The Role of Endoplasmic Reticulum Calcium Pumps during Cytosolic Calcium Spiking in Pancreatic Acinar Cells*

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Carl C. H. Petersen§, Ole H. Petersen§, and Michael J. Berridge

From the Agricultural and Food Research Council Laboratory of Molecular Signalling, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3RE, United Kingdom and the Medical Research Council Secretory Control Group, The Physiological Laboratory, University of Liverpool, Liverpool L69 3BX, United Kingdom

Many cell types show repetitive short lasting cytosolic calcium spikes with long interspike periods when stimulated with submaximal concentrations of agonists linked to the phosphoinositide signaling pathway. In pancreatic acinar cells these spikes have been shown to be evoked by constant levels of inositol trisphosphate through a mechanism of calcium-induced calcium release and do not depend acutely on the presence of extracellular calcium. However, the processes involved in the interspike period have remained unclear. Here we report that the endoplasmic reticulum Ca\textsuperscript{2+}-ATPases play a significant role, not only in resequestering calcium after a spike, but also in regulating the long interspike period. Decreasing the activity of the endoplasmic reticulum calcium pumps leads to shorter interspike intervals and thus higher spiking frequencies, while the duration of each spike increases. The endoplasmic reticulum Ca\textsuperscript{2+}-ATPases are able to entirely suppress a response that can subsequently be evoked by partial inhibition of the pumps. This suggests that during the interspike period there is a considerable amount of calcium released from intracellular stores, which is rapidly buffered by the endoplasmic reticulum calcium pumps and the cytosolic calcium-binding proteins. A calcium spike will be initiated by calcium-induced calcium release only when the buffering is saturated.

Many cell types respond to submaximal concentrations of agonists linked to the phosphoinositide signaling pathway by frequency-modulated spiking of the cytosolic free calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{c}) rather than a graded increase in [Ca\textsuperscript{2+}]\textsubscript{c}.

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§ To whom correspondence should be addressed. Tel: 44-223-336643; Fax: 44-223-324387.

The abbreviations used are [Ca\textsuperscript{2+}]\textsubscript{c}, cytosolic free calcium concentration; InsP\textsubscript{3}, inositol 1,4,5-trisphosphate; Ins(2,4,5)P\textsubscript{3}, inositol 2,4,5-trisphosphate; ER, endoplasmic reticulum.

EXPERIMENTAL PROCEDURES

Fragmented mouse pancreas was digested by pure collagenase, washed, and pipetted to produce single acinar cells as previously de-
The role of ER calcium pumps during calcium spiking was studied using a two-voltage pulse protocol. The effects of thapsigargin on spiking frequency for 

**RESULTS**

The aim of this series of experiments was to assess the role of the ER Ca\textsuperscript{2+}-ATPase in the spiking mechanism. In order for repetitive calcium spiking to occur, a functional pump is required for the uptake of calcium into the intracellular stores so that further spikes can be generated. We therefore applied very low concentrations of thapsigargin (500 pm), a specific inhibitor of the ER Ca\textsuperscript{2+}-ATPase (Thastrup et al., 1990; Lytton et al., 1991), aiming to induce only partial inhibition of the pump. Fig. 1A suggests that 500 pm thapsigargin indeed produces only slight inhibition of the pump. Comparing the duration of similar amplitude spikes evoked by 5 pm Ins(2,4,5)P\textsubscript{3} before (Fig. 1A, i and ii) and after (Fig. 1A, ii) the application of 500 pm thapsigargin in the same cell, we find that the duration of the spikes increases by 60 ± 15% (mean ± S.E.) (n = 4) when thapsigargin is present. The spike duration is thus controlled, at least in part, by the activity of the ER Ca\textsuperscript{2+}-ATPases.

**DISCUSSION**

The results presented here demonstrate that partial inhibition of the ER Ca\textsuperscript{2+}-ATPase in the presence of Ins(2,4,5)P\textsubscript{3} increases the spiking frequency (or initiates spiking in cells with subthreshold levels of Ins(2,4,5)P\textsubscript{3}). The simplest explanation of the data is that considerable amounts of stored intracellular calcium are released in the interspike period but are rapidly buffered and resequestered into the calcium stores. By partially inhibiting the calcium pump, the cytosolic buffers are more rapidly saturated, leading to an earlier explosive release of calcium and thus a higher spiking frequency. The events leading to repetitive cytosolic calcium spiking can be explained by the adaptation of previously published models (Berridge, 1993). In Fig. 2, the left-hand side of the model depicts an intracellular calcium store (with an InsP\textsubscript{3} receptor and a calcium pump) at five stages (i-v) during the spiking cycle. The right side of Fig. 2 depicts changes in the cytosolic free calcium concentration with time and shows an idealized spiking pattern.

The following sequence of events emphasizes the importance of active (calcium stores) and passive (cytosolic calcium-binding
ATPase would lead to a more rapid filling of the cytosolic cal-
spike period, the buffers
tered by the ER Ca2+-ATPases. This process can then be re-
calcium-binding proteins and Ca2+-ATPases on other calcium
stores. (ii) As the calcium release continues during the inter-
spike period, the buffers (B) begin to fill. (iii) The decrease in
available calcium buffer (due to local saturation of cytosolic
binding proteins and the filling of other calcium stores)
slows the rate at which free calcium ions are removed from
the cytosol, and at a given time the release rate overtakes the
maximum rate of buffering. At this point the Ca2+ begins to
rise slowly. (iv) Due to positive feedback (calcium-induced cal-
release) on the calcium release channels an explosive rise
of Ca2+ is initiated. (v) As [Ca2+] reaches its maximum, fur-
ther release is inhibited, allowing the calcium to be reques-
tered by the ER Ca2+-ATPases. This process can then be re-
formed, repeating repetitive calcium spikes.

The model predicts that partial inhibition of the ER Ca2+-
ATPase would lead to a more rapid filling of the cytosolic cal-
ium buffers, initiating a spike earlier than in the case for
uninhibited calcium pumps, thus increasing the spiking fre-
cuency, as our experiments demonstrate. In fertilized mouse
eggs Kline and Kline (1992) noted a transient increase in cal-
cium spiking frequency and decrease in spike amplitude fol-
lowing the addition of 20 μm thapsigargin. Although Kline and
Kline (1992) drew no conclusion from this particular observa-
tion in their study of fertilization-induced calcium spikes, the
temporary increase in frequency may have resulted from a
partial inhibition of the calcium pumps corresponding to the
condition we achieved by administering a much lower concen-
tration of thapsigargin. The model we propose could then ac-
count for their unexplained observation.

A recent paper by Camacho and Lechleiter (1993) has shown
that expression of extra calcium pumps in the ER of Xenopus
oesocytes increases the frequency of calcium oscillations. Su-
perficially this appears to contradict the data presented here.
However, the calcium oscillations described by Camacho and
Lechleiter (1993) are of a very high frequency and do not have
an interspike interval. Their data show that the calcium stores
must be refilled and [Ca2+] returns to a low level before the
next spike can be initiated. Higher activity calcium pumps on
the intracellular stores decrease the duration of each calcium
spike (as we also observed (Fig. 1A, i and ii)), thus allowing
higher spiking frequencies to be observed since their system
shows no interspike interval. The data from our paper show
that the ER calcium pumps have a much wider role than
merely resquestering the calcium after a spike. In fact the ER
Ca2+-ATPase is intimately involved in generating long inter-
spike periods and is thus able to control cytosolic calcium spik-
ing. In the most impressive case the ER calcium pumps are able
to entirely suppress a response, which can subsequently be
evoked by partial inhibition of the pumps (Fig. 1B, ii).

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Fig. 2. A simple one-pool model to illustrate the importance of active and passive calcium buffering in the generation of calcium spikes. The model predicts that partial inhibition of the ER Ca2+-ATPase would lead to a more rapid filling of the cytosolic calcium buffers. This initiates a spike earlier than in the case for uninhibited calcium pumps, thus increasing the spiking frequency, as our experiments demonstrate. A more detailed description of the model is in the discussion.