Volume 267, number 2, 301–304 FEBS 08609 July 1990

Intracellular aluminium inhibits acetylcholine- and caffeine-evoked Ca^{2+} mobilization

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Received 17 May 1990

The effect of intracellular aluminium on Ca^{2+} signalling in single internally perfused mouse pancreatic acinar cells was investigated by measurement of the Ca^{2+}-dependent Cl^- current using the patch-clamp whole-cell recording configuration. Acetylcholine (ACh) normally evoked a pulsatile Ca^{2+}-dependent Cl^- current, but when $AlCl_3$ (1 mM) was present in the internal perfusion solution the ACh responses were virtually absent. When aluminium was acutely infused into the internal perfusion solution, the ACh-evoked Ca^{2+} signals and also the caffeine-evoked responses quickly disappeared, but the Ca^{2+} ionophore, ionomycin (100 nM), could still induce a large increase in the Cl^- current. It is concluded that intracellular aluminium can abolish receptor-activated intracellular Ca^{2+} release probably by inhibition of Ca^{2+}-induced Ca^{2+} release

Aluminium; Ca^{2+}-activated Cl^- current; Caffeine; Acetylcholine

1. INTRODUCTION

Aluminium is neurotoxic [1–5], but little is known about the molecular mechanisms involved in its actions. Recently two reports have suggested that the intracellular inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$)/Ca^{2+} signalling system [6] may be disrupted by aluminium [7,8]. We have therefore tested the hypothesis that intracellular aluminium may interfere with Ca^{2+} signals evoked by activation of receptors linked to inositol lipid hydrolysis. In pancreatic acinar cells activation of muscarinic receptors evokes an oscillatory increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) due to pulsatile intracellular Ca^{2+} release from caffeine-sensitive stores close to the cell membrane triggered by Ca^{2+} outflow from an $Ins(1,4,5)P_3$-sensitive store [9]. The intracellular Ca^{2+} signals can be monitored in single internally perfused cells by measurement of the Ca^{2+}-dependent Cl^- current [9,10] using the patch-clamp whole-cell recording configuration [11]. We now show that intracellular infusion of an $AlCl_3$-containing solution (10 μM–1 mM) via a fine tube inserted into the patch-clamp pipette [9,12], rapidly abolishes acetylcholine (ACh) and caffeine-evoked Ca^{2+}-dependent Cl^- current responses. This effect is not due to interference with the Ca^{2+} activation of Cl^- channels since an increase in $[Ca^{2+}]_i$ mediated by the Ca^{2+} ionophore, ionomycin (100 nM), can still increase the Cl^- current in the presence of intracellular aluminium. We conclude that intracellular aluminium can inhibit receptor-activated intracellular Ca^{2+} release and suggest that the main effect is inhibition of the caffeine-sensitive Ca^{2+}-induced Ca^{2+} release.

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2. MATERIALS AND METHODS

Fragments of mouse pancreas were digested by pure collagenase (Worthington, 200 units·ml^{-1}, 20–30 min, 37°C) in the presence of 1 mM Ca^{2+}. After wash with physiological saline, the cell suspension was gently pipetted to obtain further separation and then again washed with control solution. Only single cells were used for the experiments. 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 [11]. In most of the experiments a fine polythene tube (portex) was inserted into the patch-clamp pipette allowing infusion of an $AlCl_3$-containing solution into the pipette tip by applying pressure to the tube [9,12]. 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. The intracellular (pipette) solution contained (mM): KCl 140, $MgCl_2$ 1.13, ATP (Na^+ salt) 5, Hepes 10, glucose 10; pH was 7.2. In some experiments (those in which 1 mM $AlCl_3$ was used) 0.25 mM EGTA was present. The fluid in the infusion tube was the intracellular pipette solution with $AlCl_3$ (10 μM or 1 mM). A fresh 0.1 M $AlCl_3$ solution was prepared for each experiment. This was diluted with the intracellular solution under vigorous stirring to give a final concentration of 1 mM or 10 μM. All experiments were carried out at room temperature (22–25°C).

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00145793/90/$3.50

3. RESULTS

Fig. 1 shows typical control responses to external application of ACh. As previously described [9,10], ACh evokes repetitive pulses of inward Cl^- current at a membrane potential of −30 mV. When the membrane potential is clamped at 0 there is hardly any effect since this is close to the Cl^- equilibrium potential (E_{Cl-}). The ACh effect is blocked by the muscarinic antagonist atropine (Fig. 1) and by a high intracellular concentration of the Ca^{2+} chelator EGTA [10]. We have previously shown that the reversal potential for the ACh-evoked current response varies with E_{Cl-} over a wide range of values [10]. The ACh-evoked inward current is therefore due to opening of Ca^{2+}-dependent Cl^- channels and this current can be used to assess $[Ca^{2+}]_i$ close to the membrane [9,10]. When the pipette solution contained 1 mM $AlCl_3$ the ACh responses were abolished (Fig. 1) or markedly reduced. Four experiments of this type (Fig. 1c) were carried out and in two cases ACh failed to evoke any effect and in the two other cells the responses were very small and transient. In the absence of aluminium 500 nM ACh invariably evoked sustained (Fig. 1b) or quasi-sustained oscillating responses that continued as long as ACh was present.

We have previously shown that caffeine, a well known potentiator of Ca^{2+}-induced Ca^{2+} release through the ryanodine receptor channel in muscle sarcoplasmic reticulum [13,14] can markedly potentiate the intracellular Ca^{2+} release evoked by low concentrations of ACh and Ins(1,4,5)P_3 [9]. In rat chromaffin cells caffeine alone can induce cytoplasmic Ca^{2+} fluctuations in cells that are initially silent [15]. We have also demonstrated that when pancreatic acinar cells are exceptionally perfused internally with a solution not containing the normally used low EGTA concentration (0.25 mM), then caffeine alone evokes repetitive pulses of Ca^{2+}-dependent Cl^- current [16]. In the following experiments reported here we did not normally include EGTA in the intracellular pipette solutions, mainly because EGTA is also a powerful chelator of Al^{3+} [17]. Caffeine (1 mM) evoked responses of the type shown in Fig. 2b in all the 10 cells tested during control conditions whether applied before or after a period of ACh stimulation. The effect of intracellular $AlCl_3$ infusion via a tube inserted into the patch pipette is shown in Fig. 2c which starts with a control response to ACh. Thereafter the intracellular tube is opened and pressure applied enabling the $AlCl_3$ (10 μM)-containing solution to enter the pipette tip and therefore the cell interior. Within 2.5 min from the start of the aluminium infusion the ACh-evoked response has been abolished. The ACh stimulation is thereafter discontinued and after an interval of about 2 min ACh is reapplied but without any effect. Thereafter caffeine is applied, but also without effect. Finally it is shown that in the same cell

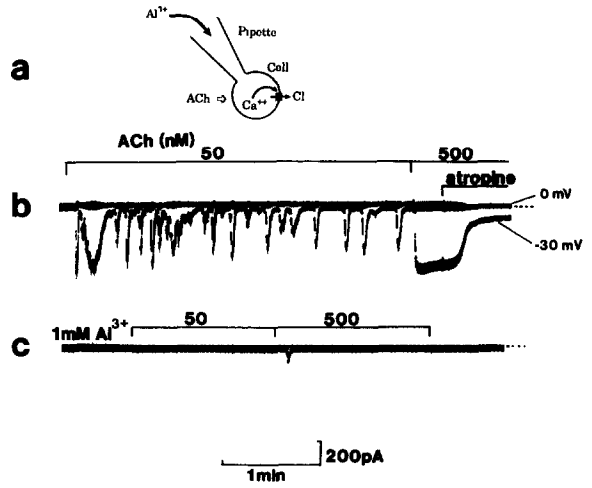

Fig. 1. Effect of acetylcholine (ACh) on Ca^{2+}-dependent Cl^- current in single internally perfused mouse pancreatic acinar cells in the absence and presence of 1 mM $AlCl_3$ in the pipette solution. Acinar cells were voltage-clamped at a holding potential of −30 mV and repetitive depolarizing voltage jumps of 100 ms duration to 0 mV applied throughout the experiments. The Cl^- equilibrium potential (E_{Cl-}) ~ 0. Because of compression of pen-recording traces all records seem to show currents at −30 and 0 mV simultaneously. Dotted horizontal lines indicate zero current level. (a) shows a cartoon explaining the experimental arrangement. (b) shows control responses to 50 and 500 nM ACh. Atropine (0.1 μM) abolished the effect of 500 nM ACh. (c) shows that in the presence of aluminium in the pipette solution ACh failed to evoke any responses.

ionomycin (100 nM) evokes a large and sustained inward current response. Four experiments of the type shown in Fig. 2c were carried out all with similar results. Fig. 2d shows the result of an experiment in which intrapipette infusion of a solution containing 1 mM $AlCl_3$ first changed the quasi-sustained oscillating response to 500 nM ACh to a clearly pulsatile pattern and thereafter abolished the Ca^{2+} signal. Similar results were obtained in 4 separate cells.

4. DISCUSSION

The results shown in Figs 1 and 2 indicate that intracellular aluminium application can block ACh-evoked cytoplasmic Ca^{2+} signals. There are many steps from receptor activation by ACh to the cytoplasmic Ca^{2+} signal and aluminium could in principle act on several of these [7,8], but in view of the finding that aluminium also inhibits caffeine-evoked intracellular Ca^{2+} release (Fig. 2c), the simplest hypothesis to account for our results is that aluminium inhibits opening of the caffeine-sensitive Ca^{2+}-induced Ca^{2+} release channel.

The ACh-evoked intracellular Ca^{2+} release in exocrine acinar cells seems to be a two-stage process in which Ins(1,4,5)P_3-evoked opening of heparin-sensitive Ca^{2+} channels in one endoplasmic reticulum store gives rise to a slight increase in $[Ca^{2+}]_i$ that directly activates

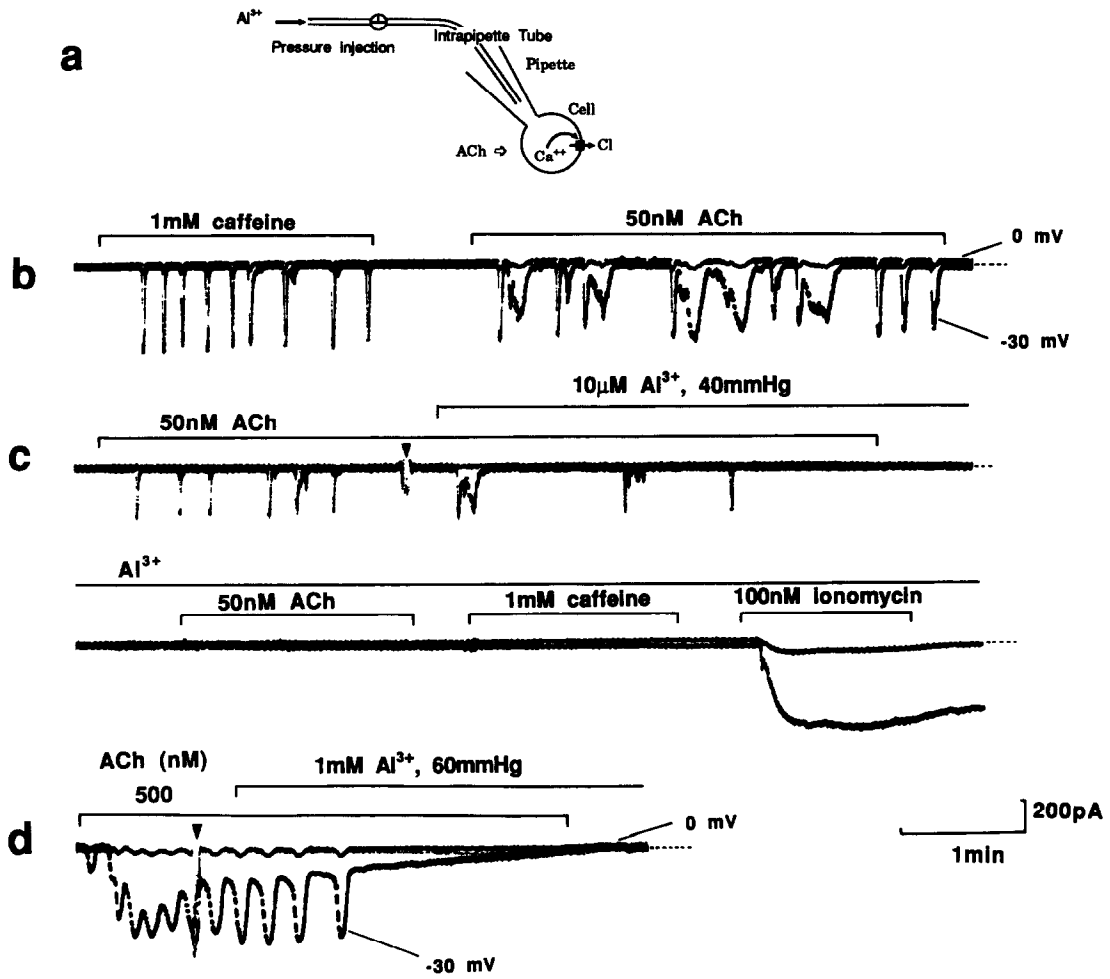

Fig. 2. Effect of ACh and caffeine on Ca^{2+}-dependent Cl^- current. (a) shows the arrangement of infusion tube for aluminium, patch-clamp pipette and whole-cell recording configuration. In (b) control responses (in the absence of an $AlCl_3$-containing solution in the tube) to caffeine and ACh (applied externally) are shown. The two traces in (c) are from the same cell and show an uninterrupted experimental run starting with a control ACh response (in all experiments with an $AlCl_3$-containing solution in the infusion tube the responses seemed weaker than in real control cells – we cannot exclude some leak of $AlCl_3$ into the cell even when the tube is closed). At the arrowhead the tube is opened and thereafter pressure injection of intracellular solution containing 10 μM $AlCl_3$ occurs which causes abolishment of the ACh-evoked Cl^- current pulses within 2.5 min. ACh stimulation is then discontinued. A renewed ACh challenge fails to elicit a response and caffeine is also ineffective. Finally, 100 nM ionomycin is applied to the bath evoking a large sustained inward current response. The record shown in (d) is from a separate experiment. ACh (500 nM) causes an oscillating response. Intrapipette infusion of an $AlCl_3$ (1 mM)-containing solution slows down the Cl^- current pulses and then abolishes the response. In this experiment EGTA (0.25 mM) was present in the pipette solution, but since Al^{3+} was present in excess of EGTA the chelator has relatively little effect on the Al^{3+} concentration.

a caffeine and Ruthenium red-sensitive Ca^{2+} channel in a separate Ins(1,4,5)P_3-insensitive store to evoke the major Ca^{2+} release [9,18,19]. It is known that the Ca^{2+} release channel in muscle which is specifically activated by micromolar or submicromolar Ca^{2+} concentrations [20,21] can be inhibited by millimolar concentrations of either Mg^{2+} or Ca^{2+} [21]. This inhibition is therefore not specific and may well be exerted also by other ions such as Al^{3+}. If this is the main effect of intracellular aluminium, the affinity of this inhibitory site must be substantially higher for Al^{3+} than for Mg^{2+} or Ca^{2+}, but this would be consistent with the generally held view that the toxic effects of Al^{3+} are due to the ability of this ion to displace Mg^{2+} at intracellular binding sites [22] and the finding that Al^{3+} can effectively compete for Mg^{2+}-binding sites in the presence of about 10^8-fold molar excess of Mg^{2+} [23]. The chemistry of aluminium is, however, complicated since it can be hydrolyzed extensively to form solutions of more or less stable polynuclear hydroxide complexes [24]. We cannot therefore know in exactly what form aluminium acts inside the acinar cells.

Acknowledgements: We thank the MRC for support and Alan Higgins for technical assistance.

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