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Electroporation can cause artefacts due to solubilization of cations from the electrode plates

Aluminium ions enhance conversion of inositol 1,3,4,5-tetrakisphosphate into inositol 1,4,5-trisphosphate in electroporated L1210 cells

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1. In electroporated L1210 cells, Ins(1,3,4,5)P_4 causes Ca^{2+} release, owing to its conversion into Ins(1,4,5)P_3, but this does not happen in cells permeabilized by digitonin treatment [Cullen, Irvine, Drøbak & Dawson (1989) Biochem. J. **259**, 931–933]. 2. If the assay medium is subjected to electroporation by using a commercially available electroporation apparatus and then the cells are added and permeabilized with digitonin, the cells behave as if they had been electroporated. 3. Electroporation causes the release of high concentrations of Al^{3+} into the experimental medium, and addition of these concentrations of Al^{3+} into the experimental medium mimics the effect of electroporation on the conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3. 4. It is concluded that the difference between electroporated and digitonin-permeabilized L1210 cells in this experimental system can be attributed to dissolution of Al^{3+} from the electroporation cuvette. Al^{3+} contamination may thus be a serious problem when using this apparatus.

INTRODUCTION

In recent investigations into the possible physiological effects of Ins(1,3,4,5)P_4 on intracellular Ca^{2+} homoeostasis, we found, using permeabilized cells, that under some experimental circumstances there was a rapid conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3, with a consequent release of intracellular Ca^{2+} [1]. The conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3 apparently happened in L1210 mouse lymphoma cells which had been permeabilized by electroporation, but not in cells permeabilized by digitonin treatment. At the time, we suggested that electroporation led to the stimulation of Ins(1,3,4,5)P_4 3-phosphatase activity. This enzyme has been reported in a variety of cell types [2–4]. However, the situation was rather confused, since electroporation of cells previously permeabilized with digitonin (or digitonin treatment of electroporated cells) still promoted conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3.

Although we have demonstrated effects of Ins(1,3,4,5)P_4 on Ca^{2+} movements in L1210 cells permeabilized with digitonin [5], a recent hypothesis for the action of Ins(1,3,4,5)P_4 [6] predicts a very close structural relationship between the Ins(1,3,4,5)P_4 receptor and the Ins(1,4,5)P_3 receptor. This would be likely to be preserved in a more intact state in cells permeabilized by electroporation than in cells permeabilized with digitonin, where much of the plasma membrane is damaged. Accordingly, we returned to the problem of electroporation and the conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3, since if it could be overcome it might result in an experimental system where larger effects of Ins(1,3,4,5)P_4 could be observed. As a result of these investigations, we now report a most dramatic and disturbing complication which can arise in a commonly used electroporation system. In short, the use of Bio-Rad Gene Pulser cells for electroporation causes solubilization of high concentrations of Al^{3+} from the electrode plates, which gives rise, in our system, to accelerated conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3, but which could clearly seriously interfere with a whole range of studies which have been, and are being, conducted using this apparatus.

MATERIALS AND METHODS

Measurement of Ca^{2+} uptake with a Ca^{2+}-sensitive electrode, growth of L1210 cells and cell harvesting were all conducted essentially as previously described [1]. Ins(1,3,4,5)P_4 was purified by h.p.l.c. to be free of Ins(1,4,5)P_3 contamination.

Electroporation was carried out with a Bio-Rad Gene Pulser, by using (in the first instance) electroporation cells supplied by the manufacturer. Cells at a density of 10^8/ml were suspended in an assay mixture (1 ml) containing 100 mM-KCl, 10 mM-Hepes/KOH, pH 7.0, 1 mM-dithiothreitol, 3 mM-$MgCl_2$, 2.5 mM-ATP, 10 μg of creatine kinase, 10 mM-phosphocreatine, 50 μM-GTP and 5 mM-succinate (K^+ salt). Cells were treated to five pulses of 0.8 kV (2 kV/cm) with a capacitance of 25 μF.

Permeabilization with digitonin was performed by adding 10 μg of digitonin (in 1 μl of dimethyl sulphoxide) to cells suspended in the above assay medium.

Measurement of Ins(1,3,4,5)P_4 degradation was, as previously [1], carried out by adding 2 μM-Ins(1,3,4,5)P_4, spiked with [^{3}H]Ins(1,3,4,5)P_4 (20000 d.p.m.), to the permeabilized cells in the incubation medium. After 30 s, 700 μl samples were transferred to Microfuge tubes containing 300 μl of ice-cold 10% (w/v) trichloroacetic acid and 10 μM-phytate. After centrifugation, the supernatants were extracted with 4 × 2 ml of diethyl ether, then neutralized with NH_3, freeze-dried and stored at −20 °C. After reconstitution in water, the samples were spiked with a [^{32}P]Ins(1,4,5)P_3 standard. Inositol phosphates were then separated by h.p.l.c. as previously described [1].

Metals in solutions were analysed by using inductively coupled plasma-optical-emission spectrometry on an ICAP 61E Atom Scan 25 sequential plasma spectrometer (Thermo-Jarrell-Ash). In the first instance, analyses were limited to Al, Ca, V, Fe, Co, Ni and Sn.

RESULTS

Fig. 1 shows, in agreement with our previous observations, that addition of Ins(1,3,4,5)P_4 (2 μM) to L1210 cells permeabilized

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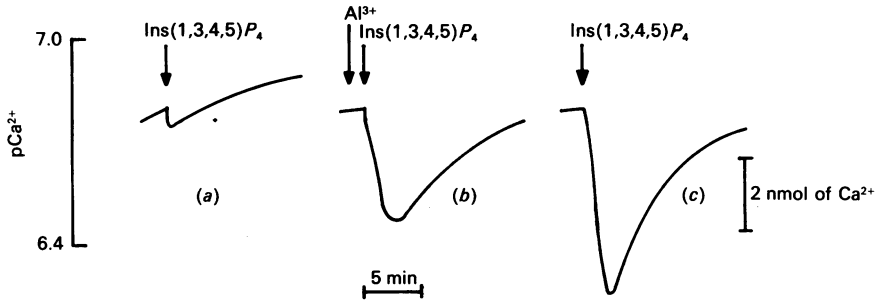

Fig. 1. Effect of Ins(1,3,4,5)P_4 on Ca^{2+} efflux in permeabilized cells

Incubation conditions were as described in the Materials and methods section. (*a*) Response to the addition of 2 μM-Ins(1,3,4,5)P_4 for cells permeabilized with digitonin. (*b*) Response to 2 μM-Ins(1,3,4,5)P_4 after addition of 50 μM-$AlCl_3$ to digitonin-permeabilized cells. (*c*) Response to 2 μM-Ins(1,3,4,5)P_4 for cells permeabilized by five shocks in a Bio-Rad Gene Pulser cuvette. Ca-electrode traces shown are typical of at least three in each condition.

Table 1. Metal cation analysis of experimental media after electroporation

The values given are from inductively coupled plasma-optical-emission spectrometry of experimental media which were placed in a Bio-Rad Gene Pulser cuvette and then subjected to the indicated number of pulses at 800 V using a 25 μF capacitance. The values are means ± S.E.M., with n at least 5 in each case.

No. of pulses	$[Al^{3+}]$ (μM)	$[Ca^{2+}]$ (μM)
1	54±0.7	8.3±1.75
5	276±8.5	9.2±1.00
10	582±81.5	7.9±1.3

by electroporation (Fig. 1*c*) causes a rather slow, but very extensive, release of Ca^{2+} from intracellular stores, but has no apparent effect when added to digitonin-permeabilized cells (Fig. 1*a*). Given our previous finding that addition of digitonin, either before or after electroporation, still resulted in a trace similar to that in Fig. 1(*c*), it seemed likely that the difference in Ins(1,3,4,5)P_4 response was due to a stimulatory effect of electroporation rather than an inhibitory effect of digitonin. One possibility was that electroporation caused a change in the experimental medium, rather than in the cells. Accordingly, we subjected a sample of assay medium to the electroporation procedure before addition of cells, which were then permeabilized by digitonin. Somewhat to our surprise, a trace identical with that shown in Fig. 1(*c*) resulted (results not shown). The material from which the electrode plates are made in Bio-Rad Gene Pulser cells appears to be aluminium or an aluminium-based alloy, and we therefore tried the effect of adding $AlCl_3$ to the incubation medium. Results of an experiment of this sort are shown in Fig. 1(*b*), where 50 μM-$AlCl_3$ has been added to medium containing digitonin-permeabilized cells. It is clear that under these circumstances Ins(1,3,4,5)P_4 addition causes a substantial release of Ca^{2+}, similar in size and time-course to that observed for electroporated cells (Fig. 1*c*). Using similar experimental conditions, we have found that Ca^{2+} release after Ins(1,3,4,5)P_4 addition is observed at Al^{3+} concentrations down to about 10–20 μM, although the effect increases greatly as the Al^{3+} concentration is increased to 200 μM.

To confirm that the effects of electroporation on Ca^{2+} release could be attributed to Al^{3+} from the electrode plates, we firstly analysed experimental media which had been subjected to an electroporation procedure in the absence of cells. By using inductively coupled plasma-optical-emission spectroscopy it was possible to measure a wide range of plausible (and less plausible) metals, as listed in the Materials and methods section. Of the metals tested, only Ca and Al were found at measurable concentrations, and it is clear from the data shown in Table 1 that, although the concentration of Ca^{2+} remains essentially

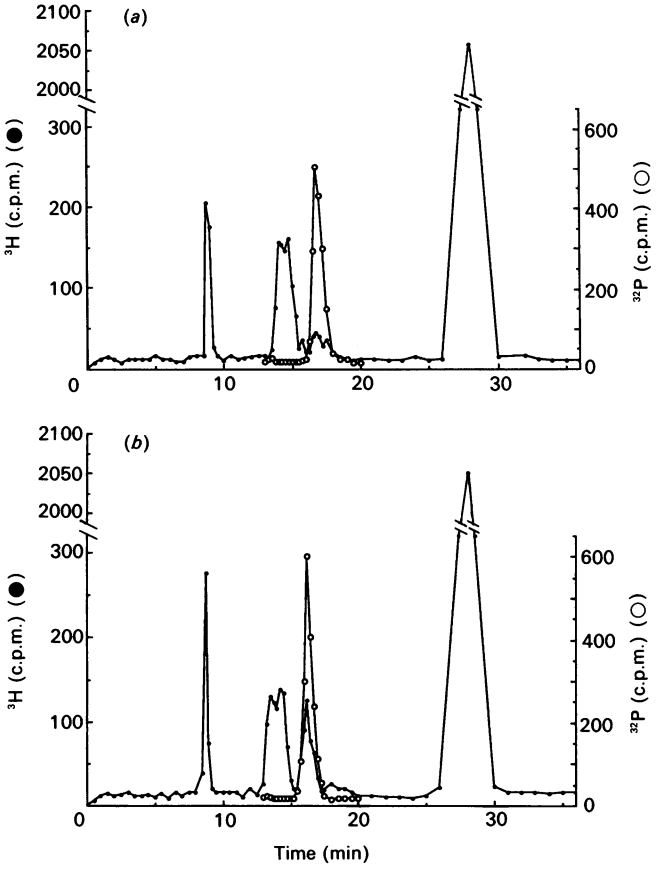

Fig. 2. H.p.l.c. analysis of the inositol phosphates produced during degradation of added Ins(1,3,4,5)P_4

In both cases cells were permeabilized with digitonin: ○ symbols mark the elution position of the [^{32}P]Ins(1,4,5)P_3 marker. (*a*) Control; (*b*) in the presence of 100 μM-$AlCl_3$. Conditions of the incubation were as for Fig. 1, with the sample for analysis being removed 30 s after addition of [^{3}H]Ins(1,3,4,5)P_4. Analyses were performed as described in the Materials and methods section. Determinations were carried out in duplicate in each of two independent experiments, and the results shown are typical individual experiments.

constant during electroporation, that of Al^{3+} rises quite dramatically with the number of pulses, exceeding the sort of levels described above to be effective in causing Ca^{2+} release after Ins(1,3,4,5)P_4 addition.

Secondly, we analysed the products of Ins(1,3,4,5)P_4 metabolism in digitonin-permeabilized cells in the absence and presence of 50 μM-Al^{3+}. The results are shown in Fig. 2. As found previously [1], in the absence of Al^{3+} the major product is Ins(1,3,4)P_3. However, in the presence of Al^{3+} there is a very clear increase in ^3H radioactivity running with the [^{32}P]Ins(1,4,5)P_3 spike. The h.p.l.c. profile looks very similar to that previously reported for Ins(1,3,4,5)P_4 metabolism by electroporated cells [1], and there seems little doubt, on the basis of these results, that Al^{3+} in the experimental medium is a sufficient condition for stimulation of the conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3, with consequent release of Ca^{2+}.

DISCUSSION

The results described above have implications at two levels. Firstly, it appears that Al^{3+} is capable of stimulating the conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3, at least in permeabilized L1210 cells. The most likely (although not the only possible) mechanism for this is by stimulation of the 3-phosphatase activity known to occur in several cell types. This might not be a direct effect. Hodgson & Shears [7] have shown that hepatocyte cytosol contains an inhibitor of 3-phosphatase activity, and it is possible that Al^{3+} might act by removing this inhibitor. Cooke *et al.* [8] have shown that Al^{3+} enhances membrane binding of InsP_5 and InsP_6, and, as these are known inhibitors of 3-phosphatase activity [9] and have been found at high concentrations in several cell types, their possible removal by Al^{3+} could be the cause of the effects that we see. It may be that Al^{3+} is inhibiting Ins(1,4,5)P_3 phosphatase to cause accumulation of that inositol phosphate. However, we could find no effect of Al^{3+} on Ins(1,4,5)P_3-stimulated Ca^{2+} release or on the Ins(1,4,5)P_3 3-kinase and 5-phosphatase activities [10]. Moreover, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 are predominantly hydrolysed by the same 5-phosphatase [11], and re-examination of the original data from Cullen *et al.* [1] has not revealed any slower formation of Ins(1,3,4)P_3 in shock-permeabilized (as compared with digitonin-permeabilized) L1210 cells. Al^{3+} has been shown to have profound effects on Ca^{2+} signalling in hepatocytes and pancreatic acinar cells [12,13]. Although in the latter case there is evidence for inhibition of Ca^{2+}-stimulated Ca^{2+} release, a stimulation by Al^{3+} of the 3-phosphatase might provide a plausible basis for the effect observed in hepatocytes on Ca^{2+} oscillations [12].

On a more general level, the finding that electroporation of cells in this apparatus generates very substantial (100–500 μM) concentrations of Al^{3+} in the experimental solution is deeply disturbing. The original apparatus described by Knight & Baker [14] used stainless-steel electrode plates, and many home-made devices use diverse electrode materials. We have recently constructed an electroporation cell using gold plates which, at least in preliminary experiments, appear relatively inert. However, it is clear that many experiments in the current literature will unwittingly have been carried out in the presence of major contamination of cations (Al^{3+} in particular) derived from the electrode-plate metal. Our experiences with the conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3 are a warning of the rather dire experimental consequences which may result.

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REFERENCES

1. Cullen, P. J., Irvine, R. F., Drøbak, B. K. & Dawson, A. P. (1989) Biochem. J. **259**, 931–933
2. Doughney, C., McPherson, M. A. & Dormer, R. L. (1988) Biochem. J. **251**, 927–929
3. Oberdisse, E., Nolan, R. D. & Lapetina, E. G. (1990) J. Biol. Chem. **265**, 726–730
4. Höer, A., Höer, D. & Oberdisse, E. (1990) Biochem. J. **270**, 715–719
5. Cullen, P. J., Irvine, R. F. & Dawson, A. P. (1990) Biochem. J. **271**, 549–553
6. Irvine, R. F. (1990) FEBS Lett. **263**, 5–9
7. Hodgson, M. E. & Shears, S. B. (1990) Biochem. J. **267**, 831–834
8. Cooke, F. M., Poyner, D., Hawkins, P., Erlebach, C. B. & Hanley, M. (1991) Biochem. Soc. Trans. **19**, 152S
9. Hughes, P. J. & Shears, S. B. (1990) J. Biol. Chem. **265**, 9869–9875
10. Shears, S. B., Dawson, A. P., Loomis-Husselbee, J. W. & Cullen, P. J. (1990) Biochem. J. **270**, 837
11. Shears, S. B. (1989) Biochem. J. **260**, 313–324
12. Schöfl, C., Sanchez-Bueno, A., Dixon, C. J., Woods, N. M., Lee, J. A. C., Cuthbertson, K. S. R., Cobbold, P. H. & Birchall, J. D. (1990) Biochem. J. **269**, 547–550
13. Wakui, M., Itaya, K., Birchall, D. & Peterson, O. H. (1990) FEBS Lett. **267**, 301–304
14. Knight, D. E. & Baker, P. F. (1982) J. Membr. Biol. **68**, 107–140

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