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Different patterns of receptor-activated cytoplasmic Ca^{2+} oscillations in single pancreatic acinar cells: dependence on receptor type, agonist concentration and intracellular Ca^{2+} buffering

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Agonist-specific cytosolic Ca^{2+} oscillation patterns can be observed in individual cells and these have been explained by the co-existence of separate oscillatory mechanisms. In pancreatic acinar cells activation of muscarinic receptors typically evokes sinusoidal oscillations whereas stimulation of cholecystokinin (CCK) receptors evokes transient oscillations consisting of Ca^{2+} waves with long intervals between them. We have monitored changes in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) by measuring Ca^{2+}-activated Cl^- currents in single internally perfused mouse pancreatic acinar cells. With minimal intracellular Ca^{2+} buffering we found that low concentrations of both ACh (50 nM) and CCK (10 pM) evoked repetitive short-lasting Ca^{2+} spikes of the same duration and frequency, but the probability of a spike being followed by a longer and larger Ca^{2+} wave was low for ACh and high for CCK. The probability that the receptor-evoked shortlasting Ca^{2+} spikes would initiate more substantial Ca^{2+} waves was dramatically increased by intracellular perfusion with solutions containing high concentrations of the mobile low affinity Ca^{2+} buffers citrate (10-40 mM) or ATP (10-20 mM). The different Ca^{2+} oscillation patterns normally induced by ACh and CCK would therefore appear not to be caused by separate mechanisms. We propose that specific receptor-controlled modulation of Ca^{2+} signal spreading, either by regulation of Ca^{2+} uptake into organelles and/or cellular Ca^{2+} extrusion, or by changing the sensitivity of the Ca^{2+}-induced Ca^{2+} release mechanism, can be mimicked experimentally by different degrees of cytosolic Ca^{2+} buffering and can account for the various cytosolic Ca^{2+} spike patterns.

Key words: acetylcholine/adenosine triphosphate/Ca^{2+} oscillation/cholecystokinin

Introduction

It is well established that Ca^{2+} plays a crucial role in stimulus-secretion coupling (Douglas, 1968). The stimulus-induced cytoplasmic Ca^{2+} signal can be evoked by Ca^{2+} entry through voltage-gated Ca^{2+} channels (Hosey and Lazdunski, 1988) or by release of Ca^{2+} from intracellular Ca^{2+} stores (Berridge and Irvine, 1984, 1989). The pancreas provides examples of both since the insulin-secreting islet cells possess voltage-gated Ca^{2+} channels which can be opened by glucose- or K^+-induced membrane depolarization (Pralong *et al.*, 1990) whereas in the exocrine pancreatic acinar cells the acetylcholine (ACh)-evoked Ca^{2+} signal is mediated by a steady inositol (1,4,5) trisphosphate [Ins (1,4,5) P_3] induced Ca^{2+} release from a non-mitochondrial store (Streb *et al.*, 1983) that in turn causes repetitive Ca^{2+}-induced Ca^{2+} release from a caffeine-sensitive store (Osipchuk *et al.*, 1990; Petersen and Wakui, 1990; Wakui *et al.*, 1990b).

Agonist-specific cytosolic Ca^{2+} oscillation patterns have been described in liver cells (Woods *et al.*, 1987; Rooney *et al.*, 1989; Sanchez-Bueno *et al.*, 1990). In single-cell microfluorimetric experiments on pancreatic acinar cells, acetylcholine (ACh) is seen to evoke sinusoidal Ca^{2+} oscillations about an elevated mean value (Yule and Gallacher, 1988) whereas cholecystokinin (CCK) evokes repetitive discrete Ca^{2+} transients at a relatively low frequency arising from a constant basal level (Osipchuk *et al.*, 1990; Yule *et al.*, 1991). Two different types of cytosolic Ca^{2+} oscillation patterns (Berridge, 1990) would therefore appear to be present in the pancreatic acinar cells. In internally perfused pancreatic acinar cells low concentrations of ACh as well as intracellular Ins (1,4,5) P_3 infusion can evoke repetitive short-lasting Ca^{2+} spikes near the cell membrane, monitored by recording the Ca^{2+}-dependent Cl^- current, but these spikes are mostly not resolved by single-cell microfluorimetry using fura 2. When a higher concentration of ACh is used or caffeine is added, the Ca^{2+} transients become broader and are now seen synchronously in both types of recording (Osipchuk *et al.*, 1990). Because of the great sensitivity of the electrophysiological method for detecting Ca^{2+} spikes near the cell membrane (Osipchuk *et al.*, 1990) we have used patch-clamp current recording from single internally perfused pancreatic acinar cells to explore the apparently different cytosolic Ca^{2+} oscillation patterns evoked by ACh and CCK and their underlying mechanisms.

We now report that under minimal intracellular Ca^{2+} buffering conditions relatively low concentrations of both CCK and ACh evoke repetitive short-lasting Ca^{2+} spikes of the same duration and frequency. In the case of ACh stimulation these short spikes only rarely initiate longer pulses of Ca^{2+} release whereas with CCK the probability that a short spike is followed by a longer and larger Ca^{2+} wave is much higher. The probability that the short ACh-evoked Ca^{2+} spikes initiate more substantial Ca^{2+} waves is dramatically increased when the intracellular perfusion solution contains a high concentration of either citrate or ATP. Our data indicate that the different Ca^{2+} oscillation patterns normally induced by ACh and CCK are not caused by separate mechanisms. We propose that the mobile low affinity Ca^{2+} buffers citrate and ATP reduce rapid Ca^{2+} reuptake and extrusion following the primary release, allowing Ca^{2+} diffusion and further Ca^{2+} induced Ca^{2+} release. CCK stimulates the formation of Ca^{2+} waves through the cell by Ca^{2+}-induced Ca^{2+} release more effectively than ACh and this effect may also be mediated

by inhibition of the active processes removing Ca^{2+} from the cytosol or by increasing the sensitivity of the Ca^{2+}-induced Ca^{2+} release mechanism.

Results

The effects of ACh with low intracellular buffer concentration

In previous studies we have investigated the effects of ACh on Ca^{2+}-dependent Cl^- currents using an intracellular perfusion solution containing 0.25 mM of the Ca^{2+} chelator EGTA and 5 mM ATP (Wakui *et al.*, 1989, 1990b; Osipchuk *et al.*, 1990). Because one of the objectives of the present study was to investigate the effects of adding relatively large amounts of Ca^{2+} buffer to the intracellular perfusion solutions it seemed useful as a control to employ solutions with minimal buffering capacity. We therefore had no EGTA in the pipette solutions perfusing the cell interior and reduced the ATP concentration to 1 mM.

Figure 1 shows a continuous recording from one cell in which the effects of 50 and 500 nM ACh are compared. At the low concentration of 50 nM, ACh always evoked repetitive spikes taking off from the baseline. These spikes were brief (a few seconds duration) and occurred at a frequency that varied from cell to cell between 2 and 6 per min. It was rare for a short spike to be followed by a longer Ca^{2+} wave and in Figure 1 there is only one example of this (occurring early in the first period of 50 nM ACh stimulation and seen more easily in the blown-up version of this part of the trace displayed in part c of Figure 4). At the higher ACh concentration of 500 nM a sustained signal with superimposed quasi-sinusoidal oscillations was observed. The individual spikes were considerably longer at this higher ACh level ($\sim$10 s) than those seen at 50 nM. Elevation of the ACh concentration therefore evokes an increase in the fraction of time during which $[Ca^{2+}]_i$ is augmented and also leads to a larger amplitude of the Ca^{2+} signal. The different Ca^{2+} oscillation patterns evoked by low and high ACh concentrations could be reproduced several times in the same cell, but there was a tendency as also illustrated in Figure 1, for the spike frequency, particularly at the low ACh concentration, to be reduced late in the experiment. The most important point is that in every one of the 11 control experiments carried out, 50 nM ACh evoked repetitive shortlasting spikes, of the type shown in Figure 1, only occasionally followed by longer and larger pulses.

The effects of CCK8 with low intracellular buffer concentration

In a previous study (Osipchuk *et al.*, 1990), the effects of a single concentration of CCK were tested during internal perfusion with solutions containing 5 mM ATP and 0.25 mM EGTA. The CCK preparation used by Osipchuk *et al.* (1990) was desulphated CCK8 which is $\sim$1000$\times$ less potent than the physiological sulphated CCK8 (Yu *et al.*, 1990) employed in the experiments reported here where the internal perfusion solution contained no EGTA and only 1 mM ATP.

Figure 2 shows a continuous recording from a single cell in which the effects of different CCK8 doses were compared. Five pM CCK8 was a threshold concentration. Out of 7 cells tested 2 did not respond, but both of these cells were sensitive to 10 pM CCK8. In 3 cells 5 pM CCK8 induced an irregular pattern of short spikes (a few seconds duration) and larger waves (always initiated by a short spike) as seen in Figure 2, whereas more regular broad waves appeared in the two other cells. 10 pM CCK8 consistently gave the type of response shown in Figure 2 in all the 5 cells tested. There were regular short-lasting spikes and $\sim$20% of these brief spikes were seen to initiate a longer ($\sim$15–30 s duration) and larger (increased amplitude) wave. The highest CCK8 concentration tested was 50 pM which gave either a quasi-sustained response or a regular dense wave pattern as seen in Figure 2. Regulation of the Ca^{2+} signal by CCK8 seems mainly to occur by control of the probability that short spikes are followed by longer and larger pulses (waves). This probability increases with increasing CCK8 concentration. In the low concentration range there is also regulation of spike frequency.

Comparison of ACh- and CCK8-evoked responses in the same cells with low intracellular buffer concentration

In three cases we compared the actions of 50 nM ACh and 10 pM CCK8 during continuous recordings from single cells.

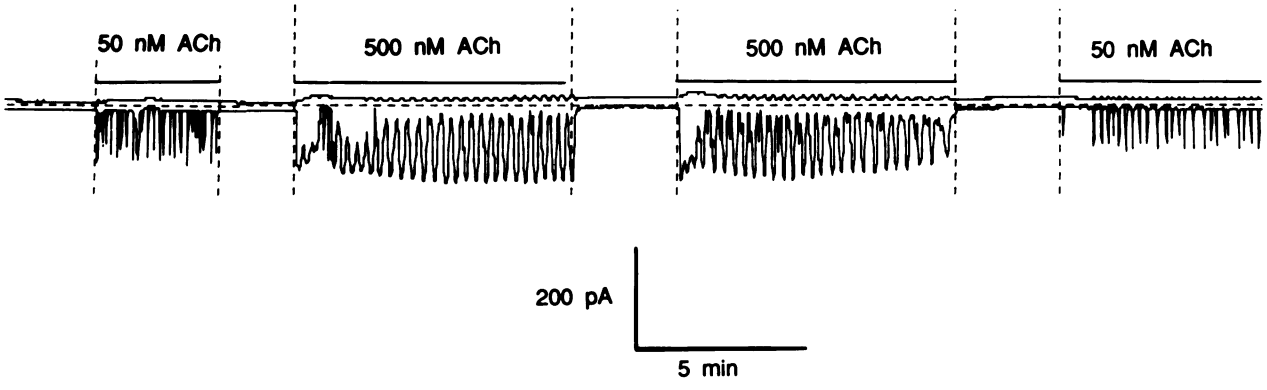

Fig. 1. The effects of ACh (50 and 500 nM) on the transmembrane current from a single mouse pancreatic acinar cell. The acinar cell was voltage-clamped at a holding potential of −30 mV and depolarizing voltage jumps of 150 ms duration to a membrane potential of 0 mV were repetitively applied throughout the experiment. Because of the compression of the current trace the record seems to show currents at −30 mV (bottom trace) and 0 mV (top trace) simultaneously. At 0 mV there are only very small current fluctuations as the Cl^- equilibrium potential (E_{Cl-}) 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) is seen. The dashed horizontal line represents the zero-current level. The initial 50 nM ACh stimulation period is also displayed for comparative purposes in Figure 4c.

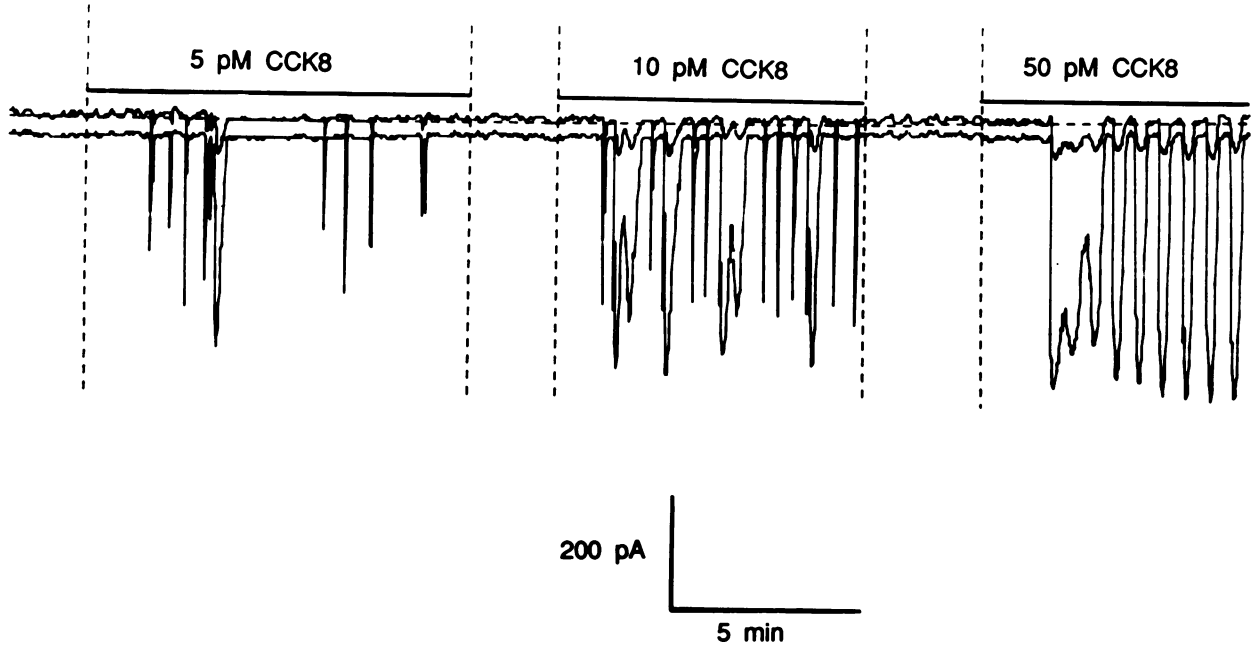

Fig. 2. The effects of CCK8 (5, 10 and 50 pM) on the Ca^{2+}-dependent Cl^- current. Continuous record from a single cell. Voltage protocol as in Figure 1.

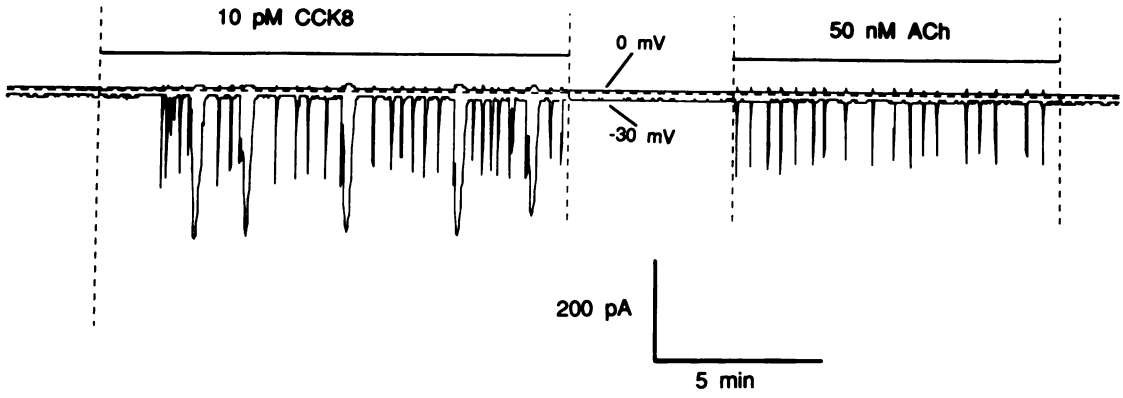

Fig. 3. The effects of CCK8 (10 pM) and ACh (50 nM) in the same cell.

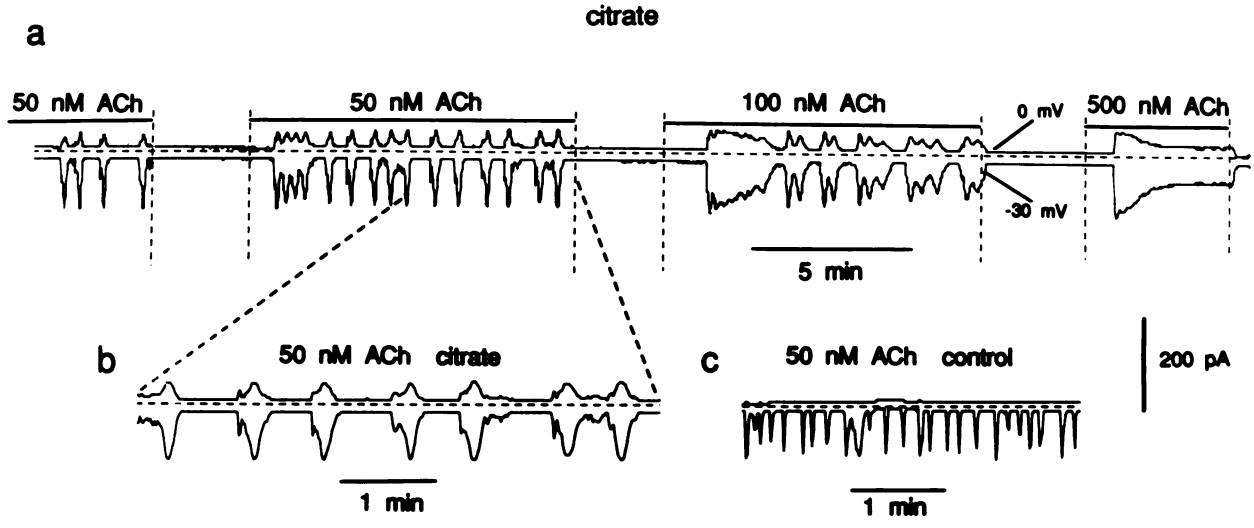

Fig. 4. The effects of ACh in an experiment where the pipette solution contained 10 mM citrate. **a**, effects of 3 ACh doses in a continuous recording from one cell. **b**, expanded record of part of the trace shown in **a**. For comparison with the pattern of ACh-evoked Ca^{2+} spiking during control conditions **c** shows part of the control trace displayed in Figure 1 on an expanded time scale (first period of 50 nM ACh stimulation). Note that E_{Cl^-} was about -5 mV in **a** and **b**, but about 0 in **c**.

Figure 3 shows that CCK8 and ACh both evoked repetitive short-lasting spikes of the same shape and with the same frequency, but in the case of the CCK8 stimulation, a substantial fraction of the short-lasting spikes initiated larger and longer waves whereas this phenomenon was not seen during the ACh stimulation period.

The effects of ACh during intracellular perfusion with solutions containing 10 or 40 mM citrate

The effects of ACh were dramatically different from control when the pipette solution contained 10 mM citrate. Figure 4 shows the result of an experiment in which 50 nM ACh evoked short-lasting spikes that were always followed by longer and larger waves. These spike−wave configurations were separated by relatively long silent intervals. For comparison Figure 4b and 4c show traces from control and citrate experiments side by side. The waves observed in the citrate experiments had durations of 10−60 s and mostly occurred at frequencies of 0.5−1.0/min. The pattern shown in Figure 4 was observed in 7 out of 13 cells tested. In 3 experiments there was no response to 50 nM ACh, but at the slightly higher concentration of 100 nM, ACh evoked the same pattern of long waves with long intervals in between. Of the remaining three experiments, one displayed the low frequency typical of citrate experiments, but the spikes were brief and in two experiments a mixture of short-lasting spikes and longer waves were seen. The latency of the ACh-evoked response was always much longer during internal citrate perfusion than under control conditions (compare Figures 1 and 4). The initial part of the ACh response in the citrate experiments often consisted of a spike initiating multiple waves, thereafter a silent interval occurred followed by repeated single spike−single wave configurations. At the higher ACh concentration of 100 nM the initial response was often a quasi-sustained one followed by repetitive clusters of waves (Figure 4a). At 500 nM, ACh evoked sustained responses (Figure 4a). The amplitude of the Ca^{2+} waves did not increase with increasing ACh concentration, but the fraction of time during which $[Ca^{2+}]_i$ was elevated increased with increasing agonist dose (Figure 4a).

As seen in Figure 4 there were outward current responses at 0 mV membrane potential in addition to the inward current responses at −30 mV during internal citrate perfusion, whereas during control conditions the responses were only seen as inward currents at −30 mV. This is simply due to the fact that when citrate was present in the pipette solution the Cl^- concentration was reduced so as to keep a constant osmolarity. While the equilibrium (Nernst) potential for Cl^- (E_{Cl-}) was about 0 mV for the control condition it was therefore negative (about −5 mV) in the citrate experiments. This change in E_{Cl-} had as expected no effect on the time course of the Ca^{2+}-dependent Cl^- currents. In some of the experiments (not shown) citrate was simply added to the control pipette solution and osmotic balance kept by adding sucrose to the external (bath) solution. In such cases E_{Cl-} was about 0 mV and there were consequently no outward current responses at 0 mV, but the pattern of the inward current responses at −30 mV was similar to the one observed in experiments with the reduced Cl^- concentration in the pipette solution.

A few experiments were also carried out with a pipette solution containing 40 mM citrate. In all three cells investigated ACh (50 nM) evoked long waves with a low frequency. In one extreme case waves lasted more than 5 min with intervals of about 5 min.

The effects of CCK8 during intracellular perfusion with 10 mM citrate

The effects of intracellular citrate were much the same on the CCK-evoked responses as they were on those induced by ACh. As seen in Figure 5 short spikes were in each case followed by longer and larger waves. As with ACh, the initial response to CCK8 was a spike followed by a very long wave and thereafter regular spike−wave complexes appeared separated by silent intervals. As for ACh, dose-dependent regulation was seen with CCK8 in such a way that with increasing agonist concentration the fraction of time during which $[Ca^{2+}]_i$ was elevated increased (Figure 5). Three experiments of the type shown in Figure 5 were carried out giving similar results.

The effects of ACh and CCK8 during intracellular perfusion with 10 or 20 mM ATP

In view of the marked difference between the ACh (50 nM)-evoked cytosolic Ca^{2+} fluctuation patterns during internal perfusion with solutions containing 1 mM ATP and those with solutions containing 1 mM ATP and citrate (Figures 1 and 4) we realized that previously published records from experiments in which internal solutions containing 5 mM ATP had been routinely used as controls often showed signs of the 'citrate effect' [Figure 1a in Wakui *et al.* (1989), Figures 1a and 2a in Wakui *et al.* (1990a) and Figure 1 in Wakui *et al.* (1990b) all show this phenomenon whereas

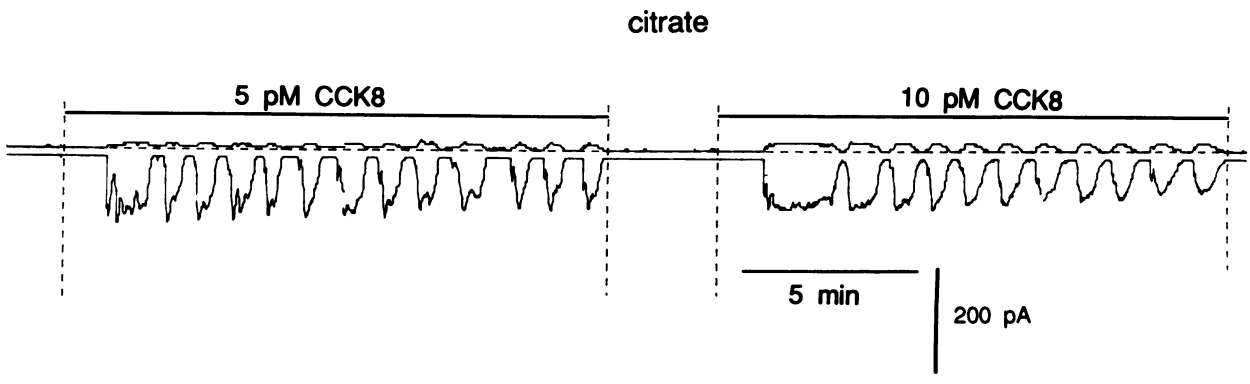

Fig. 5. The effects of CCK8 (5 and 10 pM) in an experiment where the pipette solution contained 10 mM citrate.

Figure 2 in Osipchuk *et al.* (1990) does not]. We investigated therefore the effects of increasing the ATP concentration in the pipette solutions even further. Results very similar to those seen with citrate were obtained during internal perfusion with solutions containing 10 or 20 mM ATP. The latency for ACh (50 nM) responses were long compared to control experiments (several minutes) and long waves separated by long silent intervals (in many cases several minutes) were observed during stimulation with ACh or CCK8 (5 pM) (n = 15). An extreme example of ACh-evoked long waves between long silent intervals is shown in Figure 6. In such cases a clear separation between a spike and the following wave could not be observed.

Discussion

Our data demonstrate for the first time that the pattern of receptor-activated cytoplasmic Ca^{2+} waves can be dramatically changed by varying the intracellular Ca^{2+} buffer composition. High concentrations of the diffusible low affinity chelators citrate or ATP (pK $\approx$3.5 and 4, respectively) (Durham, 1983; Sillen and Martell, 1971) markedly reduce the frequency and prolong the duration of the repetitive Ca^{2+} transients. An enhanced intracellular buffer concentration increases the probability that a short ACh-evoked Ca^{2+} spike is followed by a longer wave to such an extent that every short spike initiates a longer Ca^{2+} pulse (Figure 4). The probability that the short CCK-evoked spikes are followed by longer waves is also markedly enhanced by internal citrate perfusion (Figure 5), but since the ACh-evoked spikes only occasionally trigger longer waves in the control situation whereas this phenomenon occurs regularly during CCK8 action, the citrate effect is particularly impressive in the case of ACh stimulation. Citrate can in effect transform the ACh-evoked control pattern into one somewhat resembling that normally induced by CCK8.

It has been clearly demonstrated that there are spatial Ca^{2+} concentration inhomogeneities during receptor-activated Ca^{2+} oscillations (Kasai and Augustine, 1990; Osipchuk *et al.*, 1990; Rooney *et al.*, 1990; Jacob, 1990) and that Ca^{2+} waves progress through cells via a regenerative mechanism (Rooney *et al.*, 1990) involving Ca^{2+}-induced Ca^{2+} release (Wakui *et al.*, 1990b; Malgaroli *et al.*, 1990). The relatively high internal citrate or ATP concentrations used in our experiments provide non-saturable and essentially linear Ca^{2+} buffering, probably diminishing the spatial Ca^{2+} concentration inhomogeneity during receptor activation. The increased cytosolic Ca^{2+} buffering may promote generation of cytosolic Ca^{2+} waves by enabling Ca^{2+} to diffuse from one release site to neighbouring locations causing further Ca^{2+} release (Figure 7). From our previous work (Osipchuk *et al.*, 1990) it is known that the short cytosolic Ca^{2+} spikes evoked by low ACh concentrations are confined to the space close to the plasma membrane Cl^- channels, whereas the longer-lasting signals evoked by higher ACh concentrations or by CCK are seen in the cell at large.

If CCK is able either to evoke inhibition of Ca^{2+} reuptake into intracellular organelles and/or Ca^{2+} extrusion from the cell, or to enhance the sensitivity of Ca^{2+}-induced Ca^{2+} release (Figure 7) then this would explain why CCK-evoked Ca^{2+} spikes could more easily initiate longer Ca^{2+} waves than the same type of spikes evoked by ACh (Figure 3). The ability of CCK to produce long waves of Ca^{2+}-dependent Cl^- current is not due to a specific action

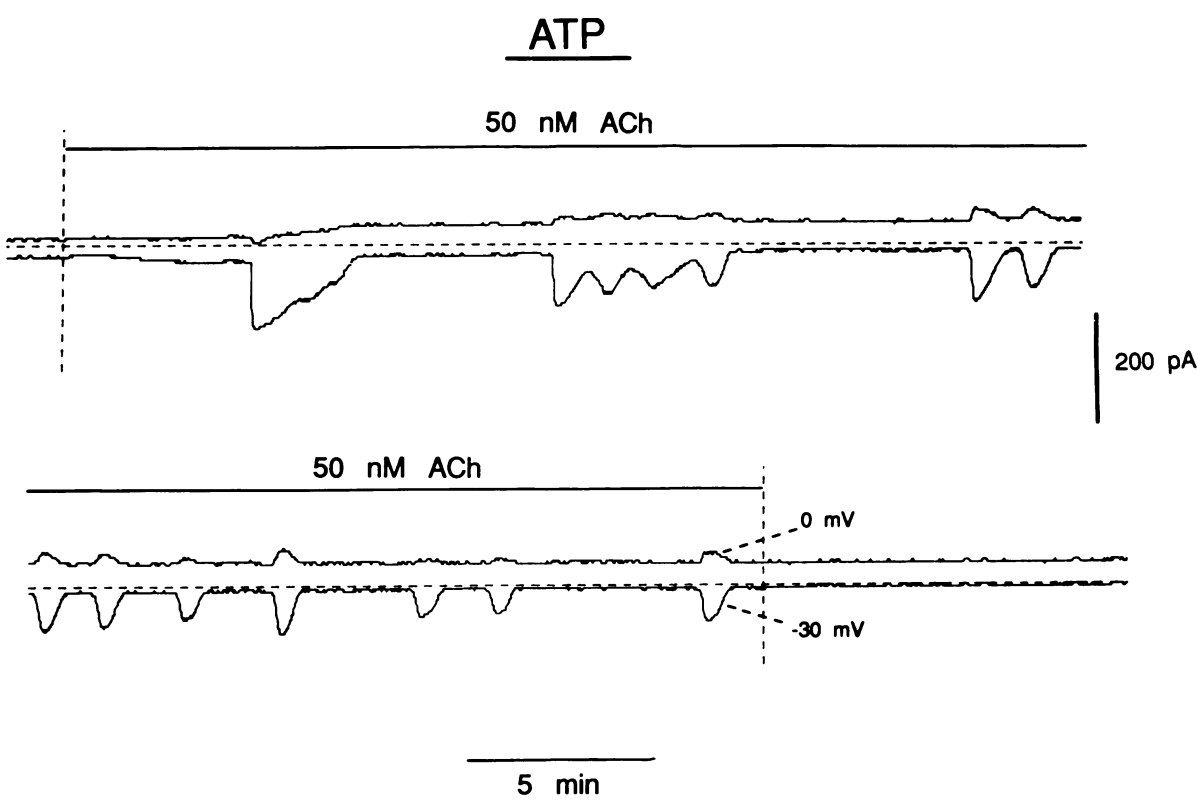

Fig. 6. The effect of ACh (50 nM) in an experiment where the pipette solution contained 20 mM ATP. The lower part of the trace is a direct continuation of the upper one.

promoting Ca^{2+} influx since CCK-evoked wave responses can be seen in the internally perfused mouse pancreatic acinar cells in the absence of extracellular Ca^{2+} (Wakui and Petersen, unpublished observations) as is the case for the ACh-induced fluctuations (Wakui *et al.*, 1989). CCK-evoked transient Ca^{2+} oscillations in intact cells have also recently been shown not to be acutely dependent on extracellular Ca^{2+} (Yule *et al.*, 1991). The CCK-evoked Ca^{2+} waves activate more Cl^- current than the spikes, probably because more Ca^{2+} is released causing a larger rise in $[Ca^{2+}]_i$ and also because a larger number of Cl^- channels are reached as the Ca^{2+} wave spreads to come into contact with larger parts of the Cl^- conducting membrane.

In secretory epithelia, such as pancreatic acini, the Cl^- channels are likely to be predominantly localized to the luminal membrane (Figure 7) (Petersen, 1986) and in this context it is interesting that a recent digital imaging study on small clusters of rat pancreatic acinar cells has shown that the initial ACh-evoked cytosolic Ca^{2+} signal is localized at the luminal pole of the cells where is activates an early Cl^- current (Kasai and Augustine, 1990). Our data presented here, based on recordings of Ca^{2+}-dependent Cl^- current, may therefore mainly reflect Ca^{2+} signalling events near the luminal membrane. Figure 7 attempts to explain the effects of citrate buffering on the spreading of the Ca^{2+} signal. Part of the Ca^{2+} primarily released close to the membrane receptors would normally be prevented from diffusing away from the release site by Ca^{2+} reuptake into the Ca^{2+} stores, and by extrusion from the cell. In the presence of citrate or excess ATP, some of the Ca^{2+} primarily released is immediately bound and can therefore more easily diffuse towards the cell interior where further Ca^{2+}-induced Ca^{2+} release can occur.

The short-lasting Ca^{2+} transients seen under control conditions could in part be due to high localized Ca^{2+} concentrations exerting a negative feedback on the Ca^{2+}

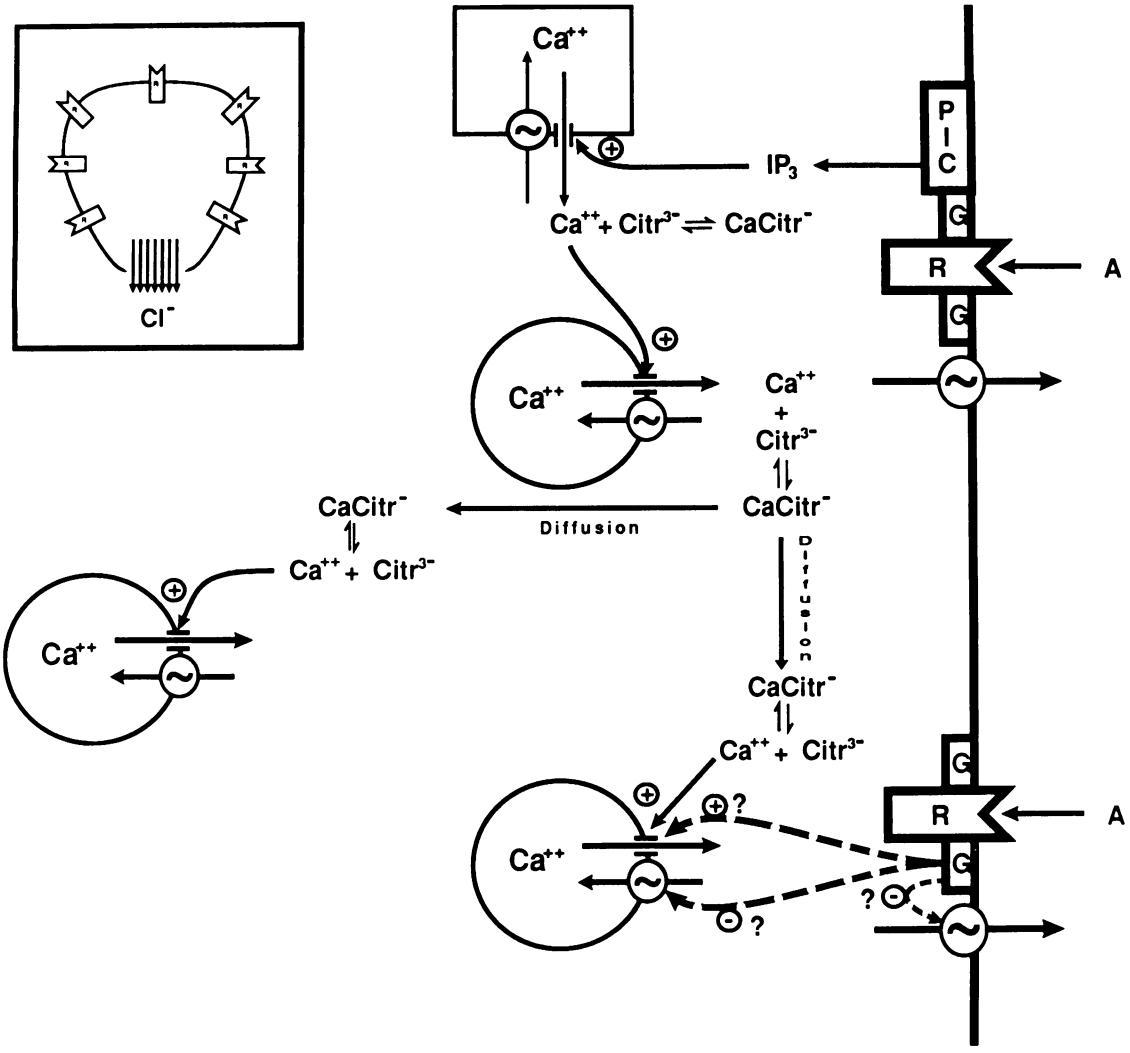

Fig. 7. Schematic diagram to explain the mechanism by which citrate ($Citr^{3-}$) buffering diminishes spatial Ca^{2+} concentration inhomogeneity and therefore promotes the formation of longer cytosolic Ca^{2+} waves. The citrate buffer reduces the availability of released Ca^{2+} for immediate cellular extrusion and reuptake and allows Ca^{2+} diffusion away from the site of release, enabling further Ca^{2+}-induced Ca^{2+} release from neighbouring pools. In addition to generating inositol trisphosphate (IP_3), agonists may also exert an inhibitory effect on the Ca^{2+} reuptake into the Ca^{2+} pools or on the Ca^{2+} extrusion pump and/or increase the sensitivity of the Ca^{2+}-induced Ca^{2+} release. This effect may be generated by separate G-proteins. CCK8 would have a stronger influence than ACh in these respects. For the sake of graphical clarity the effects of receptor activation on IP_3 generation and the possible novel actions on Ca^{2+}-induced Ca^{2+} release, Ca^{2+} extrusion and Ca^{2+} reuptake into the Ca^{2+} pools have been shown separated, but all receptor activations might have the potential to generate all the messages. The inset indicates that the receptors for ACh and CCK are likely to be present all over the basolateral membrane whereas the Cl^- channels are most likely confined to the luminal membrane.

release initiated by a Ca^{2+} rise (Petersen and Wakui, 1990). Such a negative feedback would be diminished by citrate or ATP buffering of Ca^{2+}. This would tend to broaden the transients. According to the minimal quantitative model for signal-induced Ca^{2+} oscillations (Goldbeter *et al.*, 1990) enhanced cytosolic Ca^{2+} buffering should increase the time taken for inositol (1,4,5) trisphosphate-induced Ca^{2+} loading of the Ca^{2+}-sensitive Ca^{2+} pool and therefore reduce the frequency of the Ca^{2+} waves as indeed observed in our experiments.

From our previous work (Wakui *et al.*, 1989, 1990b; Osipchuk *et al.*, 1990) it appears that the shortest Ca^{2+} transients are those evoked by intracellular Ins (1,4,5) P_3 or Ca^{2+} infusion whereas those evoked by ACh often are somewhat longer and those induced by intracellular GTP-γ-S infusion or CCK stimulation are even broader. Caffeine or ACh can dramatically potentiate the effects of Ins (1,4,5) P_3 or Ca^{2+} infusion so that more long-lasting transients are obtained (Osipchuk *et al.*, 1990; Wakui *et al.*, 1990b). This information taken together with our new evidence may suggest that the effects of receptor activation, perhaps additional to Ins (1,4,5) P_3 generation, and the effects of caffeine and citrate are all due to an increased spreading of the Ca^{2+} signal via Ca^{2+}-induced Ca^{2+} release. Such an effect could be mediated either by inhibition of Ca^{2+} pumps or by an increased sensitivity of the Ca^{2+}-induced Ca^{2+} release mechanism. The effect is unlikely to be mediated by diacylglycerol since activators of protein kinase C inhibit rather than enhance the Ca^{2+} signals (Llano and Marty, 1987; Maruyama, 1989; Swann *et al.*, 1989). It is clear from our results that CCK8 would have a stronger effect than ACh in enhancing the Ca^{2+}-induced Ca^{2+} release process and in this context it is interesting that Schnefel *et al.* (1990) have found that CCK receptors interact with a number of different G-proteins in pancreatic acinar cells. One or several of the unknown messages generated by these G-proteins could be involved in controlling the spreading of the intracellular Ca^{2+} signal. We do not, however, have any direct evidence for such a receptor-generated spreading factor and it cannot be ruled out that an agonist-specific spatial distribution of Ins(1,4,5)P_3 production could explain the agonist-specific Ca^{2+} signal patterns we have described.

Materials and methods

Fragments of mouse pancreas were digested by pure collagenase, washed and pipetted to produce single acinary cells as previously described (Wakui *et al.*, 1989, 1990b; Osipchuk *et al.*, 1990). The tight-seal, whole-cell current configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells (Hamill *et al.*, 1981; Marty and Neher, 1983) as previously described in detail for studies on pancreatic acinar cells (Jauch *et al.*, 1986). The Ca^{2+}-dependent Cl^- currents were measured with the two-voltage pulse protocol exactly as described by Wakui *et al.* (1989, 1990b). 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 cell under investigation was continuously exposed to a flow of control solution or control solution containing CCK8 (sulphated) (Sigma) or ACh (Sigma). The control intracellular pipette solution contained (mM): KCl 140, Na_2ATP 1, $MgCl_2$ 1.13 glucose 10 and HEPES 10 (pH 7.2). In experiments where 10 or 40 mM potassium citrate was present or where the ATP concentration (Na salt) was elevated to 10 or 20 mM in the pipette solution the KCl concentration was reduced so as to keep the osmolarity at the control level. In these experiments E_{Cl-} was negative and outward currents were seen at 0 mV. In some experiments sucrose was added to the extracellular (bath) solution and the KCl concentration in the pipette solution not reduced to compensate for extra ATP or citrate. In these cases E_{Cl-} remained at 0 mV. In terms of oscillation patterns there was no difference between these two protocols. The pH of all pipette solutions was 7.2. Some of the initial ATP and citrate experiments were carried out without osmotic compensation and gave results that did not seem different from those later obtained, but the cells in these early experiments swelled visibly. All experiments were carried out at room temperature.

Acknowledgements

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