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Cytoplasmic Ca^{2+} oscillations evoked by acetylcholine or intracellular infusion of inositol trisphosphate or Ca^{2+} can be inhibited by internal Ca^{2+}

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In single internally perfused mouse pancreatic acinar cells, changes in the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were monitored by measuring the Ca^{2+}-dependent transmembrane Cl^- current under voltage-clamp conditions. Cytoplasmic Ca^{2+} oscillations were induced by external acetylcholine (ACh) application, internal infusion of inositol (1,4,5) trisphosphate or its non-metabolizable analogue inositol trisphosphorothioate or by intracellular Ca^{2+} infusion. Such $[Ca^{2+}]_i$ oscillations could be rapidly inhibited by external application of the Ca^{2+} ionophore ionomycin (10–100 nM). Cytoplasmic Ca^{2+} oscillations could also be evoked by external caffeine (1 mM) application when the internal perfusion solution did not contain any Ca^{2+} chelator. In such cases intracellular Ca^{2+} infusion transiently abolished the $[Ca^{2+}]_i$ oscillations. We conclude that although Ca^{2+}-induced Ca^{2+} release is the cause of the ACh-evoked $[Ca^{2+}]_i$ oscillations, there is also a negative feed-back since Ca^{2+} can inhibit Ca^{2+} release initiated by Ca^{2+}.

Acetylcholine; Inositol trisphosphate; Ca^{2+}-induced Ca^{2+} release; Ca^{2+} inhibition of Ca^{2+} release; Cl^- current

1. INTRODUCTION

Receptor-mediated inositol lipid breakdown can evoke oscillations in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) due to inositol (1,4,5) trisphosphate (Ins (1,4,5) P_3)-induced pulsatile Ca^{2+} release from intracellular organelles [1]. Since internal perfusion of pancreatic acinar cells with Ins (1,4,5) P_3 or its non-metabolizable phosphorothioate derivative evokes repetitive pulses of internal Ca^{2+} release, as does muscarinic receptor activation, the Ca^{2+} oscillations are not due to pulsatile Ins (1,4,5) P_3 formation [2]. In pancreatic acinar cells, intracellular Ca^{2+} infusion via a patch-clamp pipette mimicks the effect of Ins (1,4,5) P_3 by evoking repetitive spikes of Ca^{2+} release from a pool close to the cell membrane [3]. This Ca^{2+}-induced Ca^{2+} release occurs most likely through a Ca^{2+}-activated Ca^{2+} release channel [4] from an Ins (1,4,5) P_3-insensitive Ca^{2+} pool [5]. We have shown that caffeine, a well-known potentiator of Ca^{2+}-induced Ca^{2+} release [4,6], evokes Ca^{2+} oscillations when applied on top of a sub-threshold acetylcholine (ACh) stimulus, but also demonstrated that caffeine applied during ACh-evoked cytoplasmic Ca^{2+} oscillations has a transient inhibitory effect [3]. This may indicate the presence of negative feedback in the system and the purpose of the experiments reported here is to test the hypothesis that Ca^{2+} may inhibit Ca^{2+} oscillations evoked by external ACh stimulation or internal Ins (1,4,5) P_3 or Ca^{2+} infusion.

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2. MATERIALS AND METHODS

Fragments of mouse pancreas were digested by pure collagenase (Worthington, 200 units/ml, 20–30 min, 37°C) in the presence of 1 mM Ca^{2+} to obtain single cells as previously described [2,3]. The tight-seal, whole-cell current recording configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells [7] but incorporating the additional feature of internal perfusion of the pipette tip [3,8] via a thin polythene tube (portex). The standard extracellular solution contained (mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.0, $MgCl_2$ 1.13, Hepes 10 and glucose 10, pH was 7.2. In the pipette solutions KCl was present at a concentration of 140 mM. The EGTA concentration was normally 0.25 mM, but in a few experiments no EGTA was present. No Ca^{2+} was added to the pipette solution but 1.13 mM $MgCl_2$ and 5 mM Na_2ATP were present. The Hepes concentration was 10 mM and pH was always 7.2. The Ca^{2+} solution perfused into the patch-clamp pipette tip by pressure application was a normal pipette solution except for the absence of EGTA and the presence of 1 mM $CaCl_2$. The free Ca^{2+} concentration was calculated to be about 100 μM. The Ins (1,4,5) P_3-containing fluid was a standard pipette solution with 10 μM Ins (1,4,5) P_3 (Sigma), but in most experiments we used 30–50 μM inositol (1,4,5) trisphosphorothioate (Ins (1,4,5) PS_3) (a gift from Dr B.V.L. Potter, University of Leicester, UK) [2].

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We monitored changes in $[Ca^{2+}]_i$ by recording the Ca^{2+}-activated Cl^- current [2,3]. Acinar cells were voltage-clamped at a holding potential of −30 mV and depolarizing voltage jumps of 100 ms duration to a membrane potential of 0 mV were repetitively applied throughout all experiments. In all experiments shown, the Cl^- equilibrium potential (E_{Cl-}) is ~0. Because of compression of pen-recording traces, all records seem to show currents at −30 and 0 mV simultaneously. Dotted lines indicate zero current level and downwards deflections represent inward current.

3. RESULTS

Fig. 1a shows that ACh (50 nM) evoked repetitive pulses of inward Cl^- current at a membrane potential of −30 mV. When the membrane potential was clamped at 0 there was no effect since this is the value of E_{Cl-}. We have previously shown that the effect of ACh is blocked by the muscarinic antagonist atropine and by a high internal concentration of the Ca^{2+}-chelator EGTA [2]. We have also shown that the reversal potential for the ACh-evoked current varies exactly with E_{Cl-} over a wide range of values. It has therefore been concluded that the ACh-evoked inward current is due to opening of Ca^{2+}-dependent Cl^- channels [2]. Internal perfusion with Ins (1,4,5) P_3 or the non-metabolizable analogue Ins (1,4,5) PS_3 also evoked a regular pulsatile Cl^- current response (Fig. 1b, c) and since this has also been shown to be blocked by a high internal EGTA concentration [2] and since both the ACh and the Ins (1,4,5) P_3 or Ins (1,4,5) PS_3-evoked current pulses are associated with simultaneous pulsatile increases in cytoplasmic Ca^{2+} concentration [3], it is clear that the ACh-evoked pulsatile Cl^- current is due to Ins (1,4,5) P_3-induced pulsatile Ca^{2+} release inside the cell [2,3].

Fig. 1d shows that intracellular Ca^{2+} infusion could also evoke pulsatile Ca^{2+} release giving rise to repetitive Cl^- current spikes confirming the recent findings of Osipchuk et al. [3]. In our previous study it was shown that caffeine alone failed to evoke any effect when applied in the absence of any other stimulation [3]. However, when the cells were exceptionally perfused internally with a solution not containing the normal 0.25 mM of the Ca^{2+} chelator EGTA, caffeine did by itself evoke oscillations in the Ca^{2+}-dependent Cl^- current (n = 7) (Fig. 1e).

We used the Ca^{2+} ionophore ionomycin to evoke Ca^{2+} entry into the cells. When ionomycin (10 nM) was applied alone, it induced a number of Ca^{2+} spikes reflected in pulses of the Ca^{2+}-activated Cl^- current (n = 4). When a higher ionomycin concentration (100 nM) was used, a sustained rise in the Ca^{2+}-dependent Cl^- current was observed following a few initial Cl^- current pulses (n = 7).

Fig. 1a–d shows that ionomycin inhibited Ca^{2+} pulses evoked by a number of external or internal stimuli. In Fig. 1a it is seen that ionomycin reversibly stopped ACh-evoked Ca^{2+}-dependent Cl^- current pulses. Similar results were obtained in 4 other experiments. In Fig. 1b and c it is demonstrated that ionomycin inhibited Ca^{2+} pulses evoked by Ins (1,4,5) PS_3 (n = 9). In the presence of external Ca^{2+} (Fig. 1b) the ionomycin-evoked inhibition occurred much faster than in its absence (Fig. 1c). In the presence of external Ca^{2+} the initial ionomycin-evoked inhibition of Ca^{2+} spikes was followed by a sustained rise in $[Ca^{2+}]_i$ which was dependent on external Ca^{2+} (Fig. 1b). In the absence of external Ca^{2+} ionomycin only evoked a slow gradual reduction in the amplitude of the Ca^{2+}-dependent Cl^- current pulses. In a separate series of 4 experiments it was shown that intracellular Ca^{2+} infusion markedly and immediately reduced the amplitude of Ins (1,4,5) P_3-evoked Ca^{2+} spikes. This effect was transient and lasted about 1 min. Fig. 1d shows that ionomycin application initially abolished Ca^{2+}-induced Ca^{2+} oscillations before resulting in a sustained rise in $[Ca^{2+}]_i$ (n = 4). Fig. 1e shows that repetitive Ca^{2+} spikes evoked by caffeine alone could be transiently inhibited by intracellular Ca^{2+} infusion. In all 7 experiments of this type Ca^{2+} infusion caused marked inhibition. In some cases the Ca^{2+} spikes were not abolished, but severely reduced in amplitude. In most experiments the inhibitory phase was longer than the one shown in Fig. 1e.

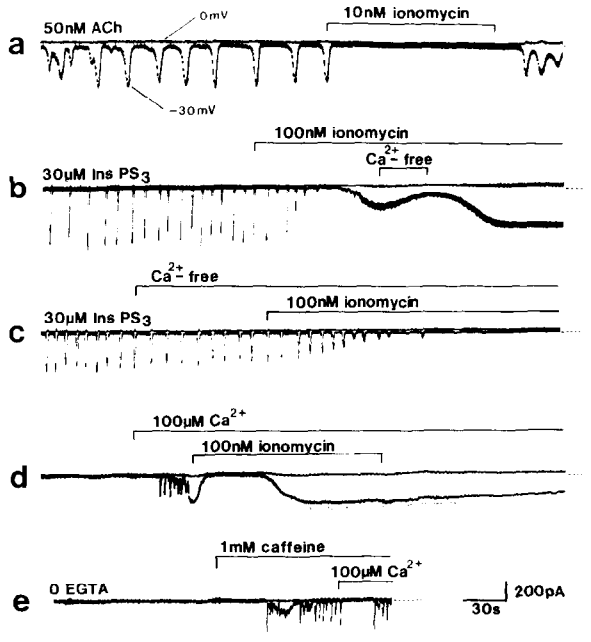

Fig. 1. Inhibitory effects of Ca^{2+} on ACh-, Ins (1,4,5) PS_3-, Ca^{2+}- and caffeine-evoked responses. (a) The inhibitory effect of 10 nM ionomycin (external) on ACh-evoked Ca^{2+}-dependent Cl^- current pulses. (b, c) The inhibitory effects of ionomycin on Ins (1,4,5) PS_3-evoked responses. This occurs more quickly in the presence of external Ca^{2+} (b) than in its absence (c). (d) The inhibition of oscillations evoked by intracellular Ca^{2+} infusion by 100 nM ionomycin applied externally. (e) The transiently inhibitory effect of internal Ca^{2+} infusion on caffeine (external)-evoked responses (no EGTA in pipette-filling solution).

4. DISCUSSION

The results show that cytoplasmic Ca^{2+} oscillations in mouse pancreatic acinar cells evoked by external ACh or caffeine application or internal infusion of either Ins (1,4,5) PS_3 or Ca^{2+} can be inhibited by Ca^{2+} brought into the cell either by external application of the Ca^{2+} ionophore ionomycin or by direct infusion via pressure application of a Ca^{2+}-rich solution into the tip of the patch-clamp pipette. This negative feedback may be of importance for oscillating Ca^{2+} release.

Very recently, Parker and Ivorra [9] have presented evidence for inhibition by Ca^{2+} of Ins (1,4,5) P_3-mediated Ca^{2+} liberation in *Xenopus* oocytes, but in this system there is apparently no Ca^{2+}-induced Ca^{2+} release and the suggestion is that Ca^{2+} directly inhibits Ca^{2+} release via the Ins (1,4,5) P_3-activated Ca^{2+} channel in the endoplasmic reticulum [9]. In the mouse pancreatic acinar cells there is strong evidence for Ca^{2+}-induced Ca^{2+} release [3] and the results shown in Fig. 1d and e indicate that the Ca^{2+}-induced inhibition of internal Ca^{2+} release occurs at the level of the caffeine-sensitive Ca^{2+}-induced Ca^{2+} release channel [4,10] rather than the Ca^{2+} channel activated by Ins (1,4,5) P_3 [11,12].

Our proposal for the generation of ACh-evoked oscillations in $[Ca^{2+}]_i$ is therefore that Ins (1,4,5) P_3 generated by receptor activation releases a small steady outflow of Ca^{2+} from the Ins (1,4,5) P_3-sensitive Ca^{2+} pool [11] which in turn causes a much larger Ca^{2+} release from an Ins (1,4,5) P_3-insensitive Ca^{2+} pool [5]. This Ca^{2+} liberation via a Ca^{2+}-activated Ca^{2+} release channel is, however, inhibited by the elevated level of $[Ca^{2+}]_i$ terminating the Ca^{2+} spike. The Ins (1,4,5) P_3-evoked steady small Ca^{2+} release continues and this will evoke a new Ca^{2+} spike via Ca^{2+}-induced Ca^{2+} release.

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