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Oscillations of cytosolic calcium in single pancreatic acinar cells stimulated by acetylcholine

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The changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) were monitored (fura-2) in single, isolated, mouse pancreatic acinar cells stimulated by acetylcholine (ACh). Responses to ACh at concentrations between 10^{-7} and 5×10^{-7} M are marked by the appearance of regular, sinusoidal, oscillations in $[Ca^{2+}]_i$. At 37°C the oscillations are transient, being seen only in the initial rising phase of the calcium signal. At 30°C regular oscillations can be maintained throughout the period of ACh application. This study reports that release of intracellular calcium and influx of extracellular calcium are both involved in the generation of these oscillatory calcium signals.

cytosolic Ca^{2+}; Fura-2; Acetylcholine; Oscillation; (Pancreas, Acinar cell)

1. INTRODUCTION

Experimental evidence has long suggested that in pancreatic exocrine acinar cells calcium has a key role to play in the coupling of cholinergic, muscarinic, receptors to secretory mechanisms (see [1]). Recent studies using the calcium-sensitive fluorescent probe, quin-2, have now directly demonstrated the elevation in cytosolic free calcium concentration ($[Ca^{2+}]_i$) evoked in suspensions of acinar cells upon stimulation by the neurotransmitter, acetylcholine (ACh) [2,3]. Patch-clamp electrophysiological recordings have unequivocally confirmed the second messenger role of calcium in activating the ion transports which support electrolyte secretion in pancreatic and other exocrine acinar cells (see [4]). The current hypothesis is that muscarinic receptor regulation of secretion in exocrine acinar cells is secondary to the receptor regulation of (i) release of calcium from intracellular stores and (ii) influx of calcium across the plasma membrane via a voltage-independent pathway [4,5].

Here, the improved calcium probe, fura-2 [6], is employed to investigate the time course and amplitude of the calcium signal induced, not in suspension of cells but in single, enzymatically, isolated mouse pancreatic acinar cells stimulated by ACh. The study reveals that when ACh is applied at submaximal concentrations (10^{-7}–5×10^{-7} M) it can give rise to oscillations of $[Ca^{2+}]_i$ of regular periodicity and large amplitude. We investigate the respective roles of calcium release and calcium influx in the generation of the oscillatory changes in $[Ca^{2+}]_i$.

2. MATERIALS AND METHODS

Pancreata were excised from adult male mice and acinar cells enzymatically isolated by collagenase digestion [7]. After dispersion and washing the cells were resuspended in a standard extracellular solution of the following composition (mM): NaCl, 140; KCl, 4.7; $MgCl_2$, 1.13; $CaCl_2$, 1.2; glucose, 10; Hepes, 10; supplemented with 0.2% foetal calf serum. Cells were loaded with fluorescent indicator by 30 min incubation in the control solution containing 1 μM fura-2 acetoxymethyl ester at 22°C. At the end of this incubation the cells were removed

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and transferred to a chamber (volume 0.15 ml) mounted on the stage of a Nikon Diaphot microscope. The cells were continuously superfused (0.5 ml/min) in a stream of media issuing from any one of four inlet tubes converging into the chamber. A system of taps just outside the chamber allowed the media flowing over the cells to be changed within 5 s. The calcium-free solutions had no calcium but 1 mM EGTA added. The temperature of the solutions was, as indicated in the text, either 37 or 30°C.

$[Ca^{2+}]_i$ was measured in single cells by dual-excitation microfluorimetry [6]. A Spex (Glen Spectra) DM 3000 CM system provided alternating excitation wavelengths of 340 and 380 nm at not less than 10 Hz. Fluorescence emitted at 505 nm was monitored by photon counting from single cells, isolated optically by means of a diaphragm. $[Ca^{2+}]_i$ was calculated from the ratio (R) of the fluorescence at the two different wavelengths according to the previously reported formula [8]:

$$[Ca^{2+}]_i = K_d\beta(R - R_{min})/(R_{max} - R)$$

where K_d = 225 nM [6], R_{max}, R_{min} and β are constants; 8.01 ± 0.37 (n = 16), 0.34 ± 0.05 (n = 9) and 3.83 ± 0.4 (n = 6), respectively. These constants were determined using the in situ calibration procedures of Schlegel et al. [8,9]. All records were corrected for autofluorescence at each wavelength (determined in unloaded cells) before ratio and $[Ca^{2+}]_i$ were calculated.

3. RESULTS

When the single pancreatic acinar cells, at 37°C, are stimulated by ACh at concentrations between 10^{-6} and 10^{-5} M, the time course and amplitude of the changes in $[Ca^{2+}]_i$ are similar to those previously described in the studies on cell suspensions, i.e. biphasic responses (see fig.1A) [2,3]. In the present study, ACh at 10^{-5} M caused a characteristic rapid rise in $[Ca^{2+}]_i$ from an average basal level of 131 ± 6.8 nM (n = 22) to a maximum of 834 ± 77 nM (n = 10) within the first minute of agonist application. Thereafter the $[Ca^{2+}]_i$ declines over the next 2–3 min to a lower, but still elevated, sustained level of 264 ± 90 nM (n = 5). $[Ca^{2+}]_i$ is only restored to prestimulus levels when ACh is removed or atropine introduced. When lower concentrations of ACh are employed the changes in $[Ca^{2+}]_i$ are more complex. At concentrations between 10^{-7} and 5×10^{-7} M the responses to this agonist are characterized by (i) very long latencies (often more than 60 s) before any pronounced or rapid change in $[Ca^{2+}]_i$ is induced, (ii) at the onset of the rapid rise in $[Ca^{2+}]_i$ sinusoidal oscillations in $[Ca^{2+}]_i$ are seen superimposed on the rising calcium signal (fig.1B). The oscillations in $[Ca^{2+}]_i$ have a regular periodicity always between 0.18 and 0.2 Hz (n = 7). The oscillations do not persist and between 30 and 150 s after the onset of the response they decline in amplitude until a more stable, sustained $[Ca^{2+}]_i$ is established. The transient nature of the oscillations in $[Ca^{2+}]_i$ makes any detailed investigation of the phenomenon difficult. It was found however, that cells superfused at the lower temperature of 30°C responded to low concentrations of ACh (variable but generally close to 10^{-7} M) with oscillations in $[Ca^{2+}]_i$ that persist throughout the period of application of the agonist. At higher concentrations of between 10^{-6} and 10^{-5} M ACh the responses to this agonist even at 30°C were identical to those described at 37°C and shown in fig.1A, i.e. biphasic. Fig.2 shows an example of the oscillations in $[Ca^{2+}]_i$ induced in a single cell at 30°C by application of 1.2×10^{-7} and 1.4×10^{-7} M ACh. The periodicity of the oscillations at 30°C in this and in all cells was between

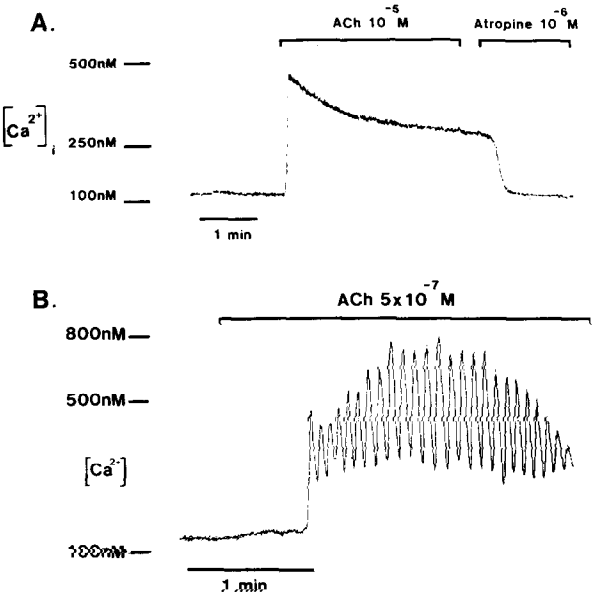

Fig.1. Changes in $[Ca^{2+}]_i$ in two pancreatic acinar cells upon stimulation by acetylcholine (ACh). The cells are superfused at 37°C. (A) ACh is applied at 10^{-5} M. The change in $[Ca^{2+}]_i$ is characteristic of the effect of ACh at concentrations between 10^{-6} and 10^{-5} M at either 37 or 30°C. The response is biphasic. There is an initial rapid rise in $[Ca^{2+}]_i$ to a maximum after which it declines to a lower but still elevated level which is sustained throughout the period of ACh application or until the muscarinic agonist atropine is applied. (B) Characteristic response of a single pancreatic acinar cell to lower concentrations of ACh (5×10^{-7} M in this instance). There is a long latency before any marked changes in $[Ca^{2+}]_i$ are evoked. The initial phase of the response is marked by sinusoidal oscillations in $[Ca^{2+}]_i$.

0.08 and 0.1 Hz (n = 24). The frequency of the oscillations was not dependent on the concentration of ACh employed. Fig.2 shows the effect of increasing [ACh] on the calcium signal. Within a narrow range the effect of increasing agonist concentration is to convert the response from one of sustained, regular, sinusoidal oscillations in $[Ca^{2+}]_i$ to one in which oscillations are seen only in the initial phase, superimposed on a rising calcium signal, and decreasing in amplitude, i.e. similar to responses at 37°C. Fig.3 shows the effect of removal of extracellular calcium from a cell in which ACh has induced regular, sustained oscillations. Upon withdrawal of extracellular calcium the oscillating calcium signals are abolished and $[Ca^{2+}]_i$ returns to prestimulus levels (n = 5). Oscillations are restored with the reintroduction of extracellular calcium.

In another series of experiments extracellular calcium was removed prior to stimulation with ACh. The calcium signal evoked by ACh is again dependent on the concentration of agonist employed. At 10^{-6}–10^{-5} M the response (not shown) at 30°C is as described for cell suspensions [2,3], i.e. a single transient calcium signal lasting several minutes. At the lower concentrations of agonist the responses, as in the calcium-containing solutions, are marked by the appearance of irregular transients or oscillations in $[Ca^{2+}]_i$. Fig.4 illustrates the different patterns of response obtained in calcium-free media. At the lowest effective concentration ACh induces either irregular, transient elevations in $[Ca^{2+}]_i$ (fig.4A) or regular sinusoidal oscillations which, in the absence of extracellular calcium, are seen to decrease in amplitude until a point is reached at which $[Ca^{2+}]_i$

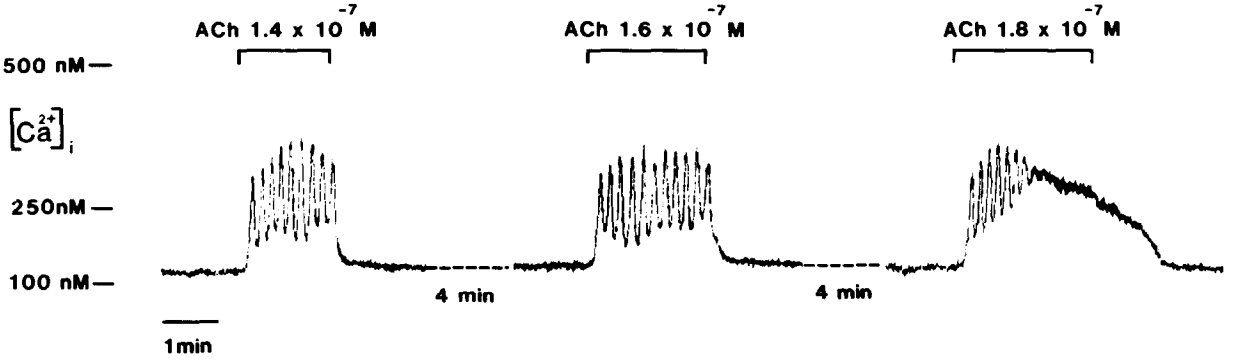

Fig.2. Single pancreatic acinar cell superfused at 30°C. Responses are shown to ACh at three different concentrations within a narrow range.

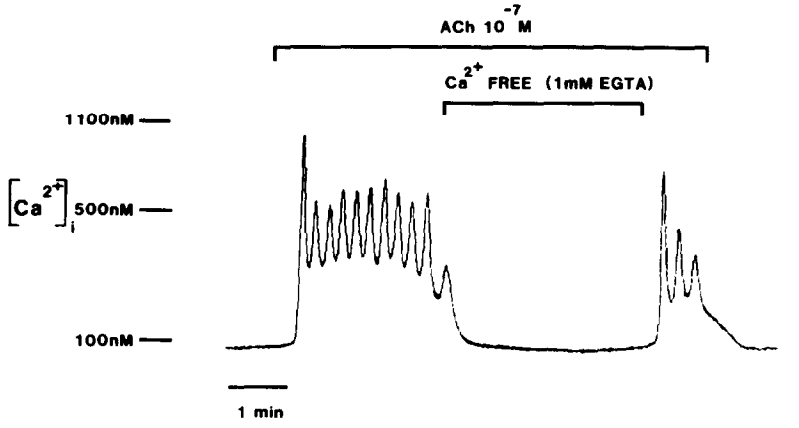

Fig.3. $[Ca^{2+}]_i$ measured in single pancreatic acinar cell superfused at 30°C. ACh (10^{-7} M) has induced oscillatory changes in $[Ca^{2+}]_i$. As indicated extracellular calcium is removed and then restored.

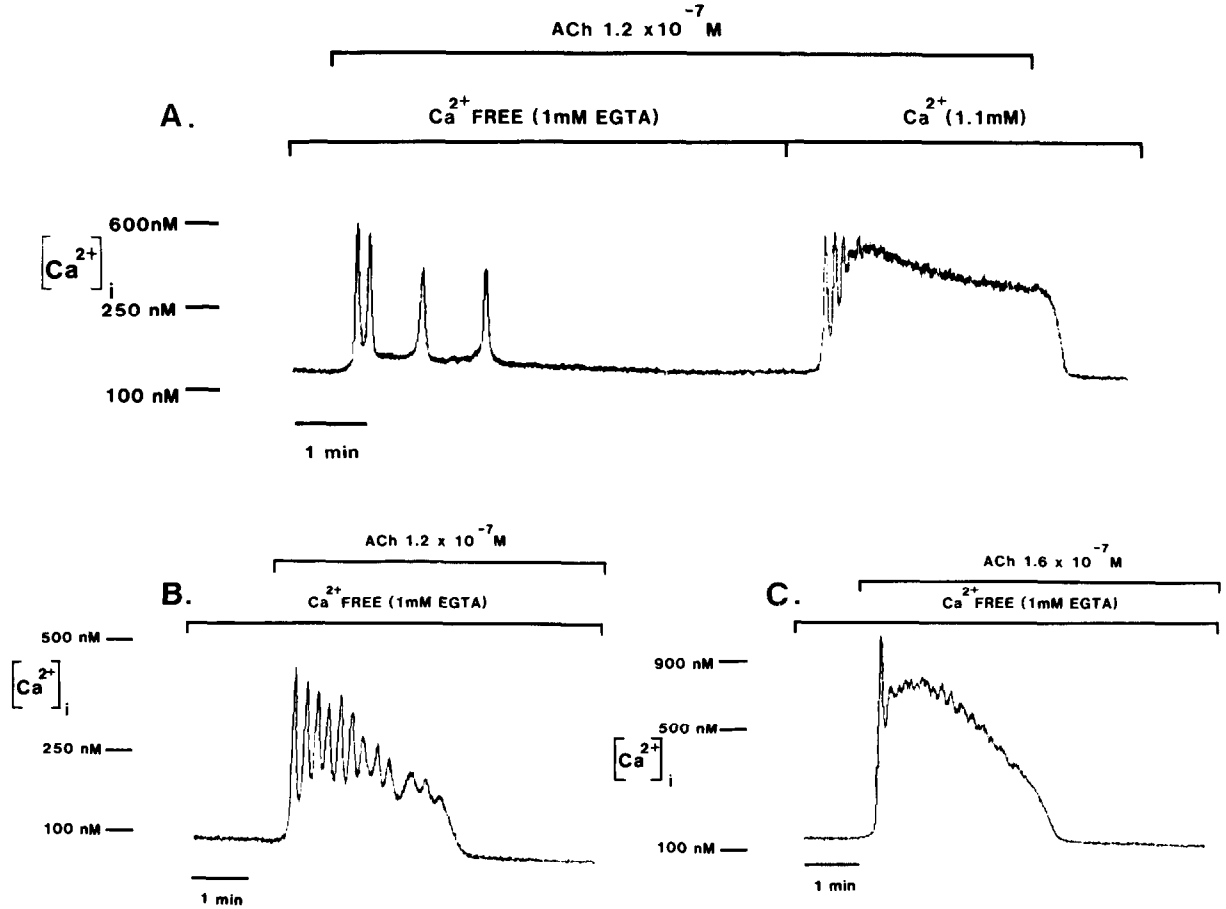

Fig.4. The traces (A–C) are from three different pancreatic acinar cells, superfused at 30°C, and show the different patterns of response ($[Ca^{2+}]_i$) when ACh is applied at low concentrations after extracellular calcium has been removed. The upper trace (A) also shows the effect upon reintroduction of extracellular calcium.

rapidly returns to prestimulus levels despite the continued presence of ACh. The effect of increasing ACh within a narrow dose range is to change progressively the response towards that evoked by the high concentrations of agonist.

4. DISCUSSION

The study clearly demonstrates an ability of ACh to induce oscillations of $[Ca^{2+}]_i$ in single pancreatic acinar cells. These agonist-induced oscillations of $[Ca^{2+}]_i$ in the pancreatic acinar cells do not however mimic those reported in rat hepatocytes [10]. In the liver cells the oscillations in $[Ca^{2+}]_i$ are due to a series of calcium spikes separated in time and with a frequency dependent on the concentration of agonist applied. It is not at all clear then if the calcium transients in different cells will reflect any common process. In pancreatic acinar cells however, the oscillations can be initiated in calcium-free solutions but they are only sustained in the presence of extracellular calcium. The oscillating calcium signals thus involve both the release of calcium from intracellular stores and, in the later stages at least, calcium influx from the extracellular to intracellular fluid. These complex responses suggest not only that there are potent feedback mechanisms regulating $[Ca^{2+}]_i$ but also that there is some functional interaction between calcium release and calcium influx. It has been suggested, for exocrine acinar cells, that this link could be a physical one between calcium stores and

the plasma membrane and that influx is achieved via refilling of depleted intracellular stores [11]. This would not in itself however, explain the oscillations in the calcium signal. Another link could be provided if the regulators of calcium release and of calcium influx were metabolically linked or associated. Inositol polyphosphates may provide such a link. Muscarinic receptor activation is associated with the generation of the inositol polyphosphates, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] in pancreatic acinar cells [12]. The role of Ins(1,4,5)P_3 in mobilizing intracellular calcium is now unequivocally established in a wide variety of tissues (see [13]). It is even reported that Ins(1,4,5)P_3 can give rise to oscillations in membrane conductance in lacrimal exocrine acinar cells [14]. Recently, a separate study on lacrimal acinar cells has provided strong evidence of a role for Ins(1,4,5)P_3, but only by acting in combination with Ins(1,3,4,5)P_4, in the regulation of calcium influx [15]. Ins(1,3,4,5)P_4 is produced when the calcium-sensitive Ins(1,4,5)P_3-3-kinase is activated to phosphorylate Ins(1,4,5)P_3, i.e. Ins(1,4,5)P_3 is the substrate for Ins(1,3,4,5)P_4 production [16,17]. The marked effects of temperature on the oscillations of $[Ca^{2+}]_i$ in the pancreatic acinar cells would be consistent with some rate-limiting enzymatic step being involved. It is possible at the low concentrations of ACh at which oscillations are observed that it takes time, hence the long latencies, for Ins(1,4,5)P_3 to reach a critical concentration required before calcium is released (see [18]). If the calcium released then stimulated the activity of the Ins(1,4,5)P_3-3-kinase then Ins(1,3,4,5)P_4 would be formed and at the same time Ins(1,4,5)P_3 would decrease resulting in cessation of calcium release. If Ins(1,3,4,5)P_4 effected refilling of the intracellular pool (see [5]) while fresh Ins(1,4,5)P_3 was being generated then the cycle would continue without depletion of intracellular calcium reserves but would be dependent on the presence of extracellular calcium.

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REFERENCES

[1] Petersen, O.H. (1980) The Electrophysiology of Gland Cells, Academic Press, New York.
[2] Powers, R.E., Johnson, P.C., Houlihan, M.J., Saluja, A.K. and Steer, M.L. (1985) Am. J. Physiol. 248, C535–C541.
[3] Williams, J.A., Korenbrot, J.I. and Ochs, D.L. (1985) Am. J. Physiol. 249, G389–G398.
[4] Petersen, O.H. and Gallacher, D.V. (1988) Annu. Rev. Physiol. 50, 65–80.
[5] Gallacher, D.V. (1988) Trends Neurosci., in press.
[6] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[7] Williams, J.A., Korc, M. and Dormer, R.L. (1978) Am. J. Physiol. 235, E517–E524.
[8] Schlegel, W., Winiger, B.P., Mollard, P., Vacher, P., Waurin, F., Zahnd, G.R., Wollheim, C.B. and Dufy, B. (1987) Nature 329, 719–721.
[9] Schlegel, W., Winiger, B.P., Waurin, F., Zahnd, G.R., G.R. and Wollheim, C.B. (1988) J. Receptor Res. 8, 493–507.
[10] Woods, N.M., Cuthbertson, K.S.R. and Cobbold, P.H. (1986) Nature 319, 600–602.
[11] Putney, J.W. jr (1986) Cell Calcium 7, 1–12.
[12] Trimble, R., Bruzzone, R., Meehan, C.J. and Biden, T.J. (1987) Biochem. J. 242, 289–292.
[13] Berridge, M.J. (1986) J. Exp. Biol. 124, 323–325.
[14] Evans, M.G. and Marty, A. (1986) Proc. Natl. Acad. Sci. USA 83, 4099–4103.
[15] Morris, A.P., Gallacher, D.V., Irvine, R.F. and Petersen, O.H. (1987) Nature 330, 653–655.
[16] Biden, T.J. and Wollheim, C.B. (1986) J. Biol. Chem. 261, 11931–11934.
[17] Zilberman, Y., Howe, L.R., Moor, J.P., Hesketh, T.R. and Metcalf, J.C. (1987) EMBO J. 6, 957–962.
[18] Miyazaki, S. (1988) J. Cell Biol. 106, 345–353.