

## EFFECTS OF HISTAMINE AND PHENIRAMINE MALEATE ON PROTEASE ACTIVITY OF SHEEP BRAIN

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**Summary:** The effects of histamine acid phosphate and pheniramine maleate, both individually and together were studied on protease activity in sucrose homogenates of sheep brain. The results indicated that at pH values 3.8 and 6.8, the addition of histamine acid phosphate and pheniramine maleate individually showed significant inhibition of protease activity. But when histamine acid phosphate and pheniramine maleate were present together there was no inhibition at pH 3.8 whereas slight inhibition was observed at pH 6.8

**Key words:** histamine

protease activity  
sheep brain

pheniramine maleate

### INTRODUCTION

Proteolytic enzymic activity is known to be altered under various physiological and pathological conditions (2,4,8,14). Investigations on sheep liver, kidney and brain have indicated that the activity of dehydrogenases and esterases was altered in the presence of histamine and pheniramine maleate but when both the agents were present they antagonized their individual effect (6,12,16). Histamine is found to be widely distributed in the brain (1) and its administration into the body provokes severe headache (15) suggesting its role in the genesis of variety of headaches (5). The activities of dehydrogenases and esterases are altered in the presence of histamine acid phosphate and pheniramine maleate (6,12,16). In this paper we report the effect of histamine acid phosphate and pheniramine maleate on the protease activity in the sheep brain.

### MATERIALS AND METHODS

Sheep were killed by decapitation and the brains transferred quickly to a clean dry glass beaker jacketed with ice in thermos flask. The tissue was homogenized in cold 0.25 M sucrose to 10% (w/v) using a pyrex glass homogenizer fitted with a motor driven pestle and centrifuged at 2000 r.p.m. for 10 min to remove the cell debris. The pH value of the supernatant was determined using a Beckman pH meter fitted with a glass electrode.

The protease activity was assayed by the method of Moore and Stein (11) as adopted by Kesava Rao *et al.* (8). The assay mixture contained in a final volume of 2.5 ml, 100 M moles sodium acetate buffer, pH 3.8 or potassium phosphate buffer, pH 6.8; 12 mg of heat denatured haemoglobin; 0.5 ml of histamine acid phosphate or pheniramine maleate. Control received 0.5 ml distilled water in the place of these agents, and 1 ml of homogenate supernatant. The



incubation was carried out at 37°C for one hr. The reaction was stopped by the addition of 2 ml of 10% (W/V) trichloroacetic acid.

All samples were corrected for zero time controls. The results were analysed statistically and significant difference between the means (calculated as values) are shown. No statistical significance is indicated when the P value was 0.05. The protein content was estimated as per the biurent method. (3).

## RESULTS AND DISCUSSION

The level of protease activity was assayed at pH 3.8, since this was found to be the optimum pH value for brain proteinases (9). The measurements at pH 6.8, being the homogenate pH represented the state of close approximation to the physiological condition and the metabolic pattern operating in living cell (7).

The results indicate that at pH values 3.8 and 6.8 the addition of histamine acid phosphate and pheniramine maleate individually showed significant inhibition of protease activity. (Table I)

TABLE I: Effect of histamine acid phosphate and pheniramine maleate on protease activity of brain homogenates expressed in  $\mu\text{g}$  of tyrosine liberated/mg Protein/hr. The results represent Mean  $\pm$  SD of 4 determinations each.

pH	Control with no reagent	Histamine acid phosphate	Pheniramine maleate	Histamine acid phosphate and maleate.
3.8	27 $\pm$ 2.2	11.8 $\pm$ 1.25* (43.71%)	13.5 $\pm$ 3.37* (49.99%)	25.62 $\pm$ 4.25 (94.91%)
6.8	19.1 $\pm$ 1.12	7.87 $\pm$ 1.0* (41.17%)	9.0 $\pm$ 1.12* (47.06%)	14.1 $\pm$ 1.5 (73.86%)

\* Statistically significant difference as compared with the values of control (P<0.05).

But when histamine acid phosphate and pheniramine maleate were present together there was no inhibition at pH 3.8 whereas slight inhibition was observed at pH 6.8 (Table I). The similarity in the individual response of these two reagents on protease activity at two pH values studied suggests identity in the mechanism of their action. Paton (13) reported that antihistamines act as histamine liberators. This may account for the similar nature of action. When these two agents were present together in the brain homogenates, the response elicited in their individual capacity was almost abolished. This may be due to the fact that antihistamines have a structure that allows them to bind with histamine receptor site, without initiating a response in the tissue and thereby restoring a condition similar to the control (10). Marshall (10) reported that all the antihistamines in general act as competitive antagonists to histamine.

When percent change was compared at pH values, the percent inhibition at pH 6.8 (homogenate pH) was same as at pH 3.8 when histamine acid phosphate and pheniramine maleate were individually present (Table I), thus substantiating the competitive antagonist nature of antihistamines to histamine when they are present together.



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