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Receptor-activated cytoplasmic Ca^{2+} spikes in communicating clusters of pancreatic acinar cells

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The transmission of receptor-generated cytoplasmic Ca^{2+} signals between communicating pancreatic acinar cells has been investigated by comparing patch-clamp recordings of Ca^{2+}-dependent Cl^- current in internally perfused single cells and small multi-cell clusters. Acetylcholine (50 nM) generates shortlasting repetitive spikes of Ca^{2+}-dependent current and these spikes are not transmitted to neighbouring cells. Cholecystokinin octapeptide (5 pM) also generates repetitive spikes, but a significant proportion of these trigger longer and larger pulses of Ca^{2+}-dependent current and these waves can easily spread from cell to cell. In pancreatic acinar units it is therefore possible to observe both local Ca^{2+} signals confined to the cell of its origin as well as Ca^{2+} signals that spread through communicating junctions to all cells in the unit.

Ca^{2+} spike; Cell communication; Acetylcholine; Cholecystokinin

1. INTRODUCTION

Receptor-activated fluctuations in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) have mainly been studied in single isolated cells [1]. There are a few studies indicating synchronous repetitive Ca^{2+} signals in cells linked by junctional channels [1,2] and in a recent imaging study of small parotid acinar cell clusters a maximal dose of carbachol evoked a synchronized Ca^{2+} rise in all cells [3]. The pancreatic acinar tissue is organized in units (acini) containing hundreds of individual cells linked by junctional channels allowing electrical communication [4] as well as the passage of fluorescent probes such as procion yellow (MW 697) from cell to cell [5]. The lateral plasma membranes have a very high density of gap junctions as revealed by electron microscopy of freeze-fractured tissue [6] and patch-clamp studies on pairs of cells have shown discrete junctional channel conductances varying from about 120 to 20 pS [7]. In small isolated clusters of pancreatic acinar cells the cholecystokinin (CCK) analogue caerulein evokes regular Ca^{2+} spikes [8] suggesting that $[Ca^{2+}]_i$ fluctuations can occur synchronously in these coupled cells. Low acetylcholine (ACh) concentrations can evoke Ca^{2+} spikes detected near the plasma membrane as short pulses of Ca^{2+}-dependent Cl^- current which are not reflected in the average $[Ca^{2+}]_i$ measurements performed simultaneously in the same single cells by microfluorimetry using the dye fura-2 [9]. It was the purpose of this study to investigate whether such Ca^{2+} spikes in the vicinity of the plasma membrane are transmitted to neighbouring coupled cells and to compare the transmission of ACh-evoked Ca^{2+} spikes with those induced by CCK.

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2. MATERIALS AND METHODS

Fragments of mouse pancreas were digested by pure collagenase (200 U/ml, 20 min, 37°C) washed and pipetted. This produced a mixture of single cells and clusters of varying sizes. In previous work on Ca^{2+} oscillations [9–12] we only selected the single cells for experimentation, but in the present study we also used the small acinar cell clusters as we had done earlier when investigating Na^+-alanine cotransport in the same cells [13]. The tight-seal, whole-cell current recording configuration of the patch-clamp technique [14] was used for measurement of the transmembrane current in single cells or small cell clusters as previously described [13]. We have previously shown that small mouse pancreatic acinar cell clusters investigated by using the patch-clamp whole-cell current recording configuration are well coupled since both the current carried by the Na^+-alanine cotransporter and the membrane capacitance increased in proportion to the number of cells in the clusters [13]. The Ca^{2+}-dependent Cl^- currents were measured with the two-voltage pulse protocol as previously described [9–12]. The control extracellular solution contained (mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.0, $MgCl_2$ 1.13, HEPES 10 (pH 7.2) and glucose 10. The unit under investigation was continuously exposed to a flow of control solution or control solution containing acetylcholine (ACh) (Sigma) or cholecystokinin octapeptide (sulphated) (CCK). The intracellular pipette solution contained (mM): KCl 140, Na_2ATP 1, $MgCl_2$ 1.13, glucose 10 and HEPES 10 (pH 7.2). All experiments were carried out at room temperature.

3. RESULTS

In a previous study on single cells we showed that ACh (50 nM) evoked repetitive shortlasting spikes of

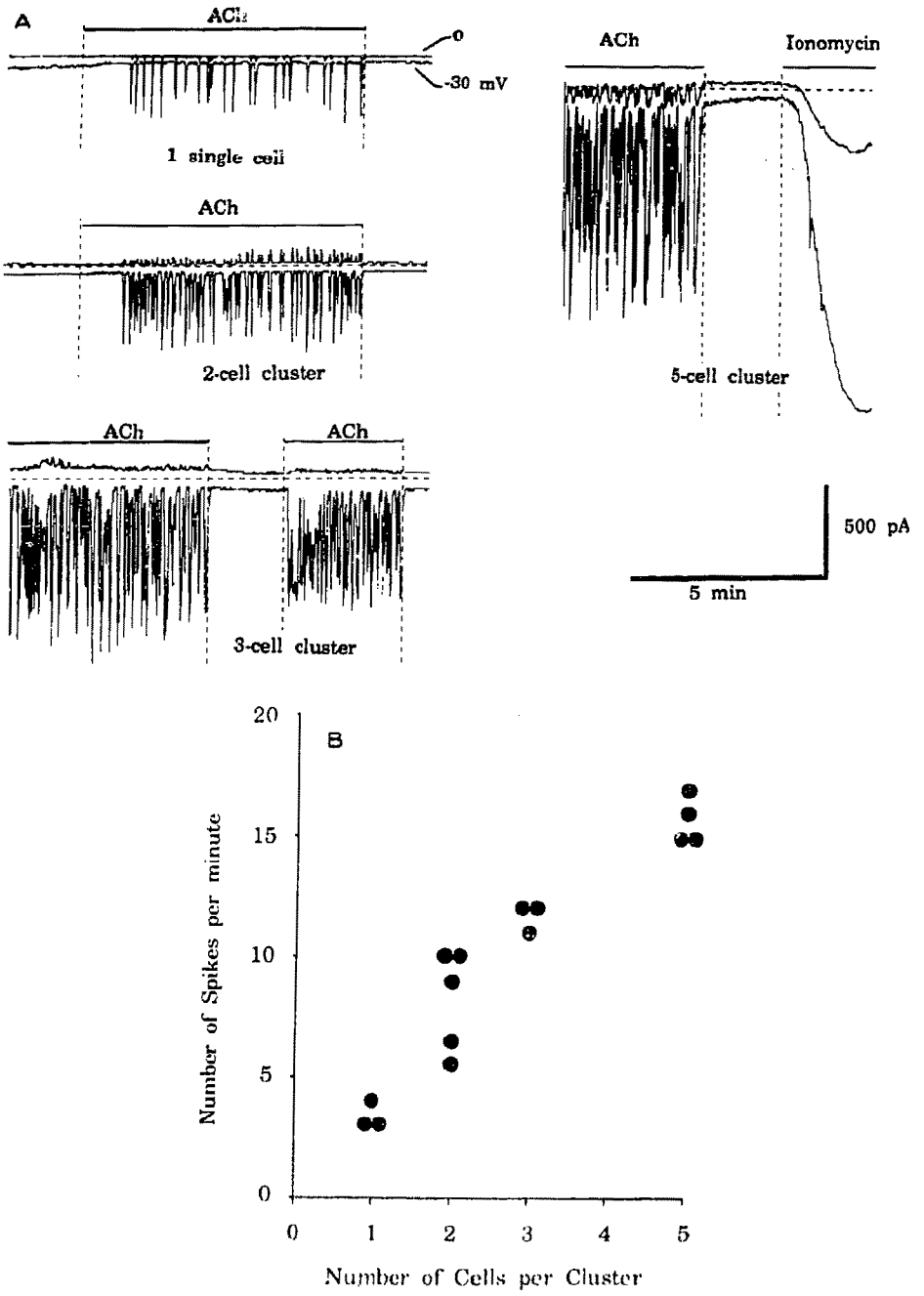

Fig. 1. (A) The effect of ACh (50 nM) on the transmembrane Ca^{2+}-dependent current in a single cell, a 2-cell cluster, a 3-cell cluster and a 5-cell cluster. In the 5-cell cluster the effect of ionomycin (200 nM) was also assessed. The acinar units were voltage-clamped at a holding potential of −30 mV and depolarizing voltage jumps of 150 ms duration to a membrane potential of 0 mV were repetitively applied throughout the experiments. Because of the compression of the current traces the records seem to show currents at −30 mV (bottom traces) and 0 mV (top traces) simultaneously. At 0 mV there are only relatively small current fluctuations as the Cl^- equilibrium potential is close to zero. At −30 mV there is a large electrical gradient favouring Cl^- efflux and during ACh stimulation when the Ca^{2+}-dependent Cl^- channels open, due to an increase in $[Ca^{2+}]_i$, inward current (downwards deflections) flows. The dashed horizontal lines represent the zero-current level. (B) Plot of spike frequency during stimulation with ACh (50 nM) as a function of acinar cluster size. Each point represents one experiment.

Ca^{2+}-dependent current and that these spikes were only rarely followed by larger and longer pulses of Ca^{2+}-dependent current [12]. Fig. 1 shows a recording of this type from a single cell where ACh (50 nM) reversibly induced repetitive shortlasting current spikes. We compared the effects of 50 nM ACh in cell clusters of varying sizes and Fig. 1A shows examples of such traces. The frequency of ACh-evoked spikes increased with increasing cluster size (Fig. 1B). The apparent spike amplitude varied much more in the multi-cell

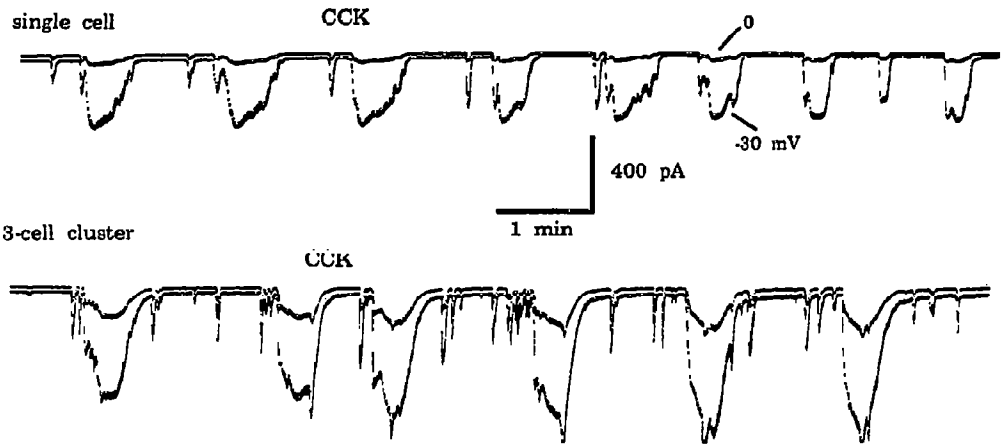

Fig. 2. CCK-evoked spikes and waves of Ca^{2+}-dependent current in a single cell and a 3-cell cluster. In both cases CCK was present at a concentration of 5 pM.

clusters than in the single cells and the maximal current amplitude increased with increasing cluster size (Fig. 1A). The maximal current evoked by 50 nM ACh did not correspond to the maximal Ca^{2+}-activated current since the Ca^{2+} ionophore ionomycin (200 nM) always evoked larger responses (Fig. 1A) (n = 8).

In our previous study on single cells we showed that CCK (5–10 pM) evoked repetitive shortlasting spikes like those induced by ACh (50 nM), but the probability of a spike triggering a larger and longer current pulse (wave) was much higher for CCK than ACh [12]. Fig. 2 shows an example where CCK (5 pM)-evoked short-lasting spikes are seen to initiate larger pulses (waves) relatively frequently. In small cell clusters (two doublets, three 3-cell and one 4-cell cluster) the spike frequency was increased as compared to the single cells and the amplitude of the waves was much larger than the maximal spike amplitude (Fig. 2).

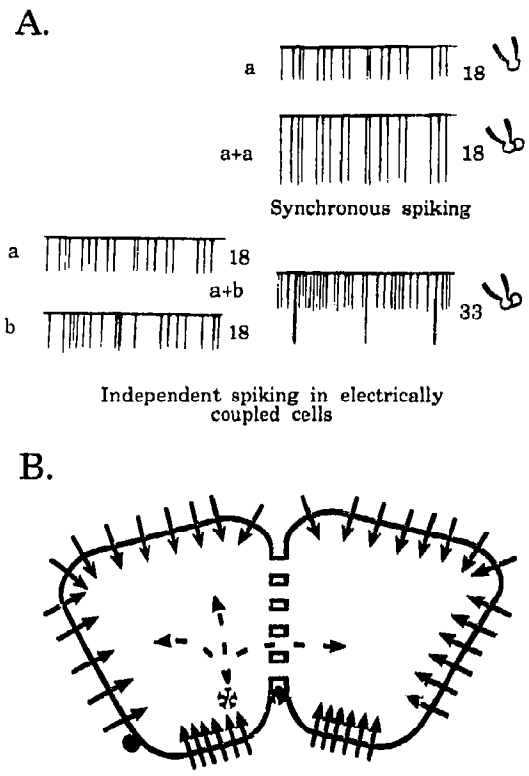

Fig. 3. (A) Schematic illustration of the theoretical Ca^{2+}-dependent current patterns for synchronous and independent Ca^{2+} spiking in electrically coupled cells. A particular spike pattern has been arbitrarily selected for a single cell a showing 18 spikes in the time frame chosen. If this pattern were also to be imposed on a neighbouring coupled cell the current trace obtained by recording from the doublet $a+a$ would be identical to the single-cell trace except that the amplitudes of the spikes would be doubled (assuming that the cells have the same size and channel density). This case seems to fit the findings relating to the CCK-evoked long waves (Fig. 2). If, however, two coupled cells have independent spiking mechanisms the patterns in cells a and b would be different but similar. We have chosen a pattern for cell b that again gives 18 spikes in the time frame. During whole-cell current recording from the double $a+b$ we would simply get the sum of the currents generated in cells a and b and this is also shown. The spike frequency almost doubles (33 spikes). It does not exactly double because some spikes occur simultaneously, in those cases giving double amplitudes. This case corresponds to the findings concerning the ACh-evoked short-lasting spikes (Fig. 1). (B) Schematic diagramme showing most likely site of receptor-activated cycloplasmic Ca^{2+} signal generation and routes of spreading. The asterisk shows the luminal site of Ca^{2+} signal initiation according to a recent imaging study [15]. In cases of low ACh concentration repetitive Ca^{2+} spikes are probably confined to this region. The broken arrows represent routes of Ca^{2+} signal spreading including passage through junctional channel to neighbouring cell. The arrows across the surface cell membranes represent Ca^{2+}-dependent ionic current mainly through Cl^- channels [10,15]. The density of Ca^{2+}-dependent Cl^- channels is particularly high in the luminal membrane [15].

4. DISCUSSION

If the short-lasting ACh-evoked Ca^{2+} spikes were transmitted to neighbouring coupled cells the amplitude, but not the frequency should increase with increasing cluster size. If on the other hand the ACh-evoked spikes are confined to the cell where they originate, the frequency, but not the amplitude, should increase with increasing cluster size (Fig. 3A). Fig. 1 shows that the frequency of ACh-evoked spikes increases with increasing cluster size indicating that spikes are generated independently of each other in the individual cells. In clusters the spike amplitude varies more than in single cells. The larger apparent spike amplitudes in the multi-cell clusters where the spike frequency is increased as compared to the single cells can be explained by superimposition of independent current spikes from electrically coupled cells (Fig. 3A). The CCK-evoked repetitive spikes also occur at a higher frequency in small cell clusters than in single cells, but this is not the case for the longer and larger waves triggered by some of the spikes which have a much larger amplitude in the clusters than in the single cells (Fig. 2) suggesting that the spikes are confined to the cells of their origin whereas the waves spread throughout the cellular network.

Fig. 3B illustrates schematically the most likely primary Ca^{2+} spike generation site and the routes of Ca^{2+} signal spreading. A recent digital imaging study of $[Ca^{2+}]_i$ changes in pancreatic acinar cells after maximal muscarinic receptor activation indicates that the Ca^{2+} signal is initiated in the luminal pole of the cell and only thereafter spreads to the basal part [15]. The repetitive Ca^{2+} spikes evoked by low ACh concentrations [9] may therefore be confined to the luminal cell pole and our results indicate that these signals do not spread to neighbouring cells. In a previous study we had concluded that CCK can more easily generate spreading Ca^{2+} waves within single cells than ACh [12]. Our new results indicate that the CCK-evoked waves can easily spread to neighbouring cells whereas the short-lasting spikes cannot.

The receptor-mediated regulation of Ca^{2+} signal spreading previously shown at the level of single cells [12] and now extended to include cell-to-cell communication gives considerable scope for fine regulation of the spatial distribution of $[Ca^{2+}]_i$ elevations. It is possible to have both local asynchronous as well as synchronous Ca^{2+} signalling according to the needs of the system.

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