LABILIZATION OF RAT LIVER LYSOSOMES BY 2-PHENETHYL ALCOHOL

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ABSTRACT

2-phenethyl alcohol labilizes rat liver lysosomes and releases the lysosomal enzymes, viz., acid phosphatase, β-glucuronidase and arylsulfatase A. The release of enzymes is dependent on the concentration of PEA. PEA shows a differential effect on the release of these lysosomal enzymes. The lysosome labilizing action of PEA is not dependent on the pH of the incubation medium.

Key Words: Mode of action of 2-phenethyl alcohol, lysosomes.

1. INTRODUCTION

The mechanism of action of 2-phenethyl alcohol (PEA) has been studied in a number of biological systems such as bacteria [1–8], yeasts [9–11], fungi [12], viruses [13–17], experimental tumor cells [18–21], mammalian cells [22–25], cell organelles [26], *in vitro* systems [27, 28] and enzyme activities [29]. The varied effects reported in literature suggest that PEA interferes with biological activities of cellular and subcellular systems by several mechanisms. Cellular processes like synthesis of macromolecules, lipid synthesis and cell membrane permeability have been suggested as its site(s) of action.

Though the exact mode of action of PEA is not understood, various lines of evidence suggest that the cell membrane may be the primary site of action of the chemical. Thus, PEA has been shown to impair the membrane function and affects the cell permeability in bacteria [4, 8], yeasts [9–11], fungi [12], tumor cells [21] and mammalian erythrocytes [25].

While the effect of PEA on cellular membranes is well documented, its effect on subcellular membranes has not been investigated in detail with the exception of a single report where PEA has been shown to release acid phosphatase from chicken liver lysosomes [26]. Isolated lysosomes provide an ideal experimental system to test the effect of PEA on subcellular membranes. In this paper we report the action of PEA on isolated rat liver lysosomes and the data presented provide conclusive evidence that it labilizes the lysosomal membrane.

2. MATERIALS AND METHODS

Chemicals: 2-phenethyl alcohol (PEA), Triton X–100, *p*-nitrophenyl phosphate, *p*-nitrocatechol sulfate, and phenolphthalein-β-D-glucuronide (sodium salt) were obtained from Sigma Chemical Co., St. Louis, U.S.A. All other chemicals were of analytical reagent grade.

Preparation of rat liver lysosomes: Inbred Wistar A/Iisc rats weighing 100 to 120 g were killed by cervical dislocation and the liver was quickly dissected out into ice-cold 0·15 M NaCl (isotonic saline). The liver was washed twice with 0·15 M NaCl, weighed, minced finely with scissors and suspended in 0·25 M sucrose. The liver was homogenized in 0·25 M sucrose (5 ml of solution per g of liver) using a Potter-Elvehjem glass homogenizer with a motor driven Teflon pestle. The homogenate was first centrifuged at 1,500 g in a Sorvall centrifuge, model RC 2–B, for 10 min at 4° C to sediment unbroken cells and nuclei. The supernatant was then centrifuged at 20,000 g for 30 min, and the pellet containing the lysosomes was suspended gently in 0·25 M sucrose to give a final concentration of 10 mg of protein per ml.

Effect of PEA on rat liver lysosomes: The effect of PEA on lysosomes was followed by measuring the release into the medium of lysosomal hydrolases. Rat liver lysosomes (0·5 mg of protein/ml) were incubated in 0·25 M sucrose containing PEA (dissolved in 50% ethanol) at various concentrations for 15 min at 37° C. All incubation mixtures, including controls, contained ethanol at a final concentration of 1%. After the incubation, the tubes were chilled in ice and centrifuged at 20,000 g for 20 min, and the resulting supernatants were assayed for acid phosphatase, β-glucuronidase and arylsulfatase A. The enzyme activity in the supernatant is expressed as percentage of total activity obtained in the presence of 0·1% Triton X–100. The data were corrected for the release of enzymes in control samples.

Enzyme assays: Acid phosphatase activity was determined by the method of Igarashi and Hollander [30] using *p*-nitrophenyl phosphate as substrate. Arylsulfatase A was estimated by the method of Jerfy and Roy [31] using *p*-nitrocatechol sulfate as substrate.

The reaction mixture for β-glucuronidase assay in 1 ml contained 30 mM acetate buffer, pH 4·5, 0·5 ml of the supernatant and 0·4 mM phenolphthalein-β-D-glucuronide. The reaction mixture was incubated at 37° C for 30 min, and the reaction was stopped by adding 5 ml of 0·2 M

glycine-NaOH buffer, pH 10·4. The absorbancy of the colour was measured at 540 nm.

Protein was estimated by the method of Lowry *et al* [32].

3. Results and Discussion

The release of acid phosphatase, β-glucuronidase and arylsulfatase A from rat liver lysosomes on exposure to 25 mM PEA is shown in Fig. 1. The release of the enzymes is rapid and linear up to 10 min. By the end of

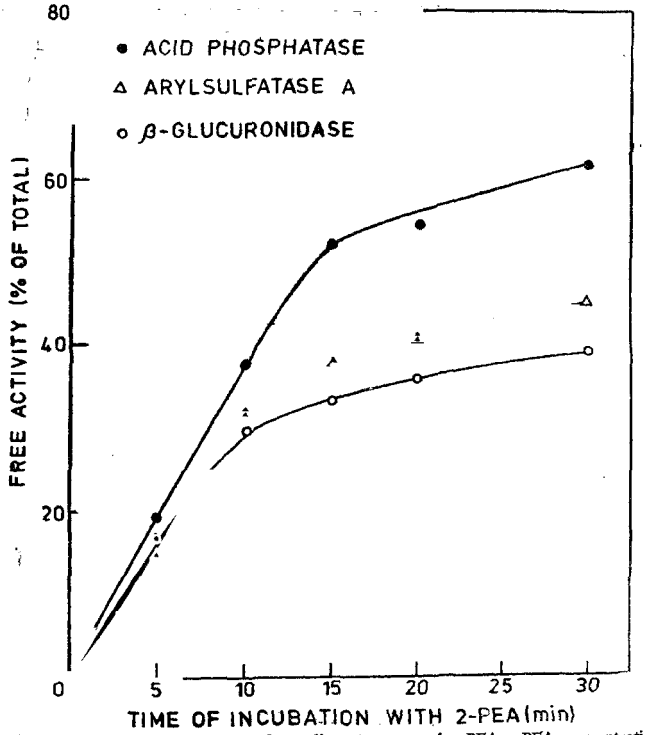

Fig. 1. Time course of labilization of rat liver lysosomes by PEA. PEA concentration, 25 mM.

30 min, 40% of β-glucuronidase, 45% of arylsulfatase A and 62% of acid phosphatase were released into the medium. These data are corrected for the release of enzymes in controls. In controls containing 1% ethanol, the release of these enzymes were 2.4, 3.4 and 8%, respectively.

The release of enzymes by PEA is concentration-dependent (Fig. 2). Concentrations up to 10 mM PEA showed negligible effect, but enzyme release is sharply increased at 25 mM. At very high concentrations (100 mM) PEA is as effective as Triton X–100 (0·1%) in releasing total acid phosphatase activity. The data in Figs. 1 and 2 point to a differential release of acid hydrolases by PEA. Thus acid phosphatase is most easily released from lysosomes, while β-glucuronidase is labilized the least, indicating that these enzymes may vary in their location or binding within the lysosomes.

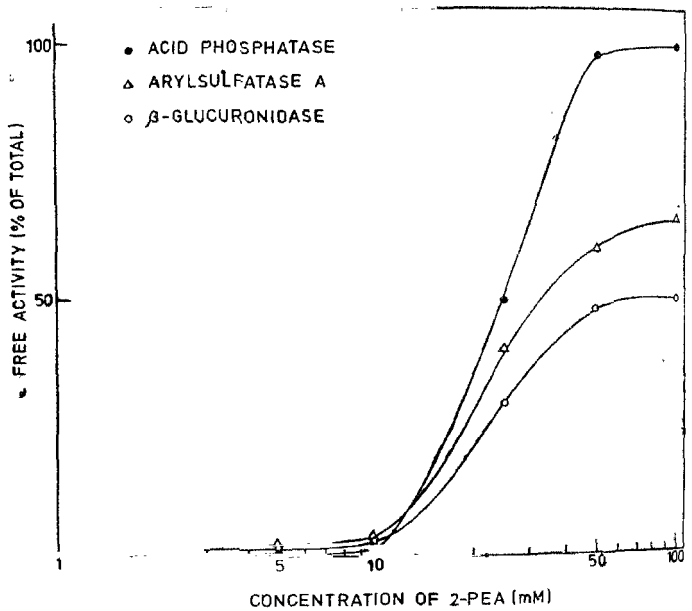

FIG. 2. Effect of PEA concentration on the release of lysosomal enzymes. Incubation time, 15 min at 37° C.

The labilization of lysosomes by PEA at varying concentrations of lysosomal protein is shown in Fig. 3. At 25 mM PEA increase in the lysosomal protein concentration (number of lysosomes) resulted in the increased release of enzymes. The release of acid phosphatase and arylsulfatase A is linear

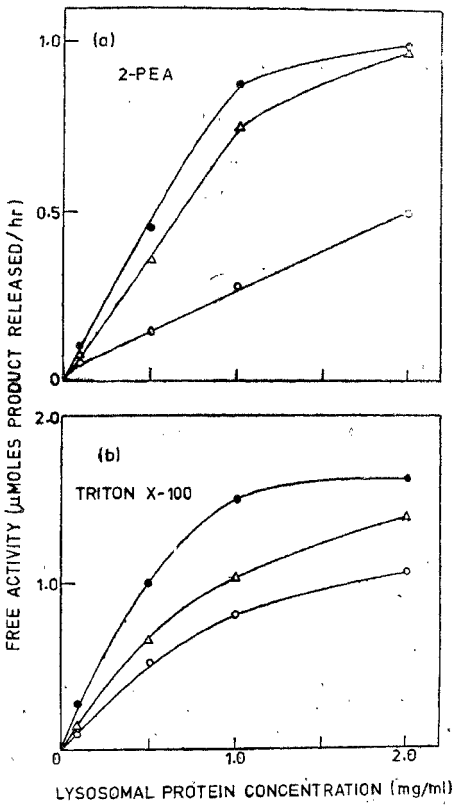

FIG. 3. Effect of lysosomal protein concentration on labilization of rat liver lysosomes by PEA (A) and Triton X-100 (B).
Acid phosphatase (●——●); β-Glucuronidase (○——○); and Arylsulfatase A (△——△).

up to 1 mg/ml of lysosomal protein, while β-glucuronidase is linear up to 2 mg/ml of lysosomal protein. Under the same experimental conditions, the solubilization of lysosomal enzymes by Triton X-100 (0·1%) is shown in Fig. 3 B.

The lysosome labilising action of PEA is not dependent on pH of the incubation medium as evidenced by the equal efficiency of the agent at pH 5·0, 6·8 and 8·0 (Table I).

TABLE I

*Influence of pH of the incubation medium on the release of lysosomal enzymes by 2-phenethyl alcohol**

Incubation medium	*Free activity (% of total)*		
	Acid phosphatase	Arylsulfatase A	β-Glucuronidase
0·25 M Sucrose–0·01M acetate (pH 5·0)	45·2	39·1	28·3
0·25 M Sucrose (pH 6·8)	50·0	42·0	29·5
0·25 M Sucrose–0·01 M Tris–HCl (pH 8·0)	49·3	47·4	33·2

* Lysosomes (0·5 mg of protein/ml) were incubated at different pH values with 25 mM PEA for 30 min at 37° C. After centrifugation at 20,000 g for 20 min at 4° C, the enzyme activities released into the supernatants were determined. Total activity in each sample was measured by incubation of lysosomes with 0·1% Triton X-100. After correction for release of enzymes in controls containing 1% ethanol, the data were expressed as percentage of total enzyme activity.

In conclusion, the data presented in this paper clearly reveal that PEA labilizes rat liver lysosomes and causes release of acid phosphatase, β-glucuronidase and arylsulfatase A. Earlier investigations on the mode of action PEA have shown that it impairs membrane function in many biological systems such as bacteria, yeasts, fungi, mammalian erythrocytes and tumor cells [4, 8-12, 21, 25]. Besides its effect on cellular membranes as shown by previous studies, the present findings show that PEA also has a profound effect on subcellular membranes.

4. Acknowledgements

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