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Primary Amines Induce Selective Release of Lysosomal Enzymes from Mouse Macrophages

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Cultured mouse peritoneal macrophages were found to release substantial amounts of lysosomal β-glucuronidase and β-galactosidase activities when exposed to millimolar concentrations of various primary aliphatic monoamines. With methylamine, ethylamine, propylamine and butylamine, lysosomal enzyme release was selective, but further increases in the aliphatic chain length resulted in the compounds becoming lytic. By contrast, structurally related primary aliphatic diamines proved to be inactive at inducing both selective and lytic lysosomal-enzyme discharge.

The persistence of macrophages at sites of chronic inflammation of both immunological and non-immunological origin has stimulated much interest in their functions in pathological situations. It is now apparent that, in addition to their role as phagocytes, these cells actively perpetuate inflammatory lesions by secreting, in response to appropriate stimuli, a wide repertoire of biologically active materials such as: components of both the classical and alternative pathways of complement (Muller *et al.*, 1978; Barber & Burkholder, 1978; Littmann & Ruddy, 1977; Bentley *et al.*, 1978), prostaglandins of various classes (Humes *et al.*, 1977; Bonney *et al.*, 1978; Hsueh *et al.*, 1980), and several neutral proteinases (Werb & Gordon, 1974; Unkeless *et al.*, 1974). Other studies have shown that mouse peritoneal macrophages can also release substantial amounts of lysosomal acid hydrolases into culture supernatants in the absence of cell death when presented with stimuli such as mouldy-hay dust (Schorlemmer *et al.*, 1977*a*), immune complexes (Cardella *et al.*, 1974), dextran sulphate (Schorlemmer *et al.*, 1977*b*) and supernatants of concanavalin A-stimulated lymphocytes (Pantalone & Page, 1977). The biochemical mechanisms underlying this response are largely unknown, although Schorlemmer *et al.* (1977*c*) have suggested that the ability of a substance to trigger lysosomal-enzyme release from macrophages may be related to its ability to activate the complement system by the alternative pathway. Such activation results in the cleavage of component C3, thereby generating fragment C3b, which has also been shown to be a potent stimulator of enzyme secretion (Schorlemmer *et al.*, 1976), and since (as mentioned above) macrophages appear to synthesize and secrete complement components of the alternative pathway, they could comprise a self-activating system. We now report that several primary aliphatic monoamines, such as methylamine, can also induce the selective release of lysosomal enzymes by an apparently similar process. By contrast, structurally related primary aliphatic diamines, such as 1,2-diaminoethane, proved to be inactive in this respect.

Experimental

Materials

Tissue-culture medium 199 was obtained from Wellcome Research Laboratories, Beckenham, Kent, U.K. Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], streptomycin, penicillin and heat-inactivated foetal-calf serum were obtained from Flow Laboratories Ltd., Irvine, Ayrshire, Scotland, U.K. Plastic tissue-culture-grade Petri dishes (30mm diam.) were obtained from Sterilin Ltd., Teddington, Middx., U.K. *p*-Nitrophenyl β-D-galactopyranoside, phenolphthalein β-D-glucuronic acid, lactic acid, NAD^+ (grade IV), methylamine hydrochloride, pentylamine, hexylamine, 1,4-diaminobutane, 1,5-diaminopentane, chloroquine phosphate, quinine hydrochloride and amantadine hydrochloride were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Ethylamine hydrochloride, propylamine, butylamine, 1,2-diaminoethane, 1,3-diaminopropane and 1,6-diaminohexane were obtained from BDH, Poole, Dorset, U.K.

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Methods

Peritoneal cells were collected from inbred male Balb/c mice by lavage of the peritoneal cavity with 3ml portions of medium 199 containing 20mM-Hepes, pH 7.2, 100μg of streptomycin/ml, 60μg of penicillin/ml and 10% (v/v) heat-inactivated foetal-calf serum. The cell suspension was adjusted to a concentration of 2×10^6 cells/ml and 2ml portions were dispensed into 30mm-diameter plastic Petri dishes. After incubation at 37°C for 2h the non-adherent cells were removed, and the remaining macrophage monolayers rinsed three times with medium 199, before adding fresh medium 199 supplemented with 10% heat-inactivated foetal-calf serum. The macrophages were cultivated overnight at 37°C to give a uniform monolayer of well-spread cells composed of more than 95% macrophages. The cells were then rinsed three times with medium 199 (without serum) before being exposed to various amines in serum-free or serum-containing medium 199. At the end of the incubation period the supernatants were removed and the cells lysed with 2ml portions of 0.1% Triton X-100 in 0.9% NaCl. Both supernatant and cell fractions were analysed for activities of various lysosomal and cytoplasmic enzymes.

Lactate dehydrogenase was assayed by determining the rate of oxidation of lactic acid with the automated method of D. W. H. Riches & D. R. Stanworth (unpublished work); β-glucuronidase was assayed by the method of Talalay *et al.* (1946), with phenolphthalein β-D-glucuronic acid as substrate; and β-galactosidase was assayed by the method of Conchie *et al.* (1959), with *p*-nitrophenyl β-D-galactopyranoside as substrate. All enzyme assays were conducted under conditions giving a linear release of reaction product with time.

Results and discussion

When either serum-free or serum-containing cultures were exposed to methylamine in the concentration range 10–50mM, purified mouse peritoneal macrophages showed marked morphological changes characterized by the formation of many phase-lucent vesicles, and a tendency to assume a rounded shape, in contrast with their normal well-spread appearance. Biochemically such treatment also resulted in the dose-dependent release of the lysosomal enzymes β-glucuronidase and β-galactosidase from the macrophages into the culture supernatants, with almost 80% of the total culture activities appearing in the supernatants after exposure to 50mM-methylamine for 5h at 37°C (Fig. 1*a*). Since the total culture activities of both β-glucuronidase and β-galactosidase remained constant at all concentrations of methylamine studied (4.61 ± 0.26nmol of phenolphthalein liberated$\cdot h^{-1} \cdot$culture^{-1} for β-glucuronidase, and 73.0 ± 3.6nmol of *p*-nitrophenol liberated$\cdot h^{-1} \cdot$culture^{-1} for β-galactosidase), it is assumed that the lysosomal-enzyme activities detected in the culture supernatants were derived from their storage location in intracellular lysosomes rather than being newly synthesized and secreted directly into the external medium. The selective nature of the release process, which occurred in the absence of cell death, is shown by the failure of the cytoplasmic enzyme lactate dehydrogenase to appear in culture supernatants at any concentration of methylamine tested.

The time course of lysosomal-enzyme release from mouse macrophages after exposure to a single dose of 50mM-methylamine is shown in Fig. 1(*b*).

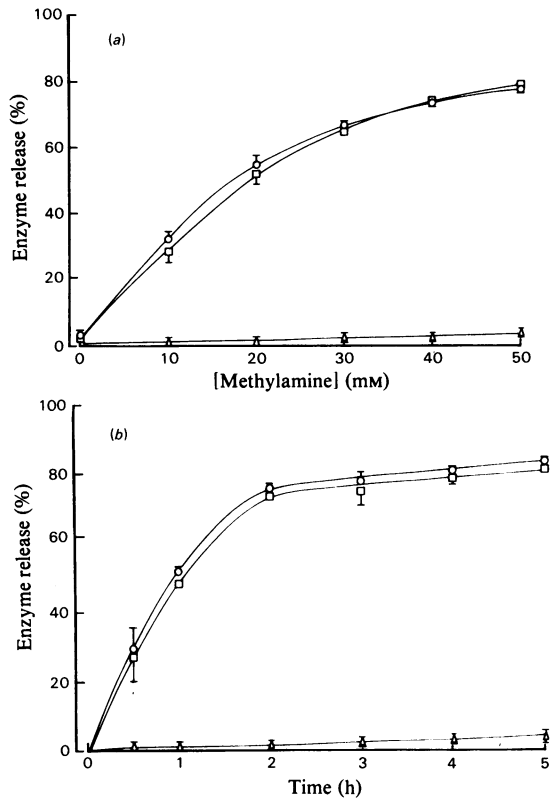

Fig. 1. *Dose-dependency and kinetics of release of lysosomal enzymes by methylamine*

(*a*) Dose-dependent release of lysosomal enzymes from mouse macrophages after exposure to methylamine for 5h in serum-free medium 199 at 37°C. (*b*) Time-dependent release of lysosomal enzymes from mouse macrophages after exposure to a single dose of methylamine (50mM) in serum-free medium 199. ○, β-Glucuronidase; □, β-galactosidase; △, lactate dehydrogenase. Each point represents the mean ± S.D. for four observations.

Significant ($P<0.001$) amounts of both β-glucuronidase and β-galactosidase were found in culture supernatants after only 30min incubation, whereas maximal enzyme release, which was in the order of 80%, occurred after 2h of incubation. The absence of significant ($P<0.1$) amounts of lactate dehydrogenase activities in the culture supernatants at any point during the experiment confirmed that the release of lysosomal enzymes was indeed a selective rather than a lytic process.

Further experiments were carried out to investigate whether any structure–function relationships existed for lysosomal-enzyme release by other weak bases. Methylamine, ethylamine, propylamine and butylamine were all found to be effective stimulants of selective lysosomal-enzyme release when used at 20mM (Fig. 2*a*). However, further increases in the length of the aliphatic chain, as in pentylamine and hexylamine, resulted in a pronounced tendency for these compounds to become lytic, presumably due to the development of detergent-like properties. Similarly, other weak bases, such as NH_4Cl, when used at 50mM, chloroquine and quinine, when used at the somewhat lower concentration of 0.3mM, and amantadine, when used at 5mM, were also found to exert a selective release of lysosomal acid hydrolases after their incubation with mouse peritoneal macrophages (Fig. 2*b*). By contrast, structurally related primary aliphatic diamines of increasing hydrocarbon chain length, from 1,2-diaminoethane through to 1,6-diaminohexane, were totally inactive as inducers of acid hydrolase discharge and as lytic agents when used at 20mM (Fig. 2*c*).

The mechanism of lysosomal-enzyme release effected by these compounds is far from clear, since primary amines appear to have many diverse effects on cells. D'Arcy Hart & Young (1978) reported that phagosome–lysosome fusion is enhanced in mouse macrophages that have been exposed to chloroquine, whereas Davies *et al.* (1980) have shown that several primary amines can inhibit fibroblast transglutaminase, an enzyme that appears to be required for the receptor-mediated endocytosis of α_2-macroglobulin. More significantly, Ohkuma & Poole (1978) have found that several weak bases, including methylamine, rapidly diffuse into macrophages as uncharged species and accumulate in the lysosomes, resulting in a marked osmotic swelling of the lysosomes (vacuolation) with a concomitant increase in the intralysosomal pH. Thus, if the increase in intralysosomal pH is related to the release of lysosomal enzymes, the ineffectiveness of primary aliphatic diamines at inducing lysosomal enzyme release might be accounted for by their relatively slow rate of lysosomal accumulation in comparison with the rapid rate of uptake of primary aliphatic monoamines.

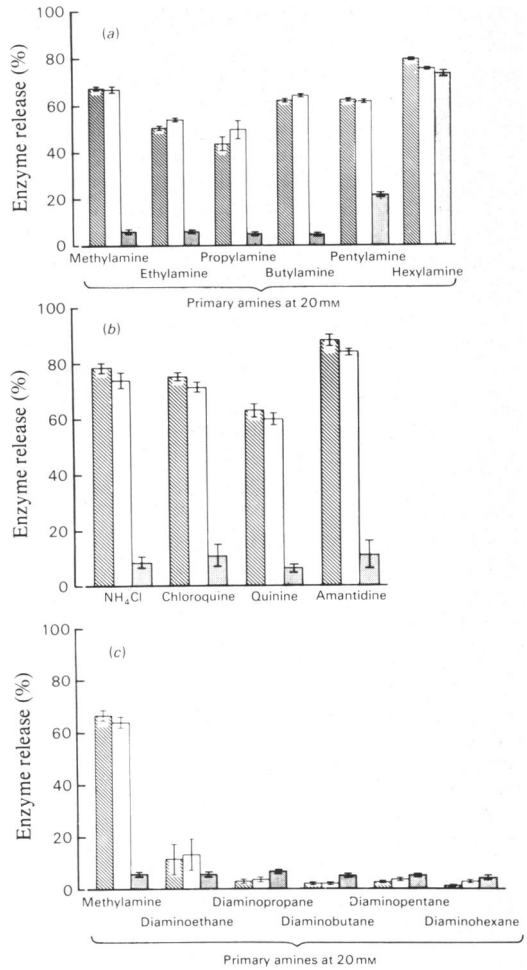

Fig. 2. *Structure–function (lysosomal-enzyme release) relationships shown by various weak bases*

(*a*) Effects of various primary aliphatic monoamines on the release of lysosomal and cytoplasmic enzymes from mouse macrophages; (*b*) effects of various weak bases on the release of lysosomal and cytoplasmic enzymes from mouse macrophages; and (*c*) effects of various aliphatic diamines on the release of lysosomal and cytoplasmic enzymes from mouse macrophages. Macrophages were exposed to the various amines for 5h at 37°C in serum-free medium 199. Control cultures, without amines, were found to release less than 5% of their total available lysosomal and cytoplasmic enzymes. ▨, β-glucuronidase; □, β-galactosidase; ▩, lactate dehydrogenase. Each point represents the mean ± S.D. for four observations.

However, an alternative explanation of the observations reported here may in some way be related to the known effects of some primary amines on the complement system (Pillemer *et al.*, 1940; D. W. H.

Riches & D. R. Stanworth, unpublished work). As mentioned above, Schorlemmer *et al.* (1977*c*) have drawn attention to the fact that most of the substances that have been found to induce selective lysosomal-enzyme release from macrophages are also capable of activating the alternative pathway of complement, thereby producing fragment C3b, which has also been shown to have a capacity to induce selective lysosomal-enzyme release (Schorlemmer *et al.*, 1976). Hence it is conceivable that these primary amino compounds can initiate the breakdown of endogenous macrophage component C3 in such a way as to produce a fragment capable of triggering lysosomal-enzyme release by by-passing the complete activation of the alternative complement pathway. Further work is required to elucidate further the nature of this release process, particularly in relation to the well-established effects of primary amines on both the lysosomal system of macrophages and the serum complement system.

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