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THE EFFECTS OF NONIDET P40 ON THE FUNCTION OF RAT PERITONEAL MAST CELLS *in vitro*

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1 Treatment of purified rat peritoneal mast cells at 37°C with concentrations of the non-ionic detergent nonidet P40 (NP40) up to 0.005% (v/v) failed to reduce their viability.

2 There was a marked reduction in the histamine releasing capacity of NP40-treated mast cells upon challenge with a variety of selective (adrenocorticotrophic hormone 1–24 (Synacthen), rabbit anti-rat IgE antiserum, adenosine triphosphate (ATP) and the calcium ionophore, A 23187) and non-selective (rabbit anti-rat mast cell antiserum plus complement) histamine liberators.

3 Nonidet P40 (0.005%) was found to reduce the activity of a mast cell membrane 'ecto-enzyme', calcium-activated ATPase, by about 45% when present at the time of its assay.

Introduction

The use of detergents to solubilize plasma membrane constituents has been studied by many workers. Pearlstein & Seaver (1976) showed that plasma membrane proteins could be extracted from intact normal or transformed fibroblasts with low concentrations of nonidet P40 (NP40), without promoting cell lysis. Similar studies have been performed with saturated bile salt detergents, such as glycocholate and taurocholate, which have been shown to solubilize 'ecto-enzyme' activity and phospholipids from human erythrocytes (Coleman & Holdsworth, 1976) and pig lymphocytes (Holdsworth & Coleman, 1976), again without release of cytoplasmic constituents. However, in these studies no account was taken of the functional activity of the cells after detergent treatment. The first report of such a study was from Cooper & Stanworth (1977), who showed that treatment of rat peritoneal mast cells with sublytic concentrations of sodium glycocholate not only solubilized the ecto-enzyme Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase (Ca^{2+}-ATPase), but also reduced the histamine releasing capacity of the cells. More recently, Irulegui, Morris, Batchelor & Stanworth (unpublished observation) have investigated the effects of both NP40 and glycocholate treatment on the structure and receptor functions of mouse peritoneal macrophages.

In the present study, purified rat peritoneal mast cells have been treated with sublytic concentrations of NP40 before challenge *in vitro* with a variety of selective [adrenocorticotrophic hormone peptide (1–24) (Synacthen), rabbit anti-rat IgE antiserum, adenosine triphosphate (ATP) and the calcium ionophore, A 23187] and non-selective (rabbit anti-rat mast cell antiserum) histamine liberators, in order to investigate the effects of NP40 treatment on the release processes.

The effect of NP40 treatment on the activity of mast cell Ca^{2+}-ATPase was also investigated, in the light of the earlier observations of Cooper & Stanworth (1977) on the effect of bile salt detergents on this ecto-enzyme.

Methods

Purification of mast cells

The method of Cooper & Stanworth (1974) was used except that all solutions were prepared in 20 mM Hepes containing 0.9% w/v NaCl solution (saline) and 0.5 mM glucose, pH 7.4 (Hepes medium), instead of 0.1 M phosphate-buffered saline, pH 7.3.

Methods of mast cell challenge by histamine liberators

For each of the histamine liberators used in this study, the following procedure was adopted for the stimulation of the NP40-treated mast cells.

About 2×10^4 mast cells (0.1 ml) were added to 0.9 ml Hepes medium containing 0.5% (w/v) bovine serum albumin (BSA), 0.5 mM Ca^{2+} and the required concentration of liberator, 4.26×10^{-6}M Synacthen,

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1:15 dilution of rabbit anti-rat IgE antiserum (Rba RIgE), 0.5 mM ATP, 0.75 μg/ml A 23187 (kindly supplied by Dr W.E. Brocklehurst of the Lilly Research Centre, Wildesham, Surrey) or 1:20 dilution of decomplemented rabbit anti-rat mast cell antiserum (Rba RMC) + 5% fresh guinea-pig serum. The mast cells and liberators were pre-warmed to 37°C before addition of the cells; then the reaction was allowed to proceed for a further 5 min at 37°C (15 min at 37°C for ATP), before the cells were separated from the supernatant fluid by centrifugation in a refrigerated MSE major for 2 min at 800 *g*. The supernatants were decanted into 1 ml 10% (w/v) trichloracetic acid (TCA), while 2 ml 5% (w/v) TCA was added to the cell pellets.

Estimation of histamine

Histamine was assayed by the automated spectrofluorimetric method of Evans, Lewis & Thomson (1973).

Enzyme assays

Lactate dehydrogenase (LDH) The activity of this enzyme was assayed by the colorimetric method of King, as described by Varley (1967) except that the final assay volumes were scaled down by a factor of 5 to give the necessary sensitivity, and incubation was allowed to proceed for 60 min.

Ca^{2+}-activated ATPase The activity of Ca^{2+}-ATPase was measured according to the method of Cooper & Stanworth (1976), based on the hydrolysis of ATP.

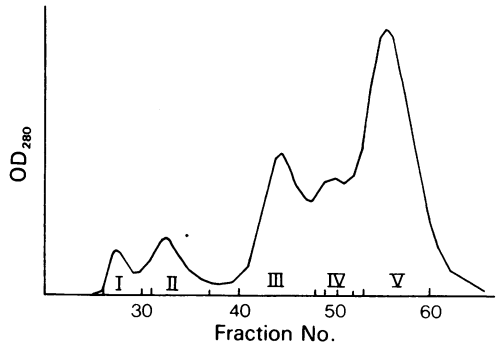

Figure 1 Ultrogel AcA 34 chromatography of rat myeloma IgE serum; peak III contained IgE. The column (84 × 2.5 cm) was eluted with PBS at 20 ml/h.

Microscopic examination of mast cell viability

Mast cell preparations were incubated at 37°C for 2 min before the addition of 0.25% (w/v) trypan blue (final concentration). After a further 5 min incubation at 37°C, an aliquot of the cells was placed within a modified Fuchs-Rosenthall chamber for analysis under the phase contrast microscope.

Production of antisera

Rabbit anti-rat IgE antiserum (RbaRIgE) Rat IgE was purified from rat myeloma IgE serum (kindly donated by Dr Bazin) by gel filtration on Ultrogel AcA 34 (Figure 1) followed by ion-exchange chromatography on DEAE-Sephadex A-50 (Figure 2). Immu-

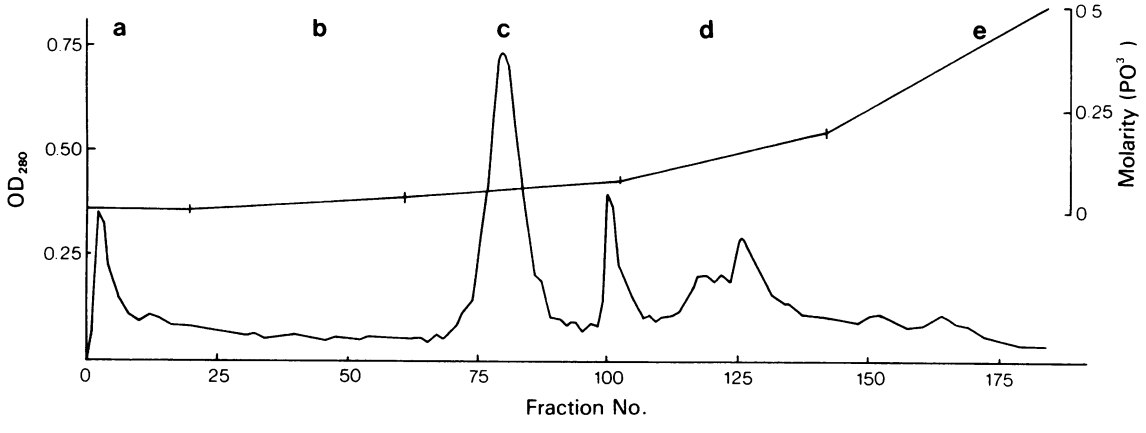

Figure 2 DEAE-Sephadex A.50 ion-exchange chromatography of peak III from AcA 34 chromatography of rat myeloma IgE serum (Figure 1). Fraction volume = 7 ml. Elution media: (a) 0.01 M phosphate, (b) 0.01–0.04 M phosphate, (c) 0.04–0.08 M phosphate, (d) 0.08–0.2 M phosphate, (e) 0.2–0.5 M phosphate, all at pH 6.5. The column (20 × 1 cm) was eluted at 10 ml/h.

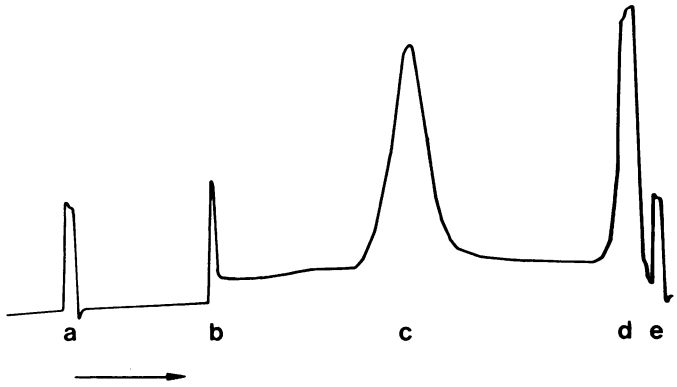

Figure 3 Schlieren trace of purified rat myeloma IgE in the MSE Centriscan 75. Speed: 55,000 rev/min, temperature: 22°C. (a) Reference line; (b) meniscus; (c) myeloma IgE antibody; (d) cell bottom; (e) reference line.

noelectrophoretic analysis of fractions showed that rat IgE was localised predominantly in the fraction which eluted at 0.06 M phosphate buffer pH 6.5. This fraction was concentrated by ultrafiltration with simultaneous dialysis and the contents analysed by ultracentrifugation in the MSE Centriscan 75, confirming it to be of a high degree of purity (approximately 90%) and to sediment with an $S_{20,w}$ value of 7.95s (Figure 3). About 30 μg of the purified rat IgE was emulsified in complete Freund's adjuvant (CFA), before being injected intramuscularly into the hind leg and subcutaneously in the neck of a rabbit. This injection procedure was repeated twice at fortnightly intervals before a bleed was taken from the ear vein, 14 days after the last injection.

Subsequent injections were performed at two-monthly intervals, bleeds being taken approximately 2 weeks after each injection. The antiserum was absorbed with normal rat serum and de-complemented by heating for 30 min at 56°C before use.

Rabbit anti-rat mast cell antiserum (RbaRMC) About 2×10^6 purified mast cells were washed 4 times in Hepes medium, emulsified with CFA and injected subcutaneously in the scruff of the neck of a rabbit. The injection schedule was continued as above, for rat IgE, and the serum collected was de-complemented by heating for 30 min at 56°C as before.

Results

Assessment of mast cell viability

In order to study the effects of NP40-treatment on mast cell function it was necessary to define a concentration range of NP40 which did not cause cell lysis. Purified mast cells were incubated for 10 min at 37°C in Hepes medium containing 0.5% BSA and various concentrations of NP40. By the three criteria, spontaneous histamine release, trypan blue uptake and LDH release, it was shown that a concentration range of 0.001%–0.005% (v/v) NP40 did not affect mast cell viability (Figure 4), in as much as the degree of release or uptake recorded was not greater than that shown by control (non-detergent treated) mast cells.

Effect of NP40 treatment on selective histamine release processes

Purified mast cells were incubated for 10 min at 37°C with NP40 (0 to 0.005% (v/v)), as above, washed and then challenged with a single dose of histamine liberating agent. The dose-response curves thus obtained revealed two patterns of detergent concentration-dependent inhibition. On the one hand (see Figure 5a) there was little effect on histamine release initiated by Synacthen or RbaRIgE until NP40 concentrations in excess of 0.002% (v/v) were employed. On the other hand (see Figure 5b) at a detergent concentration as high as this, a substantial amount of inhibition had been achieved when ATP or A 23187 was used as releasing agent; the NP40 treatment having the most marked effect on A 23187-induced histamine release. However, the releasing capacity of all liberators was ultimately inhibited by at least as much as 90% after treatment of the mast cells with 0.005% (v/v) NP40.

Effect of NP40 treatment on non-selective histamine liberators

Purified mast cells were treated with NP40 (0 to 0.005% (v/v)), as before, washed and then challenged with de-complemented RbaRMC (diluted 1:20) plus

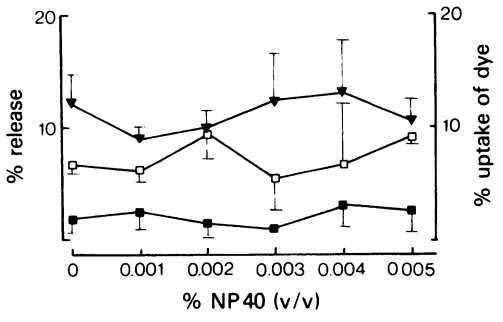

Figure 4 The effect of NP40 (0 to 0.005%) on rat peritoneal mast cell viability: (▼) LDH release ($n = 4$); (□) trypan blue uptake ($n = 2$); (■) spontaneous histamine release ($n = 6$).

5% fresh guinea-pig serum. The NP40 treatment also reduced the histamine release effected by this non-selective liberator. But, as will be seen from Figure 5c, it was necessary to employ detergent concentrations in excess of 0.003% (v/v) before appreciable inhibition was achieved; a maximum of around 70% inhibition being observed after treatment of the mast cells with 0.005% (v/v) NP40.

Effect of NP40 on the activity of Ca^{2+}-ATPase

The mast cell membrane enzyme, Ca^{2+}-ATPase, was assayed in the presence of various concentrations of NP40 (Figure 6). This procedure was found to reduce the activity of the enzyme in a dose-dependent fashion, 55% of the enzyme activity remaining at a detergent concentration of 0.005% (v/v). Interestingly, low concentrations of the detergent (<0.001% NP40) appeared to stimulate the activity of Ca^{2+}-ATPase.

Discussion

It has been reported that NP40 treatment can solubilize membrane proteins from intact fibroblasts (Pearlstein & Seaver, 1976) and can reduce the activity of Fc and C3 receptors on mouse macrophages (Irulegui *et al.*, unpublished observations). In the present study we have shown that treatment of mast cells with sublytic concentrations of this detergent dramatically reduces the histamine releasing capacity of the cells; greater than 90% inhibition of histamine release effected by selective liberators (Synacthen, RbaRIgE, ATP and A 23187) being observed after treatment of the mast cells with 0.005% (v/v) NP40 (Figure 5a and b). The most marked effect of NP40 treatment was reflected in mast cells subsequently challenged with the calcium ionophore. A previous study undertaken in our laboratory (Cooper & Stanworth, 1977) had

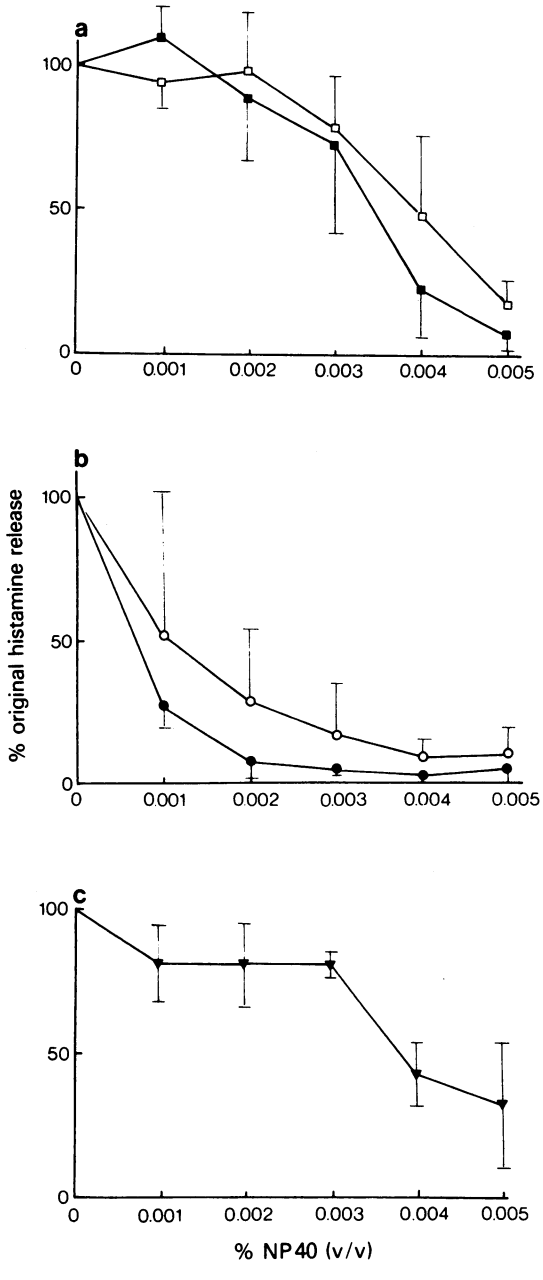

Figure 5 The effect of NP40 treatment on the histamine releasing capacity of rat peritoneal mast cells. (a) (■) Synacthen (4.26×10^{-6} M)-induced histamine release ($n = 5$); (□) RbaRIgE (diluted 1:15)-induced histamine release ($n = 4$). (b) (●) A 23187 (0.75 μg/ml)-induced histamine release ($n = 3$); (○) ATP (0.5 mM)-induced histamine release ($n = 3$). (c) (▼) Rba RMC (diluted 1:20) + 5% fresh guinea-pig serum-induced histamine release ($n = 3$). Vertical lines show s.e. mean.

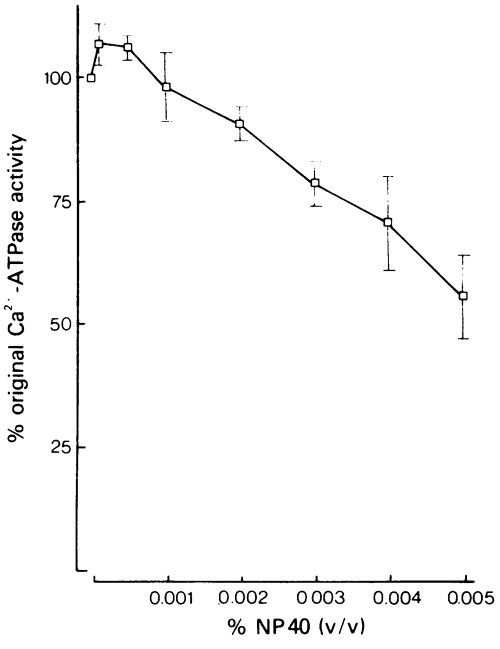

Figure 6 The effect of NP40 on the activity of mast cell calcium-activated ATPase ($n = 4$).

revealed that histamine release initiated by this reagent was not affected by pre-treatment with the bile salt detergent, sodium glycocholate in contrast to release effected by antigen, Synacthen or ATP. These earlier findings were attributable to the solubilization by the glycocholate of a Ca^{2+}, Mg^{2+}-activated ATPase, which we have shown to be located on the outer surface of the plasma membrane of rat peritoneal mast cells (Cooper & Stanworth, 1976). Clearly, therefore, the action of NP40 is different from that of glycocholate; and, indeed, we have found no evidence that it removes mast cell membrane enzymes such as the Ca^{2+}-ATPase, 5'-nucleotidase or alkaline phosphodiesterase. However, whereas glycocholate did not affect the activity of the Ca^{2+}-ATPase *per se*, treatment with NP40 (0.005%) inhibited this enzyme by about 45% (Figure 6). Hence, the non-ionic detergent (NP40) has the ability to impair *in situ* the activity of a mast cell ectoenzyme which recent findings (Batchelor, Cooper & Stanworth, 1979) have suggested might be involved in the histamine release process.

However, this does not readily explain our observations that NP40 treatment reduces histamine release effected artificially by incubation of mast cells with the calcium ionophore, A 23187. But, as the intercalation of amphiphiles into membrane bilayers is thought to involve phospholipids (Sheetz & Singer, 1974), it is probable that the NP40 occupies sites within the mast cell membrane phospholipid which the calcium ionophore occupies during its triggering of the cells. Nonidet P40 may, therefore, affect the activity of membrane protein constituents such as Ca^{2+}-ATPase by likewise altering their phospholipid environment.

Perhaps not surprisingly, NP40 treatment appeared to have a less marked effect on a mast cell membrane antigenic constituent as reflected by our findings that the lytic release of histamine initiated by Rba RMC antiserum was not inhibited as strongly as were the selective release processes.

Thus, we have provided further examples of the exploitation of non-lytic detergents as probes in determining the role of mast cell plasma membrane constituents in histamine release processes.

References

BATCHELOR, K.W., COOPER, P.H. & STANWORTH, D.R. (1979). The possible role of membrane-located adenosine triphosphatases in mast cell triggering. In *The Mast Cell*, International Symposium, Davos. ed. Pepys, J. & Edwards, A.M. Tunbridge Wells: Pitman Medical.

COLEMAN, R. & HOLDSWORTH, G. (1976). The release of membrane components prior to haemolysis during extraction of intact erythrocytes with bile salts. *Biochim. biophys. Acta*, **426**, 776–780.

COOPER, P.H. & STANWORTH, D.R. (1974). A simple and reproducible method for isolating rat peritoneal mast cells in high yield and purity. *Prep. Biochem.*, **4**, 105–114.

COOPER, P.H. & STANWORTH, D.R. (1976). Characterisation of calcium-ion-activated adenosine triphosphatase in the plasma membrane of rat mast cells. *Biochem. J.* **156**, 691–700.

COOPER, P.H. & STANWORTH, D.R. (1977). Effect of removal of calcium-activated adenosine triphosphatase from rat mast cells by treatment with sodium glycocholate. *Biochem. J.*, **168**, 583–585.

EVANS, D.R., LEWIS, J.A. & THOMSON, R.S. (1973). An automated fluorimetric assay for the rapid determination of histamine in biological fluids. *Life Sci.*, **12**, 327–336.

HOLDSWORTH, G. & COLEMAN, R. (1976). Plasma membrane components can be removed from isolated lymphocytes by the bile salts glycocholate and taurocholate without cell lysis. *Biochem. J.*, **158**, 493–495.

PEARLSTEIN, E. & SEAVER, J. (1976). Non-lytic, non-ionic detergent extraction of plasma membrane constituents from normal and transformed fibroblasts. *Biochim. biophys. Acta*, **426**, 589–597.

SHEETZ, M.P. & SINGER, S.J. (1974). Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4457–4461.

VARLEY, H. (1967). *Practical Clinical Chemistry*, 4th Edition, pp. 279–280. New York: William Heinemann.

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