

Alkaline earth ions and the secretion of enzymes from human neutrophil leucocytes

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The ionophore A23187 induces enzyme release from neutrophils (Zabucchi, Soranzo, Rossi & Romeo, 1975), indicating that entry of Ca^{2+} into the cell initiates secretion. However, A23187 produces greater release of lysosome than of β -glucuronidase suggesting that Ca^{2+} entry into the cell may selectively mobilize the 'specific' granules (Wright, Bralove & Gallin, 1977). Spontaneous enzyme release occurs in the absence of stimulus or ionophore and is increased by Ca^{2+} (Goldstein, Horn, Kaplan & Weissmann, 1974).

Neutrophils (95% pure) were obtained from venous blood by the method of Böyum (1968) with hypotonic lysis to remove red cells. Lactic dehydrogenase (LDH), β -glucuronidase and lysosome were assayed as described previously (Wroblewski & LaDue, 1955; Talalay, Fishman & Huggins, 1946; Boasson, 1938). ^{89}Sr -uptake was measured by the method described by Foreman, Hallett & Mongar (1977).

Spontaneous enzyme release amounting to $2.1 \pm 0.3\%$ (mean \pm s.e. mean, $n = 3$) of total cell content for β -glucuronidase and $5.5 \pm 2.0\%$ for lysosome occurred in 60 min of incubation of 37°C at a pH of 7.5, in the absence of extracellular Ca^{2+} or Sr^{2+} . Under these conditions, release of LDH was $5.8 \pm 1.5\%$. Extracellular Ca^{2+} , 1 mM increased spontaneous lysosome release to $7.1 \pm 2.1\%$ and β -glucuronidase release to $3.3 \pm 0.6\%$. Sr^{2+} , 10 mM increased release of lysosome to $22.4 \pm 4.3\%$ and of β -glucuronidase to $4.8 \pm 0.8\%$. Increases caused by Ca^{2+} and Sr^{2+} were significant: $P < 0.01$, Wilcoxon test. The rates of spontaneous lysosome and β -glucuronidase release in the presence of Sr^{2+} , 10 mM are similar to the rate of ^{89}Sr -uptake by neutrophils (Figure 1). At a Sr^{2+} concentration of 10 mM, increase of pH from 7.5 to 8.5 increased secretion of lysosome from $22.4 \pm 4.3\%$ to $40.2 \pm 6.5\%$; β -glucuronidase release increased from $4.8 \pm 0.8\%$ to $10.5 \pm 1.0\%$. Addition of Sr^{2+} , 10 mM or raising pH did not increase LDH release. Changing pH from 7.5 to 8.5 produced a twofold increase in ^{89}Sr -uptake.

Spontaneous enzyme secretion induced by Sr^{2+} may be the result of entry of Sr^{2+} into the cell, and raising extracellular pH appears to facilitate the entry of Sr^{2+} into the cell.

References

- BOASSON, E.H. (1938). On the bacteriolysis by lysosyme. *J. Immun.*, **34**, 281–293.
- BÖYUM, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.*, **21**, Suppl., **97**, 77–89.
- FOREMAN, J.C., HALLETT, M.B. & MONGAR, J.L. (1977). Movement of strontium into mast cells and its relationship to the secretory response. *J. Physiol. Lond.*, **271**, 233–251.
- GOLDSTEIN, I.M., HORN, J.K., KAPLAN, H.B. & WEISSMANN, G. (1974). Calcium-induced secretion from human polymorphonuclear leukocytes. *Biochem. biophys. Res. Commun.*, **60**, 807–812.
- TALALAY, P., FISHMAN, W.H. & HUGGINS, C. (1946). Chromogenic substrates. II Phenolphthalein glucuronic acid as a substrate for the assay of glucuronidase activity. *J. Biol. Chem.*, **116**, 757–772.
- WROBLEWSKI, F. & LADUE, J.S. (1955). Lactic dehydrogenase activity in blood. *Proc. Soc. exp. Biol. Med.*, **90**, 210–217.
- WRIGHT, D.G., BRALOVE, D.A. & GALLIN, J.I. (1977). The differential mobilization of human neutrophil granules. *Am. J. Path.*, **87**, 273–284.
- ZABUCCHI, G., SORANZO, M.R., ROSSI, F. & ROMEO, D. (1975). Exocytosis in human polymorphonuclear leukocytes induced by A23187 and calcium. *FEBS Letters*, **54**, 44–48.

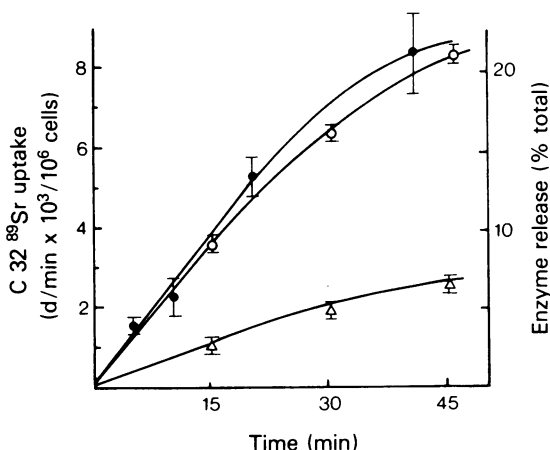


Figure 1 Rate of release of (○) lysosome and (Δ) β -glucuronidase from neutrophils in the presence of Sr^{2+} , 10 mM at pH 8.5 (●) Rate of ^{89}Sr -uptake by neutrophils with a total Sr^{2+} concentration of 10 mM. Each point is the mean (\pm s.e. mean) of three determinations.