INHIBITION OF CELL SPREADING BY LYSOSOMOTROPIC AMINES

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Received 11 July 1979

1. Introduction

Ammonia and various amines with weak-base properties have been shown to accumulate in acidic cellular vacuoles such as lysosomes, and to inhibit lysosomal degradative processes in several types of cells in vitro [1–11]. In addition these compounds may: inhibit protein secretion [12] as well as other vesicle-mediated secretory processes [13], inhibit the adsorbtive uptake of macromolecules [14–16], suppress the activity of certain receptor-dependent toxins [17,18], and prevent the focal patching of occupied cell-surface receptors normally preceding internalization and degradation of receptor–ligand complexes [19]. That all of these effects are observed only at relatively high amine concentrations (2–10 mM) may indicate that they are consequential of a generalized perturbation of cellular membrane mobility, caused by the accumulation of amines in certain compartments of the cellular membrane system.

Isolated rat hepatocytes have been shown to attach to and spread readily on substrata of adsorbed serum (fibronectin) or collagen [20–22], and scanning electron microscopy has revealed that the spreading is mediated by a continuous flow of a thin, basal layer of lamellar cytoplasm [21] directed towards the cell periphery. A general interference with cellular membrane flow might therefore be expected to cause inhibition of hepatocyte spreading, and such inhibition by lysosomotropic amines is demonstrated here.

2. Materials and methods

Isolated rat hepatocytes were prepared from the liver of 16 h fasted male Wistar rats (250–300 g) by the method of collagenase perfusion [23,24].

For the measurement of cell spreading, 10^6 cells in 2 ml medium were seeded onto 6 cm polystyrene tissue culture dishes (Falcon) pretreated with adsorbed collagen (the pretreated dishes had been allowed to stand for 10 min at room temperature with 2 ml collagen solution; Sigma type I calf skin collagen dissolved at 5 μg/ml in 0.2% acetic acid, then rinsed 3 × with 2 ml sterilised distilled water and stored dry in sterile packages before use). The medium was composed of the salts and buffers of 'suspension buffer' [23], fortified with the vitamins of Dulbecco's medium, amino acids at 10 × normal physiological concentrations [25], 20 mM pyruvate and Mg^{2+} added to 2 mM. The NaCl and NaOH concentrations were so adjusted that the final osmolarity and pH were 290 mosM and 7.6, respectively, at 37°C. The dishes were incubated for 3 h at 37°C, fixed in 1% glutaraldehyde [2] and the percentage of spread cells (cells which had lost their rounded outline) was counted microscopically. In control cultures (no amines or other inhibitors added), 90% of the cells had spread in 3 h.

Protein degradation was measured as the release of [^{14}C]valine from 24 h in vivo-labelled cells, during a 1 h incubation in shaking centrifuge tubes in unsupplemented suspension buffer [3]. The degradation rate without amine addition was 4.5%/h (mean value of several experiments).

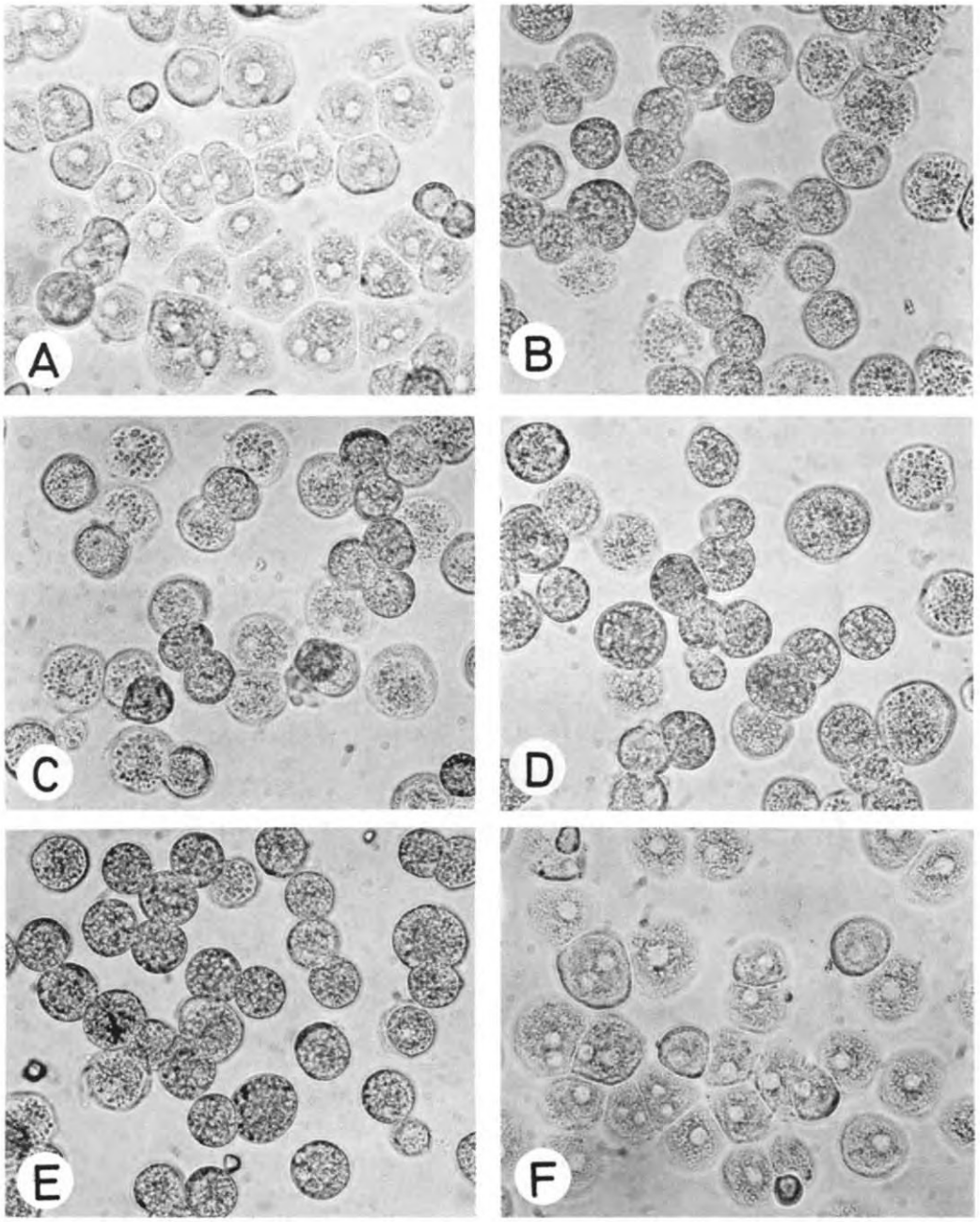

Fig.1. Morphology of spreading hepatocytes in the absence and presence of lysosomotropic amines. 10^6 rat hepatocytes in 2 ml medium were incubated in collagen-treated 6 cm tissue culture dishes for 3 h at 37°C with: (A) no additions; (B) 10 mM propylamine; (C) 10 mM methylamine; (D) 10 mM dimethylamine; (E) 10 mM trimethylamine; (F) 10 mM tetramethylammonium. The cultures were fixed with 1% glutaraldehyde and photographed at a magnification of 260 ×.

Protein synthesis (basal rate without amine addition = 0.5%/h) was measured as the incorporation of [^{14}C]valine at constant specific radioactivity during a 1 h incubation of the cells in centrifuge tubes [26,27]; the medium was suspension buffer supplemented with 20 mM pyruvate and amino acids at 10 × normal physiological concentration [25] except in the case of 2-aminobutyrate where pyruvate and amino acids were omitted during the protein synthesis measurement in order to demonstrate the stimulatory (energy-providing) function of this amino acid; an effect demonstrated with other amino acids [27], but not seen with any of the amines.

3. Results and discussion

As shown in fig.1 and table 1, of the tested amines, all with weak-base properties inhibited the spreading of hepatocytes on adsorbed collagen, a process which is normally completed within 3 h. These amines are also potent inhibitors of protein degradation (table 1) by virtue of their ability to accumulate in lysosomes [2], whereas they have little effect on protein synthesis under the experimental conditions

Table 1
Effects of amines on hepatocytic protein synthesis, protein degradation and cell spreading

Amine	Amine effect (% change)		
	Cell spreading	Protein degradation	Protein synthesis
Imidazole	−44	−56	−14
Methylamine	−45	−62	−13
Dimethylamine	−51	−63	− 5
Propylamine	−62	−69	−11
2-Aminobutanol	−66	−62	−17
Trimethylamine	−84	−60	− 6
Tetramethylammonium	−10	+ 7	− 8
2-Aminobutyrate	−10	+ 3	+14

Isolated rat hepatocytes were incubated under the conditions in section 2, and the effects of various amines (at 10 mM) on cell spreading, protein degradation and protein synthesis were tested. The amine effects are expressed in % of control values (+ means stimulation, − means inhibition). 400–500 cells were counted for the determination of cell spreading; the values for protein synthesis and degradation are the means of 3 cell samples

used (i.e., in the presence of amino acids and pyruvate). This is an important control, because the protein synthesis inhibitor cycloheximide (1 mM) inhibits hepatocyte spreading by 45%. We also find that colchicine (1 mM), an agent known to disrupt microtubules, inhibits spreading (by 42%). The effect of ammonia on cell spreading could not be studied due to its rapid metabolic conversion to alanine in the presence of pyruvate (20 mM in the test system).

The two amines shown in table 1 as having no significant effect on either hepatocyte spreading or protein degradation – tetramethylammonium and the amino acid 2-aminobutyrate – also lack weak-base properties, supporting the notion that cell spreading and lysosome function may be inhibited by related mechanisms.

The assumed mechanism of action of the weak-base amines on cell spreading is schematically shown in fig.2. It is known that the rate of membrane

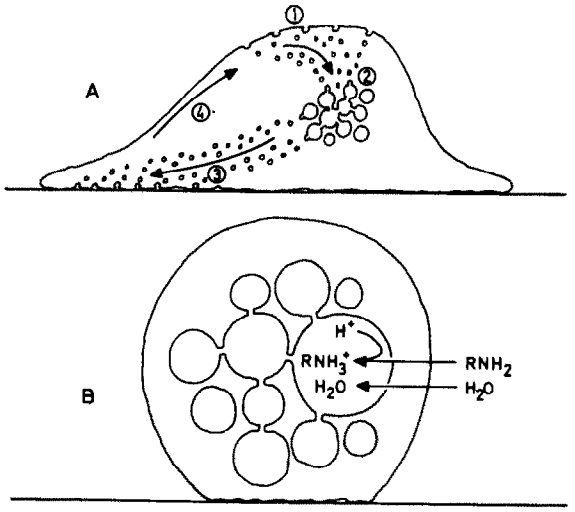

Fig.2. Possible interruption of membrane flow and cell spreading due to lysosomal swelling. (1) Endocytosis; (2) fusion of endocytic vesicles with lysosomes; (3) exocytosis (return of membrane components to the cell surface by secretion or other recycling processes); (4) lateral bulk flow or diffusion of some membrane components (including patching and capping) eventually returning to endocytic sites to complete membrane recycling. (A) Untreated, normally spreading cell; (B) amine-treated cell with accumulation of amine and water in lysosomes and other acidic vacuoles. The lysosomal apparatus is depicted here as a more or less continuous organelle, in agreement with recent findings via electron microscopic serial sectioning [28,29].

recycling is high in normal cells [30,31], and membrane material from the cell surface may pass through acidic vacuoles such as lysosomes, protein-secretory vesicles and parts of the Golgi apparatus [29,30]. The osmotic swelling of vacuoles and the neutralization of intravacuolar acidity caused by the weak-base amines may conceivably result in a functional impairment and a retardation of membrane flow through the vacuolar compartments. There is some evidence for continued influx and accumulation of material in lysosomes during ammonia blockage [9] and the degree of lysosomal swelling observed [2] is hardly possible without the acquisition of new membrane material. A relative accumulation of membrane material in intracellular vacuoles, as suggested in fig.2, would seem to be a more likely mechanism for the 'surface' effects of weak-base amines than, e.g., direct binding to cell surface components, particularly since amines with structures as dissimilar as, e.g., methylamine and imidazole both inhibit cell spreading (table 1).

Acknowledgements

The work was supported by grants from The Norwegian Cancer Society and The Norwegian Council for Science and The Humanities.

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