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The perturbation, by aluminium, of receptor-generated calcium transients in hepatocytes is not due to effects of Ins(1,4,5)P_3-stimulated Ca^{2+} release or Ins(1,4,5)P_3 metabolism by the 5-phosphatase and 3-kinase

The pursuit of the mechanism underlying toxic effects of Al^{3+} has led to the demonstration that Al^{3+} may compete for Mg^{2+}-binding sites on certain enzymes even when the free Mg^{2+} concentration is as much as 10^8-fold in excess (Miller *et al.*, 1989). Moreover, a recent publication in this journal has highlighted the possibility of an interaction between aluminium and the phosphoinositide-mediated calcium signalling pathway (Schöfl *et al.*, 1990); in hepatocytes, hormone-stimulated repetitive Ca^{2+} transients (Woods *et al.*, 1986), were perturbed by superfusion of the cells with as little as 5–10 μM-Al^{3+} (as Al^{3+} citrate). Amongst possible explanations for these effects are the proposals that Ins(1,4,5)P_3 might bind Al^{3+} particularly avidly, thereby affecting Ins(1,4,5)P_3 metabolism or Ca^{2+} mobilization (Birchall & Chappell, 1988; Schöfl *et al.*, 1990). Therefore we decided to look at possible effects of aluminium on (i) calcium movements in isolated rat liver microsomes, and (ii) Ins(1,4,5)P_3 metabolism by the 5-phosphatase and 3-kinase. It should be emphasized that in view of the uncertainties surrounding the kinetics of Al^{3+} exchange between various chelators (Miller *et al.*, 1989), the media used in our experiments were pre-equilibrated for several hours in plastic containers.

The preparation of rat liver microsomes and the measurements of Ca^{2+} uptake and release using a Ca^{2+}-sensitive electrode were as described by Dawson *et al.* (1987). Aluminium was solubilized by mixing $AlCl_3$ (10 mM) and sodium citrate (50 mM) for at least 2 h prior to use. Rat liver microsomes were preincubated for 2 h at 0–4 °C with 300 μM-Al^{3+}/1.5 mM-citrate (to give 10 μM-Al^{3+} in the final assay medium) and, separately, 0.5 mM-Ins(1,4,5)P_3 with 5 mM-Al^{3+}/25 mM-citrate for up to 5 h. Al^{3+}-pretreated microsomes behaved exactly like control microsomes (i.e. plus citrate alone) in terms of Ca^{2+} uptake, GTP-enhancement of Ins(1,4,5)P_3 sensitivity and Ins(1,4,5)P_3-stimulated Ca^{2+} release. Similarly, Al^{3+}-pretreated Ins(1,4,5,)P_3 behaved just like control Ins(1,4,5)P_3.

For the studies of Ins(1,4,5)P_3 metabolism, 1 mM-$AlCl_3$ was stirred with 25 mM-sodium citrate for 3–4 h. Ins(1,4,5)P_3 5-phosphatase was assayed in 0.5 ml aliquots of buffer containing 2 μM-[^{3}H]Ins(1,4,5)P_3 (1 nCi/ml), 100 mM-KCl, 1 mM-$MgSO_4$, 10 mM-Hepes (pH 7.2 with KOH) and saponin (0.2 mg/ml). Ins(1,4,5)P_3 3-kinase was assayed in similar buffer, except for the addition of 5 mM-Na_2ATP plus 5 mM-$MgSO_4$. Either 40 μM-Al^{3+} (or the equivalent citrate concentration as the control) was added to each of the buffers, which were preincubated overnight at 0–4 °C before being brought to 37 °C and mixed with 25 μl of a 1% (w/v) liver homogenate prepared in 0.25 M-sucrose/5 mM-Hepes (pH 7.2 with KOH). Ins(1,4,5)P_3 metabolism was then analysed as described by Shears (1989). There was no significant effect of 40 μM-Al^{3+} on either the 3-kinase or the 5-phosphatase. For example, in representative triplicate determinations (% substrate metabolized/min) the 5-phosphatase activity was 1.75 ± 0.04 (control) or 1.67 ± 0.1 (Al^{3+}); the 3-kinase activity was 0.13 ± 0.02 (control) or 0.14 ± 0.005 (Al^{3+}).

These results indicate that aluminium does not interact with Ins(1,4,5)P_3 by perturbing either its metabolism or its Ca^{2+}-releasing properties. It would therefore seem more profitable to pursue the idea that the profound effects of aluminium upon hepatic calcium transients are due to either (*a*) modulation of receptor-activated release of Ins(1,4,5)P_3, through an interaction with either phospholipase C or G-proteins (Miller *et al.*, 1989; Schöfl *et al.*, 1990) or (*b*) interactions with other aspects of the Ca^{2+} transport system such as Ins(1,3,4,5)P_4 (Irvine *et al.*, 1988) and Ca^{2+}-induced Ca^{2+} release (Berridge *et al.*, 1988).

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On the use of EGTA to assess the effect of Ca^{2+} on liver microsomal glucose-6-phosphatase

In a recent report, Waddell *et al.* (1990) claimed that: "..EGTA cannot be used as a buffer to study the effects of Ca^{2+}.." on liver microsomal glucose-6-phosphatase because they found that metal chelators such as EGTA and nitrilotriacetic acid (NTA) caused (1) disruption of the microsomal membrane and (2) loss of enzyme activity in disrupted microsomes. The intactness of the membrane, as assessed by the measurement of mannose-6-phosphatase before and after treatment of the vesicles with a detergent, fell in their hands from 96% to 65% when