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Effect of divalent cations on lysosomal enzyme release from macrophages

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Macrophages constitute the main cell type in chronic inflammatory lesions (Davies, 1976) and may secrete a variety of pro-inflammatory products including lysosomal enzymes (LE), lysosyme, plasminogen activator, collagenase and elastase (Unanue, 1976). Divalent cations play a central role in stimulus-secretion coupling in other inflammatory cells (Henson, 1974) but their role in macrophage secretion is not known. In the present study, we have investigated the effect of divalent cations on LE release from macrophages, using zymosan and the divalent cation ionophore A23187 as stimuli.

Thioglycollate-stimulated mouse peritoneal macrophages were prepared as previously described (Gordon, MacIntyre & McMillan, 1977). Release of LE (β-N-acetyl-glucosaminidase) was determined fluorometrically, by a modification of the method described for platelet LE release by Gordon (1975). As an indicator of cell lysis, release of the cytoplasmic enzyme lactate dehydrogenase (LDH) was determined photometrically, by a modification of the method of Wroblewski & La Due (1955). Zymosan suspensions were prepared as described by Weissmann, Dukor & Zurier (1971) and ionophore A23187 was dissolved in dimethylsulphoxide. To determine the role of extracellular divalent cations, macrophages were incubated with zymosan or A23187 in calcium and magnesium-free Hanks buffered salt solution and known amounts of calcium and magnesium were added.

Incubation of macrophages for 4 h at 37°C with zymosan (25–250 μg/ml) resulted in selective release of LE without leakage of LDH. Zymosan-induced release of LE occurred in the absence of extracellular divalent cations. However, release was enhanced by magnesium (0.3–5 mM) and inhibited by calcium (0.3–5 mM). In the presence of equimolar calcium and magnesium, LE release was similar to that produced in the absence of both.

Incubation of macrophages with A23187 (0.1–30 μM) for 2 h at 37°C led to release of both LE and LDH. Release occurred only when divalent cations were present extracellularly. Calcium was more potent than magnesium at facilitating release and the effect of the two ions was additive.

A23187-induced release of LE from macrophages, which occurs only in the presence of extracellular divalent cations, is essentially a lytic process. This is in marked contrast to the selective secretion induced by A23187 in other cell types (Foreman, Mongar & Gomperts, 1973; Feinman & Detwiler, 1974). Zymosan-induced secretion of macrophage LE did not require extracellular calcium or magnesium although secretion was enhanced by magnesium and inhibited by calcium. The influence of divalent cations on zymosan-induced LE secretion may be related to an effect on phagocytosis which is dependent on magnesium but not calcium (Lay & Nussenzweig, 1968; Henson, 1969). Further studies are in progress to investigate the role played by intracellular cations in mediating secretion of LE from macrophages.

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Binding of 5-hydroxytryptamine to human blood platelets

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Drummond & Gordon (1975) described the binding of tritiated 5-hydroxytryptamine ([^{3}H]-5-HT) to rat blood platelets at 4°C. Total and specific binding were measured, the latter being defined as the component of [^{3}H]-5-HT bound which was displaceable by non-radioactive 5-HT. We wished to use human platelets and to determine whether specific [^{3}H]-5-HT binding could be demonstrated. Our aim was then to characterize 5-HT binding sites and assess their relationship to platelet aggregation. The physiological response to specific [^{3}H]-5-HT binding is likely to be platelet aggregation.

Platelet rich plasma (PRP) was prepared in EDTA (Boullin, Green & Price, 1972) and cooled to 2°C. The [^{3}H]-5-HT binding technique was essentially that of Drummond & Gordon (1975); 1–200 nmol/l [^{3}H]-5-HT was incubated with PRP for 45 s to 30 minutes. Thereafter platelets were separated from plasma by centrifugation.

At 2°C [^{3}H]-5-HT was bound extremely rapidly, attaining equilibrium after 120 seconds. 30–50% of [^{3}H]-5-HT was specifically bound being displaced by non-radioactive 5-HT.

Scatchard analysis of values for specifically bound [^{3}H]-5-HT showed at least 2 binding sites: a high affinity (K_1) low capacity (C_1) site, and a low affinity (K_2) high capacity (C_2) site. Individual variations in the values of K and C were measured in several subjects. There was greater variation in K_1 and K_2 than in the capacities of the 2 binding sites (C_1 and C_2). Subject 1 (values are the mean ± s.e. of the mean of 4 determinations made on different days): K_1 $1.58 \pm 0.036.10^{-11}$ mols; C_1 1288 ± 160 molecules/platelet; K_2 $15.1 \pm 2.8.10^{-11}$ mols; C_2 7089 ± 94 molecules/platelet. The capacities of the two binding sites defined in our experiments are similar to two of the three sites described in the rat. The affinity constants of our two specific binding sites are, however greater by about four orders of magnitude.

Subsequent experiments have attempted to measure total and specific [^{3}H]-5-HT binding at 20° and 37°C. To diminish platelet 5-HT transport, PRP was preincubated with metabolic inhibitors, (1 mmol/l N-ethyl maleimide, ouabain, and NaCN), for 30–90 min prior to assessing [^{3}H] 5-HT binding. Under these conditions the values for the affinity constants and binding capacities for specifically bound [^{3}H]-5-HT were comparable to those seen at 2°C.

Preliminary results indicate that the non-displaceable component of total [^{3}H]-5-HT binding may correspond to 5-HT transport.

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