Identical regional mechanisms of intracellular free Ca^{2+} concentration increase during polarized agonist-evoked Ca^{2+} response in pancreatic acinar cells

Emil C. TOESCU,*† David V. GALLACHER† and Ole H. PETERSEN†

*Department of Physiology, University of Birmingham, School of Medicine, Edgbaston, Birmingham B15 2TT, and †MRC Secretory Control Group, Physiological Laboratory, Crown Street, P.O. Box 147, Liverpool L69 3BX, U.K.

The initial increase of intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) following agonist stimulation is spatially restricted to one pole of the cell, from where a wave of $[\text{Ca}^{2+}]_i$ spreads across the cytosol. In the present study we have investigated the dynamic properties of the agonist-activated Ca^{2+} -release mechanisms in different regions of the acinar cell and show that, during maximal agonist stimulation, the rate of $[\text{Ca}^{2+}]_i$ increase at the secretory

pole is identical with that recorded at the basal pole. Furthermore, the relationship between $[\text{Ca}^{2+}]_i$ and the apparent rate of $[\text{Ca}^{2+}]_i$ increase is similar in both regions of the cell. The data show that whereas the sensitivity to the Ca^{2+} -releasing agent is different in different regions of the cell, the process of $[\text{Ca}^{2+}]_i$ increase, once triggered, will proceed in an identical fashion, irrespective of the area of the cell.

INTRODUCTION

Activation of muscarinic and cholecystokinin receptors on pancreatic acinar cells evokes a rapid increase of the concentration of intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$), which mediates the secretory response of these cells [1]. The source of this initial $[\text{Ca}^{2+}]_i$ increase is the intracellular Ca^{2+} pools and, at least in the initial moments, the release process is due to the activation of Ca^{2+} -release channels by the intracellular second messenger $\text{Ins}(1,4,5)\text{P}_3$ (InsP_3). When analysed with digital Ca^{2+} -imaging techniques, it became apparent that, in functionally and morphologically polarized cells such as the exocrine cells, the initial increase of $[\text{Ca}^{2+}]_i$ has a particular spatial organization. The Ca^{2+} signal is generated initially at the secretory pole of the cell, from where a Ca^{2+} wave spreads across the cytosol [2–4]. Recently it has been shown that this pattern of $[\text{Ca}^{2+}]_i$ increase can be reproduced by cytosolic perfusion of InsP_3 [5,6]. Whereas it becomes more and more clear that InsP_3 -mediated Ca^{2+} release is responsible for the initial increase of $[\text{Ca}^{2+}]_i$ in the secretory pole, where the type 3 InsP_3 receptor is preferentially localized [7], the mechanisms involved in the subsequent increases of $[\text{Ca}^{2+}]_i$ in the rest of the cytosol are not yet well established. Recently, Kasai et al. [6] proposed the existence, in pancreatic acinar cells, of three different subcellular regions, which are functionally distinct with respect to the type and kinetics of Ca^{2+} release.

Analysis of the mechanisms involved in the spread of the Ca^{2+} wave is complicated by the fact that measurements of $[\text{Ca}^{2+}]_i$ following agonist stimulation will include the opposing effects of activation of both the plasma membrane Ca^{2+} pump [8] and the Ca^{2+} -entry pathway [9]. We have recently demonstrated that La^{3+} , at a concentration of 1 mM, effectively 'seals' the pancreatic acinar cells from extracellular Ca^{2+} [10]. In this condition, and in the presence of a maximal concentration of agonist, which effectively prevents the reuptake of Ca^{2+} into the internal pools, the increase of $[\text{Ca}^{2+}]_i$ depends only on the release of Ca^{2+} from intracellular pools and the passive buffering in the cytosol. Our results show that, apart from temporal differences, the rate of $[\text{Ca}^{2+}]_i$ increase is identical in different regions of the pancreatic

acinar cells. Thus the previously reported temporal differences in Ca^{2+} signalling between the secretory and the basolateral poles of the pancreatic acinar cells [2–6] reflect mainly differences in the sensitivity of the Ca^{2+} -releasing channels rather than differences in the kinetics of Ca^{2+} release.

MATERIALS AND METHODS

Preparation of cells

A mixture of individual cells and small clusters of acinar cells was obtained from isolated mouse pancreata by enzymic dispersion in a water bath at 37 °C as previously described [3]. Briefly, the intact glands were injected with a collagenase solution (200 units/ml; Worthington), the glands were incubated for 7–15 min and finally manually agitated to yield the isolated cell preparation. Throughout the preparation procedure and subsequently, during incubation and perfusion, a buffer solution containing (mM) NaCl (140), KCl (4.7), CaCl_2 (1.1), MgCl_2 (1.1), glucose (10) and Hepes (10), pH 7.2 (adjusted with NaOH, with an osmolality of 295 mOsm/kg) was used. In most experiments, 1 mM LaCl_3 was present during the experiment.

Ca^{2+} imaging and data analysis

After loading [1 μM fura-2 acetoxymethyl ester (fura-2/AM); 30 min at room temperature] the cells were washed and used within 3–4 h. During experiments, the cells, placed on a glass coverslip attached to an open Perspex (Lucite) perfusion chamber, were continuously perfused from a gravity-fed perfusion system at room temperature.

The images were captured using a Nikon Diaphot inverted microscope, an intensified charge coupled device camera (Photonic Science Inc.) and recorded on a MagiCal station (Applied Imaging). Other details of the Ca^{2+} imaging were described previously [3]. The fluorescence signals were captured, at video-frame rate (0.16 s intervals between two true ratio images), with a 20 $\times$ -magnification objective. In each field, comprising several

Abbreviations used: $[\text{Ca}^{2+}]_i$, intracellular free Ca^{2+} concentration; InsP_3 , $\text{Ins}(1,4,5)\text{P}_3$; CRA, Ca^{2+} -releasing agent; fura-2/AM, fura-2 acetoxymethyl ester; ACh, acetylcholine; InsP_3R , InsP_3 receptor.

† To whom correspondence should be addressed.

cells, those cells responding to agonist were analysed by calculating the mean $[Ca^{2+}]_i$ value within areas defined with a light-sensitive pen on a bright-field image showing the morphology of cells. The areas used for these measurements were of a similar size to those used by us previously [3,16] and comparable with the 'trigger' zone described by Kasai et al. [6]. Owing to biological variability and to the inherently variable geometry of our perfusion method, the lag time between the start of agonist application and the initiation of Ca^{2+} response varied from one experiment to another. To compare agonist-evoked Ca^{2+} signals in different experiments, we have chosen to normalize the Ca^{2+} signal to the first time point of the $[Ca^{2+}]_i$ increase (defined as the first point of increase of $[Ca^{2+}]_i$ which is followed by two consecutive higher values of $[Ca^{2+}]_i$). Thus, each data point presented in our figures represents a mean of 19 individual values obtained from 4 separate experiments.

Fura-2/AM fluorescence was calibrated using the cells loaded in the normal way and perfused subsequently either with 10 mM EGTA or with 10 mM Ca^{2+} , in the presence of 2 mM ionomycin.

Materials

Chemicals were purchased from Merck-BDH and Sigma, except for fura-2/AM, which was obtained from Novo-Calibiochem.

RESULTS AND DISCUSSION

Previous studies [2–4] have shown that, at the single-cell level, the site of initial $[Ca^{2+}]_i$ increase following agonist stimulation is the secretory pole. Figure 1(a), showing the mean $[Ca^{2+}]_i$ increase following stimulation with a maximal concentration of the muscarinic agonist acetylcholine (ACh, 5 μ M), illustrates this effect (results represent the mean for 19 individual cells, sampled in four individual experiments). During the experimental protocol the perfusion medium also contained 1 mM La^{3+} . With this

Figure 1 Agonist-stimulated release of Ca^{2+}

(a) and (b) Increase of $[Ca^{2+}]_i$ in the secretory (■) and the basal (□) pole after stimulation of pancreatic acinar cells with 5 μ M ACh in the presence of 1 mM La^{3+} . (c) and (d) Rate of $[Ca^{2+}]_i$ increase measured as the first derivative of $[Ca^{2+}]_i$ in respect to time ($d[Ca^{2+}]_i/dt$). In (b) and (d) the traces corresponding to the basal pole (□) were shifted along the temporal axis by 0.3 s. Time $t = 0$ represent the first detectable increase of $[Ca^{2+}]_i$ as defined in the Materials and methods section. For all traces the symbols represent the mean values for 19 cells (recorded in four individual experiments), and for reasons of clarity the error bars ($< 10\%$ of the mean value) have been omitted.

Figure 2 Relationship between $[Ca^{2+}]_i$ and the apparent rate of $[Ca^{2+}]_i$ increase

Data presented are derived from the experimental values depicted in Figure 1. The values corresponding to the secretory and the basal pole are shown as ■ and □ respectively.

maximal agonist concentration, and in the presence of La^{3+} , which effectively blocks Ca^{2+} transport across the plasma membrane [10], the increase of $[Ca^{2+}]_i$ reflects only the release of Ca^{2+} from intracellular Ca^{2+} pools. The data were normalized, as described in the Materials and methods section, to the first detectable increase of $[Ca^{2+}]_i$ at the secretory pole. The initial increase in the secretory pole is followed, within 0.3 s, by a similar increase in the basolateral pole of the cells. Inspection of Figure 1(a) suggests that the Ca^{2+} -release processes taking place at the two opposite poles of the cell are very similar. Indeed, a simple translation along the time axis of the curve describing the $[Ca^{2+}]_i$ increases at the basolateral pole (Figure 1b) yields superimposable traces. Figure 1(c) shows the derived apparent mean rate of Ca^{2+} release (calculated as $d[Ca^{2+}]_i/dt$) at the two poles of the cell. Again translation along the time axis results in practically superimposable traces. The rate of Ca^{2+} release increases during the first second, reaching a peak of 900 nM Ca^{2+} /s, after which it starts to decay. The 'noise' inherent in the calculation of Ca^{2+} release rates at higher values of $[Ca^{2+}]_i$ renders the interpretation of the latter parts of the traces difficult. These results might seem to contradict some recently published data [6] which show that, in rat pancreatic acinar cells, the rate of Ca^{2+} release at the secretory pole is higher than that recorded at the basal pole of the cells. One, rather unlikely, possibility is that of species differences. However, under normal conditions the rate of Ca^{2+} increase also depends on the plasma-membrane Ca^{2+} -transport systems. On the timescale of interest here (up to 1–2 s from the initiation of the increase of $[Ca^{2+}]_i$), it is unlikely that the ' Ca^{2+} -store depletion'-activated Ca^{2+} -entry pathway is sufficiently active to influence the events [10]. In contrast, the plasma-membrane Ca^{2+} -ATPase is a powerful Ca^{2+} -transport mechanism which can be activated quite early during the agonist-evoked Ca^{2+} response [8,10]. Differences in the Ca^{2+} -sensitivity and/or activation kinetics of the plasma-membrane Ca^{2+} -ATPase between the two opposite regions of the mouse pancreatic acinar cells might therefore influence the results. To avoid these complications we have used the La^{3+} -' Ca^{2+} -sealing' technique, in which 1 mM La^{3+} acts as an inhibitor of both Ca^{2+} entry and plasma-membrane Ca^{2+} -ATPase [10]. The present findings allow us to suggest that, in terms of release of Ca^{2+} from the intracellular pools, the same mechanism is activated at both functional poles of the cells.

Comparison of Figures 1(b) and 1(d) indicate that the maximal rate of Ca^{2+} release is reached at values of cytosolic Ca^{2+} of about 0.6 μ M. Figure 2, in which the apparent rate of Ca^{2+} release in

each region of the cell is plotted against the level of $[\text{Ca}^{2+}]_i$ for each time point, analyses in more detail this relationship. Between resting levels and 600 nM $[\text{Ca}^{2+}]_i$ the apparent rate of Ca^{2+} release increases monotonically as a function of cytosolic Ca^{2+} . Further increases of $[\text{Ca}^{2+}]_i$ beyond 600 nM did not affect the rate of release, whereas values higher than 1 μM $[\text{Ca}^{2+}]_i$ inhibit the rate of Ca^{2+} release. The bell-shaped curve shown in Figure 2, obtained in an intact-cell system, is reminiscent of the Ca^{2+} -dependency of isolated InsP_3 -sensitive Ca^{2+} -release channels incorporated in lipid bilayers [11]. The decrease of the apparent rate of Ca^{2+} release seen at values of $[\text{Ca}^{2+}]_i$ higher than 1 μM (Figure 2) could be due to either of two processes: the intrinsic Ca^{2+} -induced inhibition of Ca^{2+} release [11] and/or the progressive depletion of internal pools. With the experimental protocol used here we cannot differentiate between these two possibilities. However, the most important feature is that the same relationship between $[\text{Ca}^{2+}]_i$ and the rate of Ca^{2+} release is present both at the secretory and the basolateral pole of the pancreatic acinar cells. This observation, together with the finding that, when the temporal difference for the initiation of the release process is accounted for, the rate of $[\text{Ca}^{2+}]_i$ increase at the secretory pole is identical with that recorded at the basal pole of the cells (Figure 1), indicates that the same mechanisms for $[\text{Ca}^{2+}]_i$ increase are involved at the two opposite poles, secretory and basal, of the pancreatic acinar cells.

Most of the current physiological models explaining the localization of the initial increase of $[\text{Ca}^{2+}]_i$ to the luminal pole envisage the participation of InsP_3 -mediated processes. In the present experiments we measured directly only the end result of agonist-dependent stimulation of the Ca^{2+} -mobilizing pathway, i.e., the release of Ca^{2+} from the intracellular stores, and did not address the question of the nature of the Ca^{2+} -releasing agent (CRA) involved. In addition to the well-established CRAs, such as InsP_3 and Ca^{2+} [1,12], there is now evidence indicating that cyclic ADP-ribose is also a CRA [13]. In pancreatic acinar cells, cyclic ADP-ribose evokes repetitive Ca^{2+} spikes localized in the secretory pole [14].

Since it is unlikely that the agonist receptors display a regional clustering around the secretory pole [15], it has been proposed that the polarization of the Ca^{2+} response reflects a heterogeneity of the Ca^{2+} pools with respect to their sensitivity to InsP_3 in such a way that the pools in the secretory granule area are more sensitive to InsP_3 . This view is supported by the demonstration that a minimal agonist concentration can evoke repetitive $[\text{Ca}^{2+}]_i$ spikes strictly confined to the secretory pole [5,16]. Similar localized Ca^{2+} signals can be elicited, in combined whole-cell patch clamp and $[\text{Ca}^{2+}]_i$ imaging, by cytosolic perfusion of a low InsP_3 concentration [5]. More recently, the case for the heterogeneity of InsP_3 receptor (InsP_3R) distribution in pancreatic acinar cells has been strengthened by immunocytochemical evidence that the type 3 InsP_3R is preferentially localized to the secretory pole [7]. While supporting the hypothesis of heterogeneous Ca^{2+} release, the present data highlight another aspect: the apparent dissociation between the InsP_3 -sensitivity and the Ca^{2+} -release process. The current view is that the same InsP_3R molecule contains the InsP_3 -binding domain and also forms the Ca^{2+} channel complex which gates the release of Ca^{2+} [17]. In our experiments, the increase of $[\text{Ca}^{2+}]_i$ appears to be identical at the two opposing poles of the pancreatic acinar cells, which display, nevertheless, different sensitivities to InsP_3 [5,6]. This observation suggests that the release of Ca^{2+} from the intracellular stores, which under our experimental conditions is the only mechanism underlying the increase of $[\text{Ca}^{2+}]_i$, is an 'all-or-nothing' process; the InsP_3 receptors in the two regions of the cell have, apparently, different sensitivities for InsP_3 activation of

Figure 3 Diagram of the 'all-or-none' Ca^{2+} -release model

(a) Represents the pancreatic acinar cells under three conditions: A, pre-stimulation; B, immediately following agonist stimulation; C, at least 0.3 s after B. The density of the hatched pattern within the cell reflects the concentration of the Ca^{2+} -releasing agent (CRA), whereas the stippled area indicates release of Ca^{2+} from intracellular pools. In (b) the temporal relationship between the concentration of the agonist-evoked Ca^{2+} -releasing agent ($[\text{CRA}]_i$) (on the left ordinate) and the internal $[\text{Ca}^{2+}]_i$ (right ordinate) at the secretory (SP) and basal pole (BP) is described. The T_s and T_g levels denote the threshold levels of CRA at which Ca^{2+} release occurs at the secretory and basal pole respectively. Once initiated, the release of Ca^{2+} has an identical rate in the two regions.

the Ca^{2+} -release channel, but once their respective threshold has been reached, the kinetics of Ca^{2+} release and its sensitivity to $[\text{Ca}^{2+}]_i$ is very similar (Figure 3). With this interpretation, the temporal delay between the increase of $[\text{Ca}^{2+}]_i$ at the secretory pole and that recorded at the basal is due entirely to the time taken for the cytosolic concentration of InsP_3 to increase sufficiently to activate the lower sensitivity InsP_3Rs in the basolateral regions. Since the diameter of a typical pancreatic acinar cell is 15–20 μm , and the range of action of InsP_3 is of the same magnitude (17 μm ; [18,19]), it is unlikely that the temporal difference is due to a diffusion barrier to InsP_3 . The discrepancy between the differential sensitivity to the actions of the CRAs in different parts of the cell and the similar Ca^{2+} -release properties could also explain the results obtained by internal perfusion of InsP_3 [5,6]. Local application of InsP_3 in the basolateral region of the cell induces only a localized increase of $[\text{Ca}^{2+}]_i$, because only locally is there sufficient InsP_3 to reach the threshold for Ca^{2+} release (Figure 3). In the rest of the basolateral region of the cell the concentration of InsP_3 is too low to generate release, whereas in the secretory pole the same concentration will trigger Ca^{2+} release.

Theoretically, the temporal delay, intimately related to the idea of heterogeneous sensitivity of InsP_3R , could also be explained by the existence of an 'induction factor'. This putative factor would be released at the secretory pole and would then diffuse across the cytosol, progressively sensitizing Ca^{2+} pools to InsP_3 . Since the same pattern of Ca^{2+} wave propagation is seen following both agonist stimulation and internal perfusion with InsP_3 [5,6], it is unlikely that this factor is exclusively dependent on specific agonist stimulation. More likely it would be a factor co-released by InsP_3 , similar to the release of the Ca^{2+} -influx

factor [20]. No experimental data are currently available to allow a critical examination of this hypothesis.

However, irrespective of the fine detail of the regulation of InsP_3R , the present observations are consistent with the hypothesis that the Ca^{2+} pools most sensitive to InsP_3 are situated at the secretory pole and that, this location, which would allow for agonist-evoked Ca^{2+} signals strictly localized to this pole, is bound to have an important physiological role. Current models of rodent pancreatic-fluid secretion invoke the existence of Ca^{2+} -dependent chloride channels at the secretory pole [2,21]. Recently, direct capacitance measurements showed that increases of $[\text{Ca}^{2+}]_i$ at the secretory pole are associated with exocytotic events [22]. The coexistence within the same receptor structure of a powerful 'all-or-none' Ca^{2+} -release process with a differential InsP_3 -sensing molecular device, spatially segregated, would provide the cells with a flexible and adaptable mechanism of Ca^{2+} -mediated signal transduction.

This work was funded by grants from the Medical Research Council.

REFERENCES

- Petersen, O. H., Petersen, C. C. H. and Kasai, H. (1994) *Annu. Rev. Physiol.* **56**, 297–319
- Kasai, H. and Augustine, G. J. (1990) *Nature (London)* **348**, 735–738
- Toescu, E. C., Lawrie, A. M., Petersen, O. H. and Gallacher, D. V. (1992) *EMBO J.* **11**, 1623–1629
- Nathanson, M. H., Padfield, P. J., O'Sullivan, A. J., Burgsthaler, A. D. and Jamieson, J. D. (1992) *J. Biol. Chem.* **267**, 18118–18121
- Thorn, P., Lawrie, A. M., Smith, P. M., Gallacher, D. V. and Petersen, O. H. (1993) *Cell* **74**, 661–668
- Kasai, H., Li, Y. X. and Miyashita, Y. (1993) *Cell* **74**, 669–677
- Nathanson, M. H., Fallon, M. B., Padfield, P. J. and Maranto, A. R. (1994) *J. Biol. Chem.* **269**, 4693–4696
- Tepikin, A. V., Voronina, S. G., Gallacher, D. V. and Petersen, O. H. (1992) *J. Biol. Chem.* **267**, 3569–3572
- Tsunoda, Y. (1993) *Biochim. Biophys. Acta* **1154**, 105–156
- Toescu, E. C. and Petersen, O. H. (1994) *Pflüger's Arch* **427**, 325–331
- Bezprozvanny, I., Watras, J. and Ehrlich, B. E. (1991) *Nature (London)* **351**, 751–754
- Berridge, M. J. (1993) *Nature (London)* **361**, 315–325
- Lee, H. C. (1993) *J. Biol. Chem.* **268**, 293–299
- Thorn, P., Gerasimenko, O. and Petersen, O. H. (1994) *EMBO J.* **13**, 2038–2043
- Rosenzweig, S. A., Miller, L. J. and Jamieson, J. D. (1983) *J. Cell Biol.* **96**, 1288–1297
- Lawrie, A. M., Toescu, E. C. and Gallacher, D. V. (1993) *Cell Calcium* **14**, 698–710
- Mikoshiba, K., Furuichi, T., Miyawaki, A., Yoshikawa, S., Nakagawa, T., Yamada, N., Hamanaka, Y., Fujina, I., Michikawa, T., Ryo, Y., Okano, H., Fujii, S. and Nakade, S. (1993) *Philos. Trans. R. Soc. London B* **340**, 345–349
- Albritton, N. L. and Meyer, T. (1993) *Cell Calcium* **14**, 691–697
- Kasai, H. and Petersen, O. H. (1994) *Trends Neurosci.* **17**, 95–100
- Randriamampita, C. and Tsien, R. Y. (1993) *Nature (London)* **364**, 809–814
- Petersen, O. H. (1992) *J. Physiol. (London)* **448**, 1–51
- Maruyama, Y., Inooka, G., Li, Y., Miyashita, Y. and Kasai, H. (1993) *EMBO J.* **12**, 3017–3022