Effect of Removal of Calcium-Activated Adenosine Triphosphatase from Rat Mast Cells by Treatment with Sodium Glycocholate

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Sodium glycocholate was shown to remove a Ca^{2+} -activated adenosine triphosphatase from the external surface of the rat mast cell without causing lysis. Sensitized mast cells pretreated with sodium glycocholate showed a decrease in histamine-releasing capacity when triggered with antigen, Synacthen and ATP. Release induced by calcium ionophore A23187 was unaffected.

It has been shown (Coleman & Holdsworth, 1976; Holdsworth & Coleman, 1976) that the bile salts sodium glycocholate and sodium taurocholate are capable of removing certain plasma-membrane 'ecto-enzymes' from the outside of intact erythrocytes and lymphocytes without causing cell lysis. In our laboratory we have demonstrated that the rat peritoneal mast cell contains a Ca^{2+} -activated ATPase,† which appears to be located on the external surface of the plasma membrane (Cooper & Stanworth, 1976a), and further studies of its properties have indicated that it could be involved in the histamine-release process (P. H. Cooper & D. R. Stanworth, unpublished work). A variety of agents can initiate the release of histamine from mast cells. These include the antigen-antibody reaction (Schachter, 1973), challenge with Synacthen (Jasani *et al.*, 1973), ATP (Dahlquist *et al.*, 1974) and calcium ionophore A23187 (Foreman *et al.*, 1973). Release induced by these agents is dependent on the presence of external Ca^{2+} and is highly selective; other soluble cell constituents are not released along with the histamine (Cooper & Stanworth, 1976b). Although the mechanisms of release need not necessarily be the same, these reactions [with the probable exception of calcium ionophore A23187, which transports Ca^{2+} directly through the lipid bilayer of the plasma membrane (Pressman, 1976)] are likely to have a common pathway involving interaction with receptors on the plasma membrane followed by stimulation of the various biochemical reactions leading to histamine release (Bach, 1974). Experiments were therefore performed to establish whether removal of externally facing protein components of the mast-cell plasma membrane by sodium glycocholate had any effect on the histamine-release process itself.

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Materials and Methods

Materials

Sodium glycocholate was obtained from Calbiochem, Bishops Cleeve, Herts. CM23 3AL, U.K. ATP, bovine serum albumin (type F), Hepes, DL-lactic acid (sodium salt), histamine, *o*-phthalaldehyde, NAD^+ and NADH were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. Synacthen (corticotrophin residues 1–24) was obtained from CIBA Laboratories, Horsham, Sussex, U.K., and calcium ionophore A23187 was kindly supplied by Dr. W. E. Brocklehurst of the Lilly Research Centre, Endwood Manor, Wildesham, Surrey, U.K.

Plastic disposable apparatus (test tubes and pipettes) used throughout was obtained from Sterilin, Teddington, Middx., U.K.

Experimental

Male rats (150–200 g weight) were pre-sensitized to egg albumin by the method of Jasani (1973), and 3 weeks later peritoneal mast cells (1.8×10^6 – 2.5×10^6 cells) were isolated and purified from three or four rats as described previously (Cooper & Stanworth, 1976b), by using 20 mM-Hepes/0.9% NaCl/0.5 mM-glucose, pH 7.4. Only preparations of greater than 97% purity were used. The cells were resuspended in the same buffer containing 0–0.2% (w/v) sodium glycocholate in a final volume of 0.25 ml. After incubation for 10 min at 37°C with occasional gentle shaking, the cells were centrifuged at 750g for 2 min at 4°C. A 150 μ l sample of the supernatant was taken for further analysis, the rest discarded and the pellet

† Abbreviations: ATPase, adenosine triphosphatase; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

washed three times with 20 mM-Hepes/0.9% NaCl/0.5 mM-glucose, pH 7.4, before finally being resuspended in 0.25 ml of the same buffer. In some experiments the cells and supernatants were separated by layering the suspension over 1 ml of 15% (w/v) Ficoll in 20 mM-Hepes/0.9% NaCl/0.5 mM-glucose/0.5% bovine serum albumin (type F), pH 7.4, followed by centrifugation at 750g for 2 min at 4°C. The supernatants were carefully removed and the pellets washed three times with 20 mM-Hepes/0.9% NaCl/0.5 mM-glucose, pH 7.4, to remove the Ficoll. However, the results were not significantly changed by this procedure. The pellets and supernatants were then analysed for Ca^{2+} -activated ATPase (EC 3.6.1.3) and lactate dehydrogenase (EC 1.1.1.27) activities and histamine content as described previously (Cooper & Stanworth, 1976a). The assays were unaffected by the presence of sodium glycocholate over the concentration range used. Since macrophages contain high ATPase activity (Coffey & Middleton, 1973), the degree of contamination of the purified mast-cell fractions was also determined. Under the conditions of assay used, the macrophages were found to have 5–8 times the ATPase specific activity (based on cell protein) of the mast cells. However, taking into account the smaller size of the macrophages (maximum protein contribution 1.1%), the total contamination was low (a maximum of 9% of the total activity).

Results and Discussion

Fig. 1 shows that up to a concentration of 0.15% (w/v) sodium glycocholate there was no lysis of the cells as revealed by the low lactate dehydrogenase activity and histamine content of the supernatant. Significant lactate dehydrogenase activity was found in the supernatant when 0.2% (w/v) sodium glycocholate was used, indicating that cell lysis had occurred at this detergent concentration. Histamine release was still low, however, showing that lactate dehydrogenase release is a more sensitive indicator of cell death. The viability of the cells (>95%) was confirmed by optical-microscopic examination of the incubation mixtures and the Trypan Blue dye-exclusion test (Cooper & Stanworth, 1976b). Nevertheless, a considerable proportion of the cellular component of Ca^{2+} -activated ATPase appeared in the supernatants before lysis, and its release was concentration-dependent. Such pre-lytic release is an indication of the outward orientation of this enzyme in the plasma membrane, as shown previously (Cooper & Stanworth, 1976a). Moreover, this enzyme may be localized in the outer leaflet of the lipid bilayer, since, even at high concentration, bile salts are unable to release proteins that completely span the erythrocyte membrane (Holdsworth & Coleman, 1976).

Fig. 1. Effect of sodium glycocholate on the release of Ca^{2+} -activated ATPase (●), lactate dehydrogenase (■) and histamine (▼) from the mast cell

Each symbol represents the mean of the results of four separate experiments. The vertical bars indicate the standard deviations.

Electron micrographs of sodium glycocholate-treated preparations at pre-lytic concentration showed that considerable folding of the plasma membrane had occurred, and the microvilli found on the outside of the cell had become considerably more complex (Plate 1). This is probably due to increased instability of the membrane after removal of proteins from the external surface. Further experiments were performed to establish whether removal of such externally facing components had any effect on the histamine-release process itself. Purified mast cells were again treated with 0–0.2% (w/v) sodium glycocholate as described above and, after being washed three times, the resulting cell pellet was resuspended in 20 mM-Hepes/0.9% NaCl/0.5 mM-glucose, pH 7.4. The cells were challenged at 37°C with ovalbumin (100 µg/ml), Synacthen (4.15 µM), ATP (0.5 mM) and calcium ionophore A23187 (0.5 µg/ml). Incubations were carried out as described previously (Cooper & Stanworth, 1976b). Fig. 2 shows that the releasing capacity of the mast cell was decreased in a concentration-dependent fashion on challenge with antigen, Synacthen or ATP, but remained unaffected when calcium ionophore A23187 was used. The percentage

EXPLANATION OF PLATE I

Effect of sodium glycocholate treatment on the morphology of the mast cell, as revealed by transmission electron microscopy
(a) Control cells; (b) cells treated with 0.15% (w/v) sodium glycocholate.

Fig. 2. Effect of sodium glycocholate treatment on the histamine-releasing capacity of the mast cell

●, Antigen-antibody ($32.7 \pm 4.6\%$); ▲, Synacthen ($62.5 \pm 6.6\%$); ■, ATP ($78.7 \pm 8.2\%$); ○, calcium ionophore ($73.9 \pm 4.9\%$). Control activities (without detergent) are given in parentheses together with standard deviations. Each symbol on the graph represents the mean of the results of four separate experiments. The vertical bars indicate standard deviations.

decrease in histamine-releasing capacity also appeared to correlate well with the removal of Ca^{2+} -activated ATPase from the external surface of the plasma membrane by sodium glycocholate. ^{125}I -labelled immunoglobulin E was also shown not to be removed from the mast cell under the conditions used (K. Batchelor, unpublished work).

These results seem to indicate that histamine re-

lease induced by antigen, Synacthen and ATP is dependent on the presence of external proteins (including the Ca^{2+} -activated ATPase) on the plasma membrane, and demonstrates that sodium glycocholate is capable of removing a proportion of the receptors necessary for the action of such liberators. Conversely, ionophore-induced release appears to be unaffected by such treatment. This may be expected, however, since ionophores transport cations through the lipid bilayer of the plasma membrane and should not therefore be dependent on interaction with external receptor proteins (Pressman, 1976).

These preliminary studies suggest that selective elution with mild detergents such as sodium glycocholate offers a potentially powerful new approach to the study of the structure and function of the outer leaflet of plasma membranes, which should find application to other cell types besides mast cells and to the study of the role of other membrane-sited enzymes.

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