Intracellular calcium signalling in rat parotid acinar cells that lack secretory vesicles

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Secretory vesicles from pancreatic acinar cells have recently been shown to release Ca^{2+} after stimulation with Ins(1,4,5)P_3 [Gerasimenko, Gerasimenko, Belan and Petersen, (1996) Cell **84**, 473–480]. These observations have been used in support of the hypothesis that Ca^{2+} release from secretory vesicles could be an important component of stimulus secretion coupling in exocrine acinar cells. In the rat, ligation of the parotid duct causes a reversible atrophy of the parotid gland. Most notably, after atrophy the acinar cells are reduced in size and no longer contain secretory vesicles [Liu, Smith, and Scott (1996) J. Dent. Res. **74**, 900]. We have measured cytosolic free-Ca^{2+} concentration ($[Ca^{2+}]_i$) in single, acutely isolated, rat parotid acinar cells, and compared Ca^{2+} mobilization in response to acetylcholine (ACh) stimulation in cells obtained from control animals to that in cells lacking secretory vesicles obtained after atrophy of the parotid gland. Application of 50–5000 nM ACh to control cells gave rise to a typical, dose-dependent, biphasic increase in $[Ca^{2+}]_i$, of which the later, plateau, phase was acutely dependent on the extracellular Ca^{2+} concentration. An identical pattern of response was observed with cells obtained from atrophic glands. Low concentrations of ACh (10–100 nM) occasionally produced $[Ca^{2+}]_i$ oscillations of a similar pattern in cells from both control and atrophic glands. We were able to show that Ca^{2+} rises first in the apical pole of the cell and the increase then spreads to the rest of the cell in cells from control glands but not in cells from atrophic glands. However, at present we are unable to determine whether this is due to the lack of secretory vesicles or whether the separation is too small to measure in the smaller acinar cells obtained from atrophic glands. We conclude therefore, that secretory vesicles make no significant contribution to overall Ca^{2+} mobilization in rat parotid acinar cells, nor are they required for oscillatory changes in $[Ca^{2+}]_i$ to occur. However we are unable to eliminate completely any role for secretory vesicles in initiating Ca^{2+} mobilization at the apical pole of the cell.

INTRODUCTION

In salivary glands, parasympathetic nerves regulate electrolyte and fluid secretion. Acetylcholine (ACh) binding to a G-protein-linked receptor triggers a cascade of events which lead, via formation of Ins(1,4,5)P_3, release of Ca^{2+} from internal stores and Ca^{2+} influx, to activation of the Ca^{2+}-dependent Cl^- and K^+ channels and ultimately to fluid and electrolyte secretion [1–3]. When the secretory stimulus is removed, Ca^{2+} release and Ca^{2+} influx cease and Ca^{2+}-ATPases remove Ca^{2+} from the cytoplasm in order to close the ion channels and terminate the response [4,5]. The restoration of the resting cytosolic free-Ca^{2+} concentration ($[Ca^{2+}]_i$) by Ca^{2+}-ATPase activity is a highly energy-dependent process [6]. Previous studies in exocrine acinar cells have suggested that highly polarized exocrine acinar cells are able to minimize the energy cost of secretory activity by activating ion channels with transient increases in $[Ca^{2+}]_i$ which are restricted to the secretory (apical) pole of the cell [7]. Recent work therefore, has emphasized the key role of Ins(1,4,5)P_3-dependent Ca^{2+} release from Ca^{2+} stores at the apical pole of the cell in initiating and propagating the Ca^{2+} signal generated in response to secretory agonists [8,9].

Ins(1,4,5)P_3 has been shown to cause Ca^{2+} release from the endoplasmic reticulum [10] and it has been generally assumed that the endoplasmic reticulum is the primary intracellular Ca^{2+} store involved in Ca^{2+} signalling [11,12]. However, secretory vesicles are the predominant Ca^{2+}-containing membrane-bound structures at the apical pole of any exocrine acinar cell [13–15]. Secretory vesicles are ideally situated to act as a focus for Ca^{2+} mobilization in this region of the cell [16], which has been shown, in pancreatic acinar cells, to be the most sensitive to Ins(1,4,5,)P_3 stimulation [8]. Evidence that secretory vesicles could participate in Ca^{2+} mobilization has recently been obtained from pancreatic acinar cells, where isolated zymogen granules have been shown to release Ca^{2+} in response to stimulation with Ins(1,4,5)P_3 [8]. It is possible that these Ins(1,4,5)P_3-sensitive secretory vesicles, rather than the endoplasmic reticulum, could be the source of agonist-evoked Ca^{2+} release at the apical pole of the cell. Transient increases in $[Ca^{2+}]_i$ could be entirely sustained from Ca^{2+} release from the secretory vesicles, and cell-wide increases in Ca^{2+} could be propagated through the cell by Ins(1,4,5)P_3 and Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum following release of 'priming' Ca^{2+} from the secretory vesicles [8,16].

An alternative strategy to determine the role of secretory vesicles in Ca^{2+} signalling is to study Ca^{2+} mobilization in acinar cells in which they are absent. Ligation of the parotid duct is known to cause a reversible atrophy of the parotid gland [17–19]. We have shown that acinar cells from atrophied glands are approximately 50 % the size of those from control glands and that these cells do not possess amylase-containing secretory vesicles [20,21]. Any contribution that Ca^{2+} release from secretory vesicles makes to the overall Ca^{2+} signal in parotid acinar cells will be missing in cells derived from atrophic glands. We have isolated acinar cells from both control and atrophic rat parotid glands and compared the pattern of Ca^{2+}

Abbreviations used: ACh, acetylcholine; $[Ca^{2+}]_i$, cytosolic free-Ca^{2+} concentration; $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration.

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mobilization in response to the muscarinic agonist ACh in order to determine what role secretory vesicles might play in Ca^{2+} signalling.

EXPERIMENTAL

Duct ligation

Adult male Wistar rats (240–270 g) were anaesthetized using diazepam (0.27 ml/kg) and hypnorm (0.27 ml/kg). The superficial surface of the parotid gland was exposed via an incision made in the skin 5 mm from the right commissure of the lip, below, and anterior to, the right ear along the submaxilla, and a flap was raised. The right parotid main secretory duct and accompanying marginal mandibular branch of the facial nerve were identified on the surface of the masseter muscle and carefully separated from each other. The main duct was ligated using a micro-clamp, as described previously [22], at a point 5 mm distal to the hilum of the gland. The ligature was retained in place for 2 weeks after which the animals were sacrificed and the parotid gland removed. Atrophied glands weighed 100 ± 7 mg ($n = 16$), whereas glands from control animals weighed 171 ± 9 mg ($n = 21$). As previously reported, secretory vesicles could not be observed in stained sections from atrophied glands, although vesicles are clearly visible in sections from control glands [20,21]. Furthermore, we were unable to detect any amylase activity in the atrophied glands. The amylase content of control glands was 128 ± 10 units/mg of gland tissue and that in glands which had been allowed to recover from atrophy [21,23–25] 133 ± 10 units/mg of gland tissue.

Cell preparation

Acinar cells were isolated as described previously [26–28]. Briefly, adult male Wistar rats (240–270 g) were killed by ether overdose. After dissection, parotid glands were finely minced and incubated for 10 min at 37 °C in incubation medium containing 0.5 mg/ml trypsin (Sigma). After a wash with trypsin inhibitor (Sigma), the glands were incubated for a further 20 min at 37 °C in the presence of 100 units/ml collagenase (Worthington Diagnostic). The resulting cell suspension was centrifuged and re-suspended twice. Cells were loaded with fura2 by a 10–20 min incubation in the presence of 1 μM fura2 acetomethoxy ester (Molecular Probes). After loading, cells were centrifuged and re-suspended in experimental medium.

A dilute cell suspension was placed into a perfusion chamber on the stage of a Nikon diaphot inverted microscope, and the cells were allowed to adhere to the glass bottom of the chamber. Cells were superfused continuously at 0.5 ml/min from one of several parallel superfusion pipettes. The solution bathing the cells could be changed in 1–2 s. All experiments were carried out at 24 ± 2 °C. Measurements were made using $400\times$ or $1000\times$ magnification on single cells, either completely isolated or part of a small (2–8) cell clump.

The ratio of UV light emitted at 510 nm following excitation at 340 nm to that emitted after excitation at 380 nm was measured using a Cairn spectrophotometer (excitation was at 96 Hz, data were averaged on-line and collected at 4–16 Hz.). Intracellular $[Ca^{2+}]$ was calculated from this ratio using the Grynkiewiez equation and custom written software ('CAIRNALYSE', obtainable from P.M.S.).

We have previously described a technique for measuring $[Ca^{2+}]_i$ simultaneously and separately at the apical and basal poles of the cell by positioning fibre optic probes over the appropriate parts of the visual field and measuring the fluorescence transmitted via the probe rather than that from the whole visual field [29]. Using $\times 1000$ magnification, $[Ca^{2+}]_i$ could be measured in a circular area approximately 5 μm in diameter. This is sufficient to resolve a single cell into basolateral and apical poles. The position of the apical pole was judged either by the presence of secretory vesicles [7] (control glands only) or by measuring $[Ca^{2+}]_i$ in a single cell from an acinar clump (the cells touch at the apical border). The basal pole was taken as the opposite end of the cell [7].

Solutions

The incubation medium consisted of a modified Hanks balanced salt solution buffered with Hepes.

The experimental medium contained (in mM) 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$ and 10 glucose, buffered to pH 7.4 with 10 mM Hepes.

RESULTS

Dose response to ACh in acinar cells from control glands and from atrophied glands

Figure 1 shows the changes in $[Ca^{2+}]_i$ obtained from acutely isolated parotid acinar cells in response to stimulation with 500 nM ACh, first in the absence and then in the presence of extracellular Ca^{2+}. In cells obtained from both control (Figure 1A) and atrophied (Figure 1B) glands, there was an initial large peak increase in $[Ca^{2+}]_i$, 1–2 s after application of ACh, which, in the presence of extracellular Ca^{2+}, was followed by a decline to a sustained or plateau $[Ca^{2+}]_i$. On average, the plateau $[Ca^{2+}]_i$ was 90 ± 22 nM (S.E.M; $n = 4$) greater than the resting $[Ca^{2+}]_i$ in cells from control glands and 104 ± 27 nM (S.E.M.; $n = 6$) in cells from atrophied glands. The plateau phase of elevated Ca^{2+} was

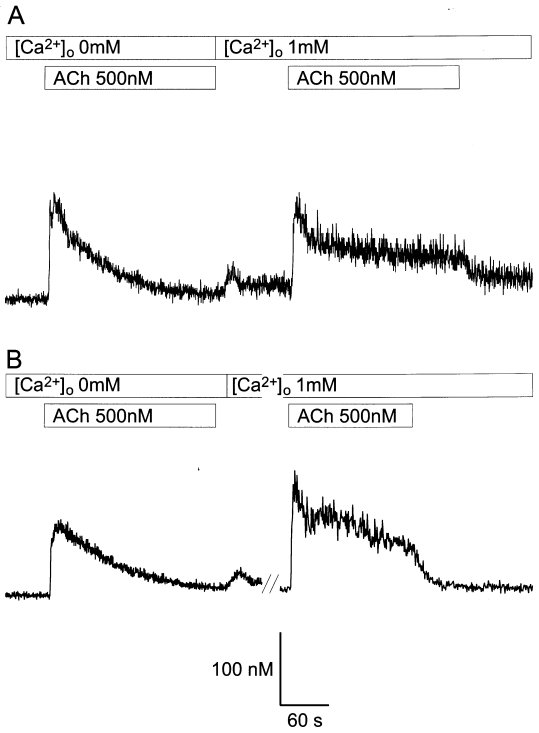

Figure 1 $[Ca^{2+}]_i$ measured in parotid acinar cells derived from control (A) and atrophied (B) glands showing the effect of stimulation with ACh (500 nM) in the absence and then in the presence of extracellular Ca^{2+}

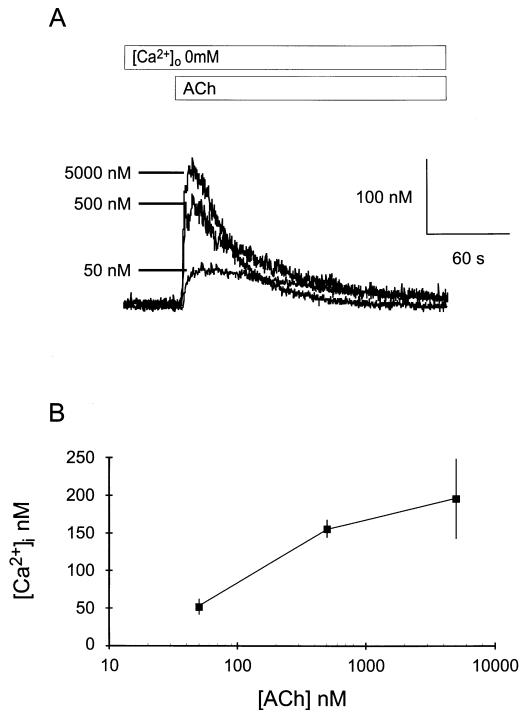

Figure 2 (A) Effect of ACh on $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} and (B) dose-dependence of ACh-induced increase in $[Ca^{2+}]_i$, in parotid acinar cells from control glands

(**A**) Time course and magnitude of the increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} after stimulation with 50, 500 and 5000 nM ACh. (**B**) Dose-dependence of the ACh-induced increase in $[Ca^{2+}]_i$. Each point indicates the mean ± S.E.M. of 3–4 experiments.

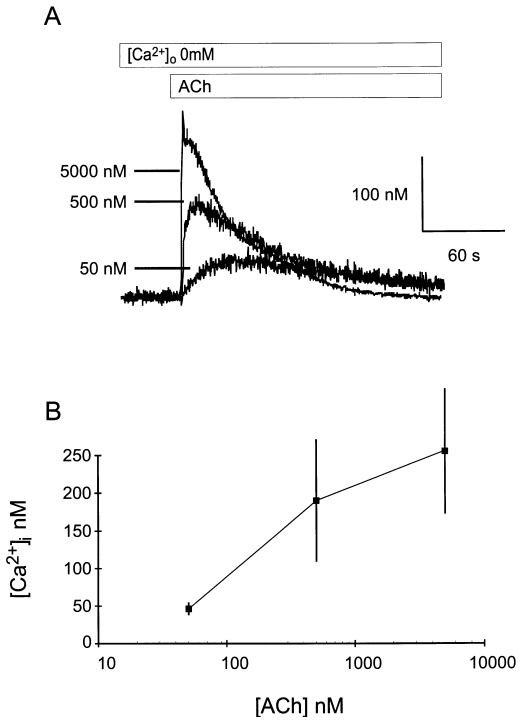

Figure 3 (A) Effect of ACh on $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} and (B) dose-dependence of ACh-induced increase in $[Ca^{2+}]_i$, in parotid acinar cells derived from atrophied glands

(**A**) Time course and magnitude of the increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} following stimulation with 50, 500 and 5000 nM ACh. (**B**) Dose-dependence of the ACh-induced increase in $[Ca^{2+}]_i$. Each point indicates the mean ± S.E.M. of 3–5 experiments.

acutely dependent on the presence of extracellular Ca^{2+}. In the absence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ returned to pre-stimulus levels within 5 min of the application of ACh. The biphasic changes in $[Ca^{2+}]_i$ in response to ACh are typical of exocrine cells [30]; our data support the widely held interpretation that Ca^{2+} release from intracellular stores is primarily responsible for the initial transient increase in $[Ca^{2+}]_i$ and that Ca^{2+} influx sustains the plateau phase of the response. Figure 2(A) shows the time course and magnitude of the increase in $[Ca^{2+}]_i$ following stimulation with 50, 500 and 5000 nM ACh in the absence of extracellular Ca^{2+}. The average peak increase in $[Ca^{2+}]_i$ is plotted in Figure 2(B). These data show the extent to which parotid acinar cells are able to mobilize Ca^{2+} from intracellular stores in response to a wide range of agonist concentrations. Identical experiments were performed with acinar cells obtained from atrophied glands. These data are shown in Figure 3. We found no significant differences in either the time course (Figure 3A) or the magnitude (Figure 3B) of the increase in $[Ca^{2+}]_i$ compared with cells from control glands after ACh stimulation at any concentration.

Table 1 shows the lag between stimulus and response and the magnitude and duration of the response to 50, 500 and 5000 nM ACh in acinar cells obtained from control glands and from atrophied glands. There was no significant difference between the control and the atrophied glands in any of these variables.

Ca^{2+} oscillations

Repetitive transient increases or oscillations in $[Ca^{2+}]_i$, sometimes superimposed on the biphasic increase in $[Ca^{2+}]_i$, have frequently been observed in exocrine acinar cells [27,31], usually in response to low concentrations of ACh (< 100 nM). Repetitive transients have been shown to proceed for extended periods of time in the absence of extracellular Ca^{2+} [30] and are therefore thought to depend on Ca^{2+} release from intracellular stores rather than on Ca^{2+} influx [32–34]. Previous studies in pancreatic acinar cells have shown that brief Ca^{2+} transients may be entirely restricted to the apical pole of the cell [7]. One possibility therefore, is that oscillations in $[Ca^{2+}]_i$ result from Ca^{2+} release and re-uptake into the secretory vesicles. If this is the case, then cells lacking secretory vesicles will be unable to produce oscillatory responses. An oscillatory component of the response to ACh was observed in only a small proportion of parotid acinar cells (≈ 10%) derived either from control glands or from atrophied glands. Oscillations in $[Ca^{2+}]_i$ were observed most frequently with ACh concentrations < 100 nM, but on occasion were also observed with 500 nM ACh. Figure 4 shows the oscillatory component of the response to 50 nM ACh in acinar cells derived from control glands (Figure 4A) and from atrophied glands (Figure 4B). In both cases, $[Ca^{2+}]_i$ oscillations were superimposed on the biphasic increase in $[Ca^{2+}]_i$, and furthermore, in both cases, the increase in baseline $[Ca^{2+}]_i$ was preceded by one or more Ca^{2+} transients.

Ca^{2+} mobilization at the apical and the basolateral poles of the cell

Most of our data represent the average increase in $[Ca^{2+}]_i$ measured over the whole cell. Using the fibre optic probes detailed in the Experimental section [29], we were able to measure changes in $[Ca^{2+}]_i$ at the apical and basolateral poles of the cell

Table 1 Time course and magnitude of the changes in $[Ca^{2+}]_i$ following ACh stimulation in the absence of extracellular Ca^{2+}

The data were obtained using parotid acinar cells from control glands and those from atrophied glands following a 2 week period of duct ligation.

	Time course (s)		$[Ca^{2+}]_i$ (nM)			
[ACh] (nM)	Lag	Duration	Base	Peak	Delta	*n*
Control						
50	17.00 ± 4.00	281.33 ± 30.90	84.67 ± 6.01	139.33 ± 6.94	54.67 ± 8.25	3
500	9.25 ± 2.66	277.75 ± 30.08	91.00 ± 8.69	244.50 ± 11.44	153.50 ± 12.16	4
5000	8.60 ± 4.63	212.00 ± 20.59	90.40 ± 4.35	285.40 ± 55.19	195.00 ± 52.96	5
Atrophied gland						
50	13.50 ± 3.93	315.00 ± 28.72	88.50 ± 4.44	133.50 ± 10.88	45.00 ± 8.68	4
500	6.33 ± 0.88	245.00 ± 35.00	99.67 ± 9.82	289.00 ± 78.34	189.33 ± 81.05	5
5000	4.33 ± 0.88	209.33 ± 17.33	92.67 ± 2.67	349.00 ± 85.04	256.33 ± 83.48	3

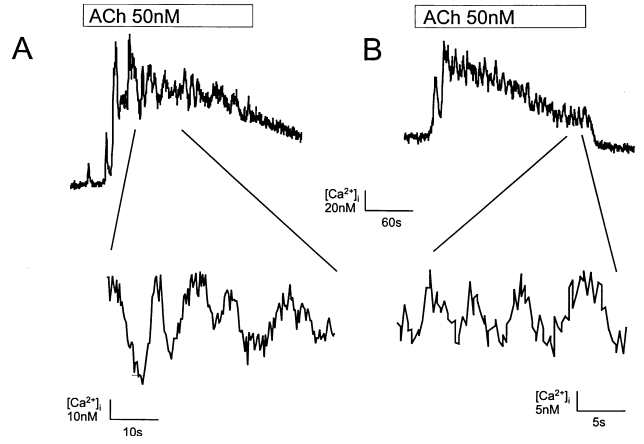

Figure 4 Repetitive transient increases in $[Ca^{2+}]_i$ stimulated by 50 nM ACh measured in parotid acinar cells derived from control glands (A) and from atrophied glands (B)

separately and simultaneously. Figure 5(A) shows the rising phase of the $[Ca^{2+}]_i$ signal obtained by stimulation with 500 nM ACh. The trace obtained from the apical pole of the cell runs exactly parallel to that from the basolateral pole, but is advanced by approximately 600 ms. Several Ca^{2+} transients occur as baseline $[Ca^{2+}]_i$ rises. These may be seen to occur first at the apical pole and then at the basolateral pole of the cell. Figure 5(C) shows the separation of the rise in $[Ca^{2+}]_i$ at the apical and basolateral poles of the cell on an expanded timescale, using averaged data collected during the rising phase of the ACh response from four experiments. These observations are similar to previous findings from pancreatic acinar cells, except that the time interval between Ca^{2+} rising at the apical and the basolateral poles was several times greater (2–3 s) in these cells. We were not able to show separation of the increase in $[Ca^{2+}]_i$ at the apical and basolateral poles of cells derived from atrophied glands. $[Ca^{2+}]_i$ appeared to increase at both poles simultaneously (Figures 5B and 5C). However, the data shown in Figure 5 were obtained at the effective limit of resolution for our system; sampling with greater time resolution simply reduced the signal-to-noise ratio. The diameter of acinar cells from atrophic glands was significantly reduced below that of cells from control glands ($9.1 \pm 0.7\ \mu$m versus $16.1 \pm 0.6\ \mu$m; S.E.M., $n = 10$), which would reduce the time separation between events at the apical and basolateral poles of the cell. Therefore we are unable to determine at this point whether the apparent lack of separation between increased $[Ca^{2+}]_i$ at the apical and basolateral poles in acinar cells from atrophic glands is a function of the absence of secretory vesicles or, rather, a function of reduced cell size such that the separation is too small to resolve.

DISCUSSION

The process of Ca^{2+} mobilization in exocrine acinar cells has two main components, calcium release from intracellular stores and Ca^{2+} influx. There are various strands of evidence which support the 'capacitance model' [35] for the control of Ca^{2+} influx, in which emptying of the intracellular stores acts as a trigger for Ca^{2+} influx. Precisely how the state of depletion of the intracellular stores is communicated to the plasma membrane remains unclear. Various soluble messengers, including 'calcium influx factor' [36] and small G-proteins [37], have been proposed, and there would also appear to be a role for the Ins(1,4,5,)P_3 metabolite Ins(1,3,4,5)P_4 in the Ca^{2+} influx process, at least in lacrimal acinar cells [38,39]. Although these messengers may be shown to be effective in certain cell types and under certain experimental conditions, unfortunately none of them qualifies as a universal signal for Ca^{2+} influx. Some years ago, Marty [16] speculated that secretory vesicles might function as an intracellular Ca^{2+} store and that the fusion of secretory vesicles with the plasma membrane that occurs in exocytosis could also serve to insert Ca^{2+} channels into the plasma membrane, thus linking depletion of intracellular Ca^{2+} stores and Ca^{2+} influx. Although clear evidence for the presence of Ins(1,4,5,)P_3 receptors on secretory vesicles [8] is consistent with this hypothesis, there is little other evidence to support it. Our data show clear evidence of agonist-stimulated Ca^{2+} influx in parotid acinar cells derived from atrophied glands in which there are no secretory vesicles (Figure 1). We conclude that release of Ca^{2+} contained in secretory vesicles is not the trigger for Ca^{2+} influx in the parotid gland, and furthermore, that Ca^{2+} influx is not dependent on the fusion of secretory vesicles with the plasma membrane during exocytosis.

The agonist-induced changes in $[Ca^{2+}]_i$ which follow Ca^{2+} release from intracellular stores may be determined by omitting Ca^{2+} from the extracellular bathing solution. The changes in $[Ca^{2+}]_i$ under such conditions must result from Ca^{2+} release from intracellular stores, including the secretory vesicles. Any contribution that Ca^{2+} release from secretory vesicles makes to the Ca^{2+} signal in control glands should be absent from the Ca^{2+}

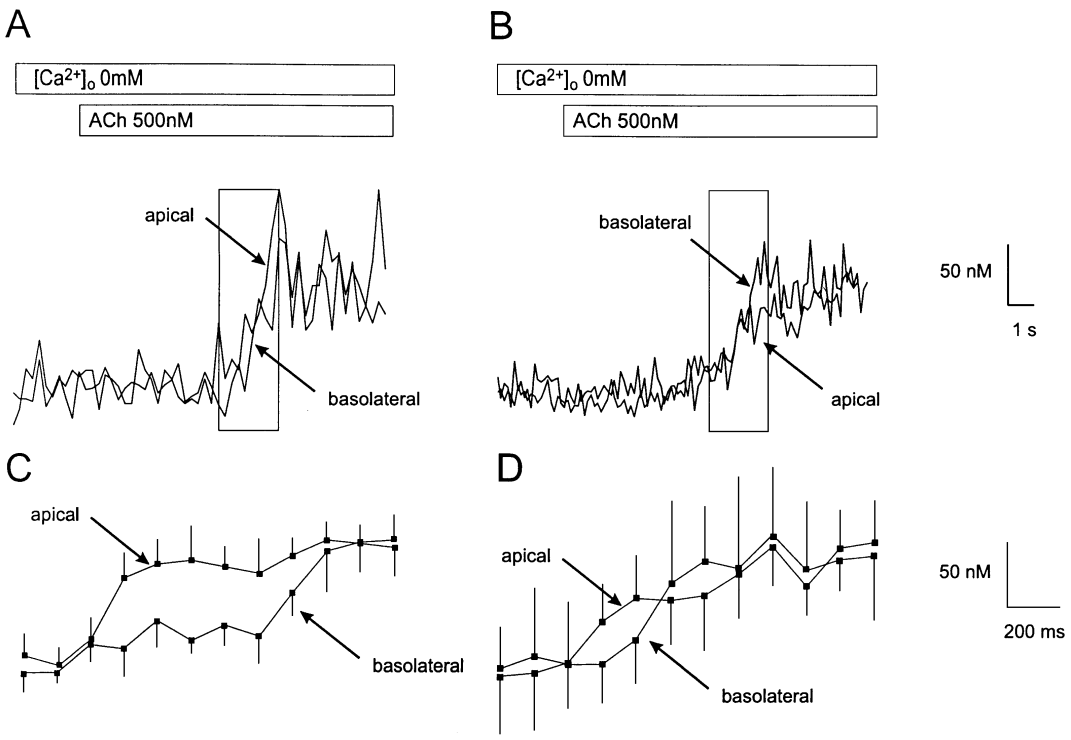

Figure 5 ACh-induced increase in $[Ca^{2+}]_i$ (A and B) and average increase in $[Ca^{2+}]_i$ (C and D) measured at the apical and basolateral poles of parotid acinar cells derived from control glands (A and C) and from atrophied glands (B and D)

The results in (**C**) and (**D**) are shown on an expanded time scale and were collected during the rising phase of the ACh response (shown by the boxed areas of **A** and **B**) from four experiments. The error bars indicate S.E.M.

signal obtained from acinar cells derived from atrophied glands. The most straightforward possibility, that the magnitude of the increase in $[Ca^{2+}]_i$ is smaller in the cells from the atrophied gland, was not observed (Figures 2 and 3). Therefore, Ca^{2+} release from secretory vesicles cannot represent any significant fraction of total Ca^{2+} release from intracellular stores.

It is possible that secretory vesicles act as a triggering event, giving rise to a Ca^{2+} signal which propagates across the cell by Ca^{2+}-induced Ca^{2+} release [40,41]. Calcium signalling mechanisms have previously been shown to contain multiple pathways for elevation of $[Ca^{2+}]_i$ [42,43], and so Ca^{2+} signalling might proceed in the absence of such a trigger signal, but with some alteration in the time course of the response, such as a delay in the onset. The data in Table 1 show that although the lag between stimulus and response decreases with increasing concentration of ACh, there was no difference between cells from control and atrophied glands. Furthermore, we could find no significant difference in the duration of the response.

Repetitive transient or oscillatory increases in $[Ca^{2+}]_i$ are thought to depend on a repeating cycle of Ca^{2+} release from, and re-uptake back into, the intracellular Ca^{2+} stores [32–34]. Various different types of $[Ca^{2+}]_i$ oscillations have previously been observed in exocrine acinar cells [44,45], including the rapid high-frequency 'spikes' [3,27,34] that we have observed in rat parotid acinar cells (Figure 4). In pancreatic acinar cells, these brief transients may be entirely restricted to the apical or secretory pole of the cell [7] where the secretory vesicles are located. It has been proposed that these brief transients result from Ca^{2+} release from the secretory vesicles [8]. An oscillatory component to the increase in $[Ca^{2+}]_i$ elicited by ACh was observed in approximately 10% of cells, either from the control glands or from the atrophied glands. Acinar cells from the atrophied glands were as likely to show an oscillatory component as were cells from the control glands. We could find no substantial difference in the pattern or frequency of transients obtained from either type of cell.

Although we have been unable to find any role for Ca^{2+} release from secretory vesicles in the Ca^{2+} influx process, in the pattern of Ca^{2+} release from intracellular stores, or in the production of repetitive Ca^{2+} transients, it is still possible that Ca^{2+} release from secretory vesicles plays some role in establishing the functional polarity of parotid acinar cells. It has been shown previously in pancreatic [7] and in lacrimal [41] acinar cells that the agonist-evoked Ca^{2+} signal is initiated at the apical pole of the cell. Although a similar polarity in parotid acinar cells has been inferred from previous studies [46], our data provide the first direct evidence that Ca^{2+} signals are also initiated at the apical pole in these cells (Figure 5). We show a separation of 600 ms between the rise in $[Ca^{2+}]_i$ at the apical and basolateral poles of acinar cells from the control group, but we were unable to show any separation between the increase in $[Ca^{2+}]_i$ between the apical and basolateral poles of the acinar cells derived from the atrophied glands. Intracellular Ca^{2+} appeared to increase simultaneously at both poles of the cell. However, cells from the atrophied glands are significantly smaller than those from control glands and, at present, we are unable to determine whether this indicates that secretory vesicles have a real function in the Ca^{2+} release process or simply that the spread of the Ca^{2+} signal across these smaller cells is too small to resolve.

Our data show that Ca^{2+} release from amylase-containing secretory vesicles in rat parotid acinar cells is not a necessary part

of the Ca^{2+} signalling process. Parotid acinar cells from atrophied glands that do not contain secretory vesicles respond to a Ca^{2+}-mobilizing agonist with Ca^{2+} release from intracellular stores and with Ca^{2+} influx in exactly the same way as acinar cells from control glands that do contain secretory vesicles. One possible interpretation of our data is that they indicate a significant difference in the process of Ca^{2+} mobilization in parotid acinar cells as compared with pancreatic acinar cells. Although our data show no role for Ca^{2+} release from secretory vesicles in the Ca^{2+} signalling process in parotid acinar cells, we cannot conclude that this is the case in pancreatic acinar cells. It has recently been reported that, following additional purification procedures in the isolation of pancreatic zymogen granules, Ins(1,4,5,)P_3 receptor activity can no longer be detected [47]. These authors conclude that any Ins(1,4,5,)P_3-mediated Ca^{2+} release from a zymogen granule preparation is most likely to be due to contamination by other membrane components such as endoplasmic reticulum. It is not clear how contamination by endoplasmic reticulum could account for all the data presented by Gerasimenko et al. [8], nevertheless, additional studies will be required to determine whether the zymogen granules play any major part in the Ca^{2+} signalling process in pancreatic acinar cells. In any event, our data indicate that Ca^{2+} release from secretory vesicles cannot be a universal component of Ca^{2+} signalling common to all acinar cell types.

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