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Effects of Lysosomotropic Monoamines, Diamines, Amino Alcohols, and Other Amino Compounds on Protein Degradation and Protein Synthesis in Isolated Rat Hepatocytes

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SUMMARY

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More than 60 different amines have been tested for their effect on protein degradation and protein synthesis in isolated rat hepatocytes. Unsubstituted monoamines and amino alcohols were generally lysosomotropic, as shown by their ability to inhibit protein degradation and to induce lysosomal swelling (vacuolation). In a nutrient-free medium these amines also inhibited protein synthesis, apparently by limiting the supply of degradation-derived amino acids and energy substrates. Some diamines were lysosomotropic; others were not. Most of the diamines inhibited protein synthesis and did so independently of whether they affected protein degradation or not. Polyamines, amino acids, and quaternary ammonium compounds were not lysosomotropic. All of the typically lysosomotropic amines inhibited protein degradation to the same extent (corresponding to approx. 75% of the overall degradation), and their maximal effects were nonadditive. This may indicate a complete and selective blockade of the lysosomal pathway of protein degradation, making these compounds useful tools in the study of intracellular protein metabolism.

INTRODUCTION

Following the discovery of ammonia as a natural inhibitor of lysosomal protein degradation in isolated rat hepatocytes (1, 2), this compound has become widely used in degradation studies (3–12). A few other amines have been examined and found to behave similarly (7, 9–12). The basis for the lysosomotropic effect of weak-base amines appears to be their ability of becoming protonated within the acidic interior of the lysosomes. This will result in intralysosomal accumulation of protonated amine, leading to osmotic swelling (vacuolation) of the lysosomes and giving rise to neutralization of the lysosomal pH (1, 9, 13).

To further explore the generality of amine lysosomotropism, and to establish a spectrum of structurally different lysosomal inhibitors, we have tested a number of relatively simple amines on suspensions of freshly isolated rat hepatocytes. Since one of our intentions was to find compounds which inhibited protein degradation with minimal side effects, particularly on protein synthesis (7), both processes were measured. The results presented in this communication show that inhibition of protein synthesis is a general, but secondary, effect of lysosomal blockade which can be largely overcome by the inclusion of amino acids and an energy substrate (pyruvate) in the medium.

MATERIALS AND METHODS

Hepatocyte isolation and incubation. Suspensions of isolated hepatocytes were prepared from the liver of 18-h-starved male Wistar rats (250–300 g) by the method of collagenase perfusion (14). The cells were incubated as 0.4-ml samples (20–30 mg wet wt) in rapidly shaking 15-ml centrifuge tubes for 1 h at 37°C, and reactions were stopped by the addition of 0.1 ml perchloric acid, 10% (w/v). The incubation medium was a simple suspension buffer (14), supplemented with a physiological mixture of amino acids (12.5× normal concentrations; cf. Ref. 15) and pyruvate (20 mM) where indicated. Amines to be tested were included at a concentration of 10 mM.

Protein degradation and protein synthesis. Protein degradation was measured as the release of acid-soluble radioactivity from protein prelabeled *in vivo* by an intravenous injection of 1 ml [^{14}C]valine (50 μCi) 24 h prior to cell isolation. The rate of release of [^{14}C]valine into an

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unsupplemented medium corresponded to the overall rate of protein degradation, i.e., 4–5%/h (11).

Protein synthesis was measured (in separate experiments) as the incorporation of [^{14}C]valine into cellular protein (7). The cells were incubated with a high concentration of radioactive valine (10 mM and 0.8 μCi/ml) to assure the maintenance of a constant specific radioactivity both in the cells and in the medium, thus eliminating isotope dilution effects (7) and the influence of any variations in the rate of valine transport (16). The rate of synthesis in an unsupplemented medium, calculated as previously described (8), was 0.3–0.5%/h.

In each experiment, the effect of a test substance was measured on at least three parallel cell samples (standard errors were in the range of 1–5%). Since data were accumulated from several experiments—with partial overlapping of test substances—the experiments were normalized in the following manner by using NH_4Cl as a reference: In each experiment the inhibitory effect of 10 mM NH_4Cl on protein degradation and synthesis, respectively, was defined as 63% (mean value; range, 58–70%) and 61% (mean value; range, 50–75%), and the effects of test substances were normalized to the same scale.

Cellular viability and lysosomal vacuolation. To assess the effect of test substances on cellular viability, cell samples were mixed with trypan blue (14) at the end of the 1-h incubation, and the percentage of intact (trypan blue-excluding) cells was determined with the aid of a Bürker chamber. At the same time lysosomal swelling (vacuolation), evident as a coarse granulation of the cytoplasm, was evaluated, and the cells were scored as positive or negative (± in borderline cases).

Chemicals. Amines were purchased from Sigma Chemical Co., St. Louis, Mo.; Koch-Light Laboratories Ltd., Colnbrook, Bucks., England; Fluka AG, Buchs, Switzerland; and ICN Pharmaceuticals Inc., Plainview, N.Y. [^{14}C]Valine (CFB 75; 285–295 Ci/mol; 50 mCi/liter) was from The Radiochemical Centre, Amersham, Bucks., England.

RESULTS AND DISCUSSION

Inhibition of protein degradation by amines. Previous experiments have shown that protein degradation in isolated rat hepatocytes is inhibited to the same extent by ammonia and its methylated analogue methylamine (11). In Fig. 1 the effect of ammonia on the release of [^{14}C]valine from labeled protein has been compared to that of methylamine and to those of four other weak-base amines, i.e., ethylamine, propylamine, imidazole, and 2-diethylaminoethanol. At low doses, the effectiveness of these agents is relatively variable, with imidazole being the weakest and 2-diethylaminoethanol the strongest inhibitor (Fig. 1A). Such variations may be related to differences in molecular size, lipid solubility, distribution, and dissociation constants, etc., but it is notable that the maximal effect of the six compounds, obtained at higher dose levels, is approximately the same. A concentration of 10 mM amine appears to be sufficient for producing near-maximal inhibition, and has therefore been used routinely in the subsequent investigation of other amines.

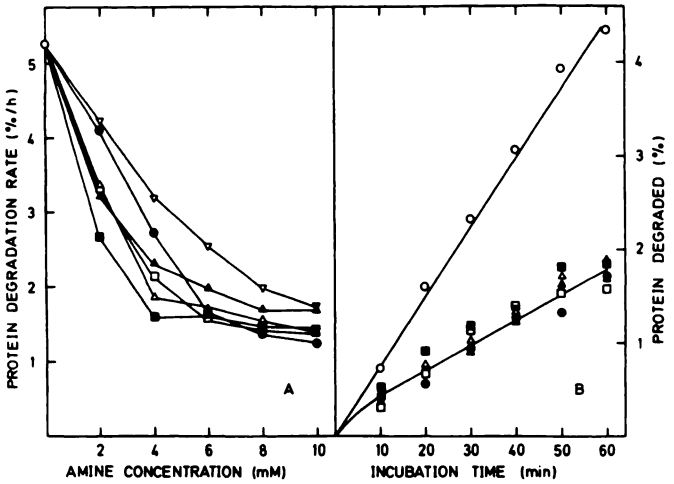

FIG. 1. *Time course and dose-response characteristics of inhibition of protein degradation by lysosomotropic amines*

Isolated rat hepatocytes were incubated for 60 min at 37°C with varying doses of (A) or 10 mM (B) lysosomotropic amines. The net release of [^{14}C]valine from labeled protein was measured and is expressed as %/h (A) or % (B). (○) Control; (●) ammonia (NH_4Cl); (△) methylamine; (▲) ethylamine; (□) propylamine; (■) 2-diethylaminoethanol; (▽) imidazole. Each point is the mean of three cell samples.

All the amines seem to exhibit a moderate lag phase of about 10–15 min before maximal inhibition is reached (Fig. 1B). This means that the actual inhibition of protein degradation may be approximately 10% higher than the value calculated from the inhibition of [^{14}C]valine release recorded at 60 min. In the testing of different amines to be reported below, no lag-phase correction has been made and the true inhibitory effects of the typically lysosomotropic amines may therefore be assumed to be approximately 10% higher than the values given in the tables.

Lysosomotropic effect of degradation-inhibitory amines. Weak-base amines appear to inhibit protein degradation by virtue of their ability to accumulate within the lysosomes, a process which can be visualized in the light or electron microscope and recognized by the appearance of swollen (vacuolated) lysosomes (2, 11, 13). Figures 2E–H demonstrate such vacuolation, as seen in the light microscope, caused by a series of degradation-inhibitory amines (propylamine, t-butylamine, triethylamine, and 2-diethylaminoethanol). The vacuolation is evident as a coarse granulation of the cytoplasm and is not seen following incubation with noninhibitory amines (cadaverine, B; and tetramethylammonium, C). With most of the amines tested the hepatocytes remain structurally intact after 1 h of incubation, as evidenced by their ability to exclude trypan blue; in contrast, Fig. 2D shows the effect of incubating cells with an amine (octylamine) which kills all the cells (100% trypan blue stainability).

Morphologically visible vacuolation is a useful characteristic for checking the lysosomotropism of test compounds, and the trypan blue exclusion test provides a quick and easy method for the simultaneous determination of lysosomotropism and the effect of amines on cellular viability.

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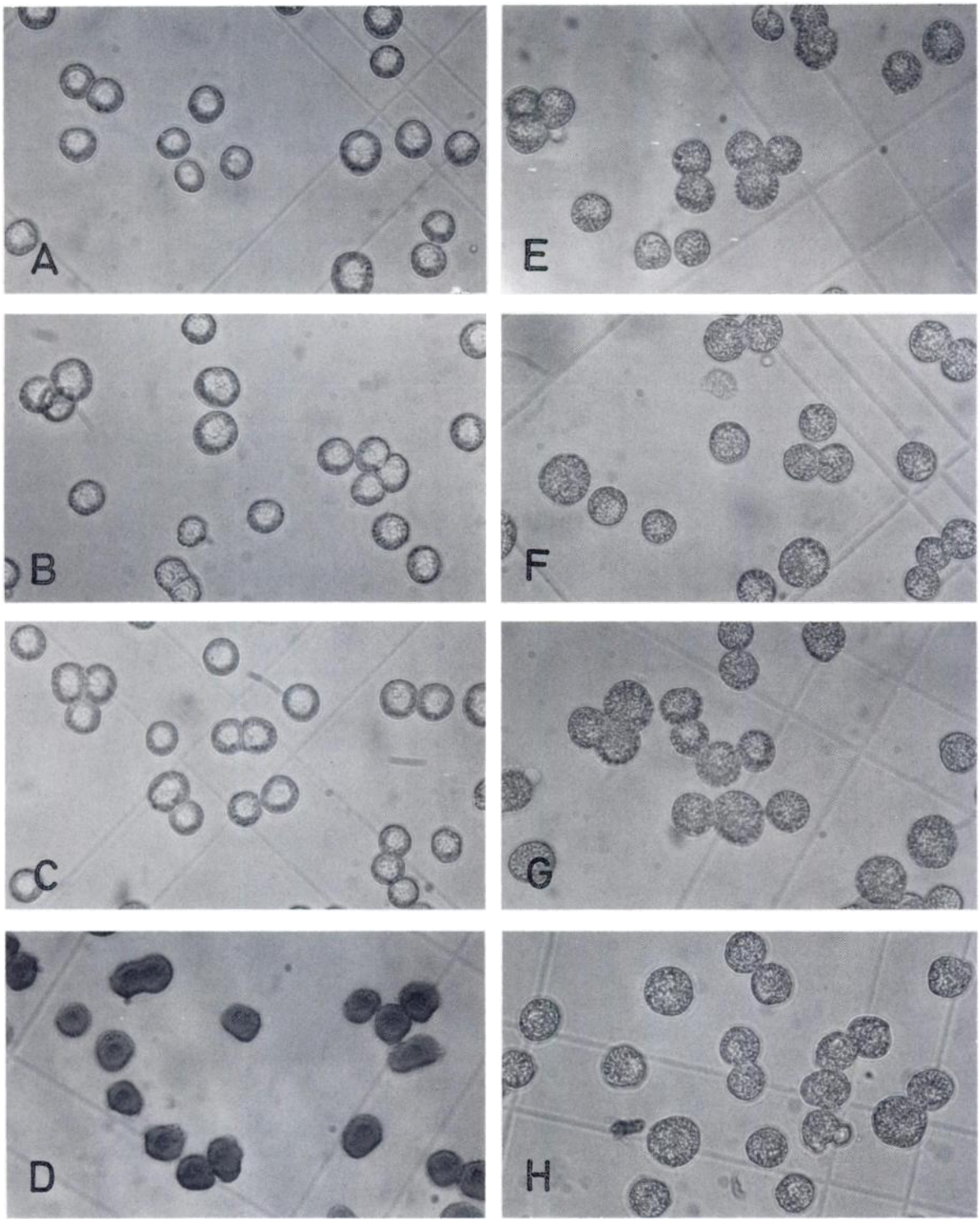

FIG. 2. *Effect of amines on hepatocyte morphology*

Isolated rat hepatocytes were incubated for 60 min at 37°C with a 10 mM concentration of the amine indicated. The cell suspension was then mixed with trypan blue (0.45% final concentration) and placed in a Bürker chamber (cf. the engraved lines visible in some of the pictures). (A) Control; (B) cadaverine (1,5-diaminopentane); (C) tetramethylammonium; (D) octylamine; (E) propylamine; (F) t-butylamine; (G) triethylamine; (H) 2-diethylaminoethanol. (A–C) No vacuolation; (D) dead cells (staining with trypan blue); (E–H) lysosomal swelling (vacuolation) evident as cytoplasmic granularity. × 260.

Relatively nonadditive effects of amines on protein degradation. The inhibitory effect of amines on protein degradation generally leveled off at 10 mM (cf. Fig. 1). Table 1 shows that the further addition of 10 mM ammonia gave very little additional inhibition, suggesting that the different amines inhibit protein degradation by the same mechanism, and almost to the maximal extent possible. Since the amines are lysosomotropic, a reason-

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TABLE 1

Inhibition of protein degradation by lysosomotropic amines in combination with ammonia

Isolated rat hepatocytes were incubated for 60 min at 37°C with a 10 mM concentration of the amine indicated, in the presence or absence of 10 mM NH_4Cl. The rate of protein degradation was measured, and the percentage total inhibition (relative to the ammonia- and amine-free control) was calculated. Each value is the mean of three cell samples; standard errors were in the range of 1–3%.

Amine	% Inhibition of protein degradation	
	Control	+ NH_4Cl
None	0	66
Methylamine	67	72
Ethylamine	70	75
Propylamine	68	76
2-Diethylaminoethanol	64	73

able hypothesis would be that they effect an essentially complete inhibition of the lysosomal pathway of protein degradation, as previously proposed (3, 6, 11). The protein degradation remaining would, by inference, reflect the activity of a nonlysosomal degradation pathway(s). The existence of dual pathways for protein degradation is further supported by the striking preference of the amine-resistant degradation for short-lived proteins and by the inability of other inhibitors to affect this type of degradation (3, 11).

Effects of amines on protein synthesis. Ammonia was previously found to inhibit protein synthesis in hepatocytes incubated in a poor medium; this inhibition was greatly reduced upon the addition of amino acids (7). Table 2 shows the effect of ammonia, two other amines (propylamine and imidazole), and an inhibitor of lysosomal proteases (leupeptin; Ref. 23) on hepatocytic protein degradation and synthesis. Both processes were inhibited by all of these compounds, leupeptin having a somewhat smaller effect on protein synthesis than the amines. The sensitivity of protein synthesis toward inhibitors of lysosomal function is rather variable, and the effect of leupeptin is not always seen (7). This variability may reflect the hormonal and nutritional state of the animal from which hepatocytes are prepared; for example, we have shown that the leupeptin effect is virtually eliminated when amino acids are included in the medium (Table 2), a situation which would be analogous to the fed state *in vivo*.

The addition of a physiological amino acid mixture produces multiple effects on hepatocytic protein metabolism (Table 2). Protein synthesis is stimulated, both as the result of an increase in the supply of precursors and by the role of amino acids in providing energy. The latter function can be as well provided for by pyruvate, which is the most effective energy substrate for hepatocytes (18). A combination of amino acids and pyruvate is therefore routinely employed when optimal conditions for protein synthesis are desired. Amino acids also inhibit protein degradation: They do so in a manner which is not additive to that of the lysosomotropic amines, suggesting that only the lysosomal pathway of protein degradation is affected, as previously concluded (18, 19). The amino acid effect is, however, additive to that of leupeptin, but not unexpectedly, as this agent inhibits lysosomal protein degradation incompletely (11).

The slightly diminished effect of ammonia on protein degradation in the presence of amino acids may be significant, and may reflect an accelerated metabolism of ammonia to, for example, urea (urea cycle intermediates such as ornithine and citrulline are included in the amino acid mixture; cf. Ref. 15). Propylamine, on the other hand, is maximally effective under all conditions—meaning that it is probably nonmetabolizable—and may therefore be a more preferable lysosomotropic inhibitor than ammonia.

The effects of the inhibitors on protein synthesis seem to be somewhat complex. A major part of the inhibition is apparently due to a reduction in the supply of amino acids deriving from protein degradation, an effect which is antagonized by amino acid addition and which seems to account fully for the inhibition of protein synthesis caused by leupeptin. Pyruvate reduces further the effect of the amines and, in particular, that of ammonia. The basis for this pyruvate effect is not known, but it may relate to a reduction in the cell's dependence upon energy substrates derived from lysosomal protein degradation, and in the case of ammonia, conversion of the latter to alanine may also play a part.

In our screening of different amines, protein synthesis was initially measured under the same conditions as

TABLE 2

Influence of amino acids and pyruvate on the inhibition of protein degradation and synthesis by amines and leupeptin

Isolated rat hepatocytes were incubated for 60 min at 37°C with ammonia (10 mM NH_4Cl), propylamine (10 mM), imidazole (10 mM), leupeptin (0.12 g/liter = 0.25 mM), amino acids (12.5×), and pyruvate (20 mM) as indicated, and the rates of protein synthesis and degradation were measured (in separate experiments). For protein degradation, the total inhibition (relative to the unsupplemented control) is given; for protein synthesis, the inhibitions by amines or leupeptin are given relative to the respective unsupplemented, amino acid-supplemented, or amino acids *plus* pyruvate-supplemented controls. Each value is the mean of three cell samples. Standard errors were in the range of 1–5%.

Medium	Protein degradation						Protein synthesis					
	Rate (%/h)	Inhibition (%)					Rate (%/h)	Inhibition (%)				
	None	None	NH_4Cl	Propylamine	Imidazole	Leupeptin	None	None	NH_4Cl	Propylamine	Imidazole	Leupeptin
Unsupplemented	6.1	0	67	68	58	55	0.39	0	61	45	42	33
+ Amino acids	3.4	44	57	70	64	69	0.66	0	34	26	27	5
+ Amino acids + pyruvate	3.1	49	56	71	57	72	0.74	0	9	18	23	8

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protein degradation, namely, in an unsupplemented medium. However, as it became increasingly apparent that all lysosomotropic amines inhibited both protein synthesis and protein degradation, it seemed likely that the former inhibition might, in most cases, be a consequence of the latter. In the presence of amino acids and pyruvate, on the other hand, the rate of protein synthesis is essentially independent of the rate of protein degradation, and under these conditions direct effects of the test compounds on protein synthesis may be detected. Therefore, several of the most active amines have been tested in both the absence and the presence of amino acids and pyruvate.

Unsubstituted aliphatic monoamines (Table 3). The *primary* amines were all lysosomotropic, as indicated by their ability to induce lysosomal vacuolation. They all inhibited protein degradation to a similar extent, suggestive of a complete blockade of lysosomal proteolysis. Their effect on protein synthesis was more variable and—for the compounds tested—to a large extent preventable by amino acids and pyruvate.

Primary amines with a chain length of up to five carbon atoms did not affect the viability of hepatocytes during the incubation period. However, with hexylamine viability was significantly reduced, and protein synthesis (in unsupplemented medium) was inhibited by 94%. Octylamine and dodecylamine killed all the cells (cf. also Fig. 2).

This lethal effect of long-chain aliphatic amines probably reflects the detergent property of their protonated forms. While the rapid and uniform killing of hepatocytes by, e.g., octylamine might seem to suggest direct plasma membrane damage, there are data indicating that other, more complex amines depend upon lysosomal function for the exertion of their toxic effects (20). These compounds are assumed to acquire their detergent properties following protonation within the lysosomes, with selective damage of the lysosomal membrane ensuing. Membrane damage occurs only when a very critical concentration of the protonated form is reached, and cell death resulting from lysosomal rupture might therefore selectively occur in cells with particularly acidic lysosomes.

The *secondary* and *tertiary* amines behave similarly to the primary ones. With dibutylamine a certain toxicity was apparent from a slight reduction in cell viability, an unusually large inhibition of protein degradation, and a particularly large inhibition of protein synthesis. Tributylamine (not shown) was only sparingly soluble in water and, moreover, killed all the cells.

The *quaternary* ammonium compounds were nonlysosomotropic (they produced no vacuolation) as expected: Since such compounds already have a positively charged nitrogen atom, they cannot be subject to protonation within lysosomes. Accordingly, tetramethylammonium did not inhibit protein degradation, and tetraethylammonium exerted only a slight, probably nonspecific,

TABLE 3

Unsubstituted aliphatic monoamines

For Tables 3–5: Isolated rat hepatocytes were incubated for 60 min at 37°C with a 10 mM concentration of the amine indicated. Protein degradation was measured in an unsupplemented medium; for protein synthesis the medium was either unsupplemented or fortified with amino acids (12.5×) and pyruvate (20 mM). Lysosomal vacuolation and cellular viability were measured in an unsupplemented medium. Measurements and calculations were as described in Materials and Methods.

Amine	Vacuolation	% Inhibition of protein degradation	% Inhibition of protein synthesis		% Intact cells
			Unsupplemented medium	+ Amino acids + pyruvate	
Primary					
Ammonia	+	63	61	7	93
Methylamine	+	62	55	13	93
Ethylamine	+	64	71	6	90
Propylamine	+	69	46	11	88
Isopropylamine	+	61	83	10	92
Butylamine	+	63	73	—	97
Isobutylamine	+	64	64	23	87
s-Butylamine	+	60	62	16	92
t-Butylamine	+	63	60	12	89
Pentylamine	+	63	81	—	93
Hexylamine	+	65	94	—	84
Octylamine	Dead	—	(96)	—	0
Dodecylamine	Dead	—	(100)	—	0
Secondary					
Dimethylamine	+	63	61	5	89
Diethylamine	+	62	61	9	91
Dibutylamine	+	71	91	—	86
Tertiary					
Trimethylamine	+	60	56	6	91
Triethylamine	+	63	58	13	94
Quaternary					
Tetramethylammonium	−	−7	8	—	92
Tetraethylammonium	−	16	16	—	91
Tetrabutylammonium	Dead	(78)	(96)	—	14

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effect on degradation and synthesis. Tetrabutylammonium killed most of the cells—probably as a result of a detergent effect.

Diamines and polyamines (Table 4). The *diamines* had very varied effects on hepatocytic protein degradation and synthesis. The compounds with a secondary or tertiary amino group (3-dimethylamino-1-propylamine, *N,N*,-diethylaminoethylamine, *N,N*- and *N,N'*-dimethylethylenediamine) were all lysosomotropic and inhibited protein degradation to the extent expected. The asymmetric diamine 1,2-diaminopropane caused similar effects, while the symmetric diamine diaminoethane produced only a marginally detectable vacuolation and a slight inhibition of protein degradation. All the other symmetric, straight-chain diamines were nonlysosomotropic and did not inhibit protein degradation significantly.

With the exception of 1,4-diaminobutane (putrescine), all the diamines inhibited protein synthesis strongly. The inhibition was reduced by amino acids and pyruvate only in the case of *N,N*-dimethylethylene diamine. As for the other diamines tested, the inhibition was unaffected by these nutrients even when lysosomotropism was evident; hence the effect of diamines on protein synthesis is independent of their effect on protein degradation.

In the cases of 1,2-diaminopropane, 1,3-diamino-2-propanol, and possibly diaminoethane, a certain nonspecific toxicity (reduced viability) was indicated. This might partly account for the observed inhibitions of protein synthesis caused by the above-mentioned substances and, in particular, for the very strong inhibition seen with 1,3-diamino-2-propanol. However, several other diamines (1,3-diaminopropane, cadaverine, 3-dimethylamino-1-propylamine, *N,N'*-dimethylethylene diamine) inhibited protein synthesis without affecting cellular viability. More specific biochemical mechanisms, such as, e.g., interference with ribosomal polyamine binding at these very high amine concentrations, may therefore be considered. Diamines do not inhibit protein synthesis in all cell types, for example, not in Ehrlich ascites cells (21), and further exploration of the cellular specificity and mechanism of action of these agents might be of interest. It is possible that in inhibiting protein synthesis diamines exert their effect from the cell surface, since they do not appear to be internalized by at least one type of cell (CHO cells; Ref. 22). Differential cellular uptake may, in general, be a plausible cause for some of the differences observed between closely related amines.

The *polyamines* spermidine and spermine were nonlysosomotropic. Spermidine did not inhibit protein degradation or synthesis appreciably, whereas spermine inhibited protein synthesis by 37%.

Amino alcohols (Table 5). Most of the tested amino alcohols were lysosomotropic and inhibited protein degradation. Their inhibitory effects on protein synthesis were, in all cases examined, partially prevented by amino acids and pyruvate and, therefore, presumably a consequence of an inhibition of protein degradation.

The amino alcohols were generally weaker inhibitors of protein degradation than the unsubstituted amines listed in Table 3. Ethanolamine, triethanolamine, and 4-amino-1-butanol produced inhibitions of less than 40%, and the effects of 3-amino-1-propanol and tris(hydroxymethyl)methylamine were of marginal significance, with respect to both lysosomotropism and inhibition of protein metabolism. At much higher concentrations, however, tris(hydroxymethyl)methylamine has been shown to produce pronounced vacuolation in the perfused liver (23).

Choline, a quaternary ammonium base, was nonlysosomotropic, as would be expected. It did, however, inhibit protein synthesis by 42% and did not affect cellular viability. This inhibitory effect may be of sufficient interest to warrant further study.

Amino acids (Table 5). Physiological α-amino acids play an important role as natural inhibitors of protein degradation (cf. Table 2); however, they act by suppressing cellular autophagy rather than by directly inhibiting lysosomal function (19, 24). Furthermore, only a limited number of the amino acids have the ability to inhibit protein degradation (19, 24).

The amino acids tested and listed in Table 5 were all nonlysosomotropic and, with the exception of L-homoserine, had no inhibitory effect on either protein synthesis

TABLE 4

Diamines and polyamines

See Table 3 for conditions.

Amine	Vacuolation	% Inhibition of protein degradation	% Inhibition of protein synthesis		% Intact cells
			Unsupplemented medium	+ Amino acids + pyruvate	
Diamines					
Diaminoethane (ethylenediamine)	±	17	64	—	85
1,2-Diaminopropane	+	64	61	71	82
1,3-Diaminopropane	−	2	51	56	93
1,3-Diamino-2-propanol	−	12	91	89	84
1,4-Diaminobutane (putrescine)	−	−9	15	—	93
1,5-Diaminopentane (cadaverine)	−	6	61	—	91
3-Dimethylamino-1-propylamine	+	57	56	76	90
N,N-Diethylaminoethylamine	+	70	72	—	92
N,N'-Dimethylethylenediamine (symmetric)	+	59	58	56	93
N,N-Dimethylethylenediamine (asymmetric)	+	56	50	23	92
Polyamines					
Spermidine	−	13	13	—	87
Spermine	−	16	37	—	91

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TABLE 5

Amino alcohols, amino acids, and some other amines

See Table 3 for conditions.

Amine	Vacuolation	% Inhibition of protein degradation	% Inhibition of protein synthesis		% Intact cells
			Unsupplemented medium	+ Amino acids + pyruvate	
Amino alcohols					
2-Aminoethanol (ethanolamine)	+	35	18	3	91
3-Amino-1-propanol	±	18	14	—	95
Isopropanolamine	+	59	44	9	94
2-Amino-1-butanol	+	62	59	17	92
4-Amino-1-butanol	+	37	28	10	89
2-Amino-2-methyl-1-propanol	+	58	56	16	94
2-Methylaminoethanol	+	55	48	16	91
2-Dimethylaminoethanol	+	61	47	16	93
3-Dimethylamino-1-propanol	+	55	60	10	91
2-Diethylaminoethanol	+	51	53	27	92
2-Dimethylamino-2-methyl-1-propanol	+	58	64	11	91
2-Amino-2-methyl-1,3-propanediol	+	58	64	5	91
Tris(hydroxymethyl)methylamine	±	9	1	—	95
Triethanolamine	+	25	42	9	93
Choline	−	0	42	—	96
Amino acids					
γ-Aminobutyrate	−	−1	−16	—	89
α(L)-Aminobutyrate	−	−1	−13	—	93
α(D)-Aminobutyrate	−	−4	−15	—	91
α-Aminoisobutyrate	−	−5	−1	—	89
DL-γ-Amino-β-hydroxybutyrate	−	−1	−1	—	91
ε-Amino-*n*-caproate	−	1	1	—	91
L-Homoserine	−	37	36	—	91
Chlorinated amines					
N,N-Dimethyl-3-chloropropylamine	+	64	77	—	92
Diethylaminoethylchloride	−	46	87	—	89
β-Dimethylaminoethylchloride	+	63	81	—	81
Heterocyclic amines					
Pyrimidine	−	21	18	—	92
Imidazole	+	56	49	14	90
Miscellaneous					
Taurine	−	1	−2	—	92
Guanidine	−	21	18	—	92

or degradation. The straight-chain aminobutyrates in fact *stimulated* protein synthesis significantly, probably by serving as energy substrates (in the presence of pyruvate, γ-aminobutyrate has been found to have no additional effect).

L-Homoserine inhibited protein synthesis and degradation by 36 and 37%, respectively. Its mechanism of action has not yet been further investigated, but if, like the physiological amino acids, it inhibits autophagy, it may become a useful tool in the study of this process.

Chlorinated, heterocyclic, and other amines (Table 5). Of the three chlorinated amines tested, two were lysosomotropic and one—diethylaminoethylchloride—was not. Nevertheless, the latter inhibited protein degradation by 46%, but microscopic examination of the cells revealed a grossly abnormal morphology, with large blebs protruding from the cell surface. This inhibition is therefore likely to reflect nonspecific toxicity, despite the maintenance of a good viability score when determined by the trypan blue exclusion method. The viability of cells treated with β-dimethylaminoethylchloride was, however, distinctly reduced, and there is therefore some reason to suspect that the unusually large inhibition of protein synthesis seen with all the chlorinated amines may, to some extent, be a manifestation of nonspecific toxicity.

Two heterocyclic amines—pyrimidine and imidazole—were tested. Pyrimidine was nonlysosomotropic and caused only moderate effects on protein degradation and synthesis. Imidazole behaved like a typical lysosomotropic amine but was possibly slightly less potent than the unsubstituted primary amines.

Two other amino compounds, taurine and guanidine, were nonlysosomotropic. The latter produced some inhibition of protein synthesis and degradation without significantly affecting cellular viability.

Amines as experimental tools. The data presented show that a wide variety of structurally different amines may share the ability to act as lysosomotropic inhibitors of protein degradation. The remarkable constancy of the extent of inhibition achieved by the typically lysosomotropic (vacuolating) amines (61.3 ± 0.8%, $N = 33$; excluding compounds which inhibited by less than 38%) strongly suggests complete or nearly complete inhibition of a distinct degradation process—most probably that occurring within the lysosomes. This idea is further supported by the observation that the maximal effects of the different amines are essentially nonadditive and that a

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variety of other degradation inhibitors with different mechanisms of action (α-amino acids, hormones, protease inhibitors, microtubule poisons) likewise fail to add significantly to the effect of amines (3, 11, 18, 19). Lysosomotropic amines would therefore seem to be useful tools for distinguishing the elements of the lysosomal pathway from other pathways of protein degradation. The latter have been estimated to be responsible for, in an unsupplemented medium, approximately 30% of the hepatocytic protein degradation (11).

Freshly isolated hepatocytes differ from most other cells in having a particularly strongly negative protein balance (1, 2), and some caution should therefore be exercised in generalizing our findings. However, several investigators have found lysosomotropic amines to be useful as inhibitors of lysosomal degradation in various cell types (1–12). In addition, such amines may inhibit the secretion of proteins (25) and the formation of catecholamine granules (26)—apparently due to their ability to accumulate within acidic vesicles. Furthermore, lysosomotropic amines have been shown to inhibit cell spreading (27) and the internalization of surface receptors (10, 22, 28, 29), effects which may be an indirect result of retardation of membrane flow (27) or a direct result of interference with an enzyme (transglutaminase) involved in the internalization of membrane components (22). Finally, other receptor-dependent processes such as those mediating the actions of hormones (29) or toxins (30) may be affected.

The inhibition of protein synthesis by the lysosomotropic amines showed a greater variability (mean ± SE = 62.9 ± 2.2%, N = 33) than did the inhibition of protein degradation, and most probably reflects the sensitivity of protein synthesis toward general metabolic conditions. In the presence of amino acids and pyruvate the variability of inhibition was—in absolute terms—small (mean ± SE = 12.3 ± 1.2%, N = 22). The fact that some inhibition of protein synthesis remained even in a nutritionally supplemented medium may suggest a slight, nonspecific toxicity of the amines or, alternatively, a restriction in the supply of key substances—other than amino acids and energy substrates—deriving from lysosomal protein degradation.

The available choice of a large number of lysosomotropic amines which are active as inhibitors of protein metabolism may facilitate the experimental investigation of interactions between lysosomal activity and other cellular processes. Furthermore, our data provide examples of closely related compounds with very different effects: aminopropane (propylamine), which (with nutritional supplementation) inhibits protein degradation only; 1,3-diaminopropane, which exclusively inhibits protein synthesis; and 1,2-diaminopropane, which inhibits both. Better knowledge of the mechanisms of action of such compounds may open up exciting new experimental possibilities.

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