its potential in Ringer solution had changed by more than 7 mV.

Potentials from the voltage-recording microelectrodes were led via preamplifiers in the Faraday cage to an 8-pole Bessel filter and recorded at 20 Hz on a PC via a CED micro 1401 interface and Spike 2 data collection programme (Cambridge Electronic Design, UK). The clamp current was recorded at 100 Hz as above. Measurements of changes in CaSM and pHSM voltages were made using the cursor tool on Spike 2.

Figures were prepared from the CED data after loading into Microsoft Excel. Spikes in the V_{Ca} or pH records generated by electronic pickup were partially erased, and the clamp current records were in some cases restricted in range. Data are presented as means ± S.E.M. of n observations. The statistical significance of observed differences was determined by a t test for means. Differences between means were considered significant when $p < 0.05$.

3. Results

3.1. Comparing the responses to caffeine and Ca^{2+} injection.

The high level of $[Ca^{2+}]_i$ reached during caffeine application is likely to allow mitochondria to take up a large fraction of the released Ca^{2+}, as previously shown for intophoretic injections [12]. In all experiments I have therefore injected cells with Ru360 to block the mitochondrial uptake pathway [16]. As shown in Fig. 2 Ru360 injection greatly increased the V_{Ca} response to caffeine. After 3 applications of 50 mM caffeine had each caused a brief increase (by respectively 6, 8 and 9 mV) in V_{Ca}, followed by an undershoot, I pressure-injected Ru360 solution in quantities sufficient to make the cell visibly green. This caused a small increase in the baseline V_{Ca}, and a doubling (to respectively 19 and 20 mV) of the following two responses to caffeine. In a total of 7 similar measurements of the effect of Ru360 injection the caffeine-induced V_{Ca} transients increased by an average 1.95 ± 0.27. I have previously shown that Ru360 had similar effects on the responses to Ca^{2+} injection (an

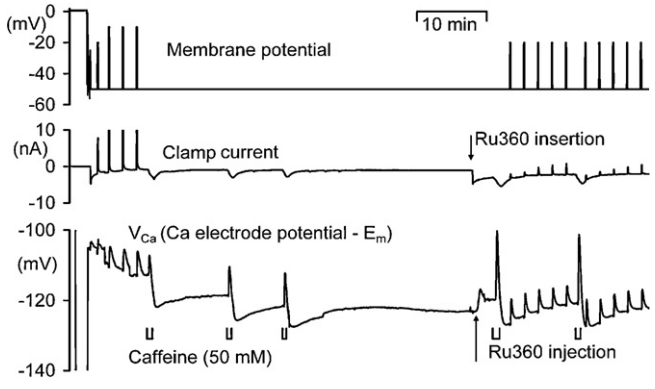

Fig. 2. The effect of Ru360 on the intracellular Ca^{2+} responses to caffeine. The membrane potential (top record) was clamped at −50 mV except when brief depolarisations were imposed. The middle record shows the clamp current; the lower shows the voltage recorded by the CaSM relative to the membrane potential. The arrow shows where Ru360 was pressure-injected, and the bars below the V_{Ca} record show where caffeine was superfused. Based on previous intracellular calibrations [16] a V_{Ca} of −120 mV corresponds to a free Ca^{2+} value of 300 nM, while −140 mV corresponds to 60 nM.