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Effect of pyridoxine deficiency on intestinal histaminase in the rat

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HISTAMINASE is one of the major enzymes responsible for inactivation of histamine in the rat,[1, 2] and the small intestine has high enzyme activity.[2, 3] It is generally considered to be a flavoprotein,[2, 4–6] with pyridoxal as the coenzyme.[7, 8] It might therefore be supposed that pyridoxine-deficient animals would show diminished histaminase activity, which would be restored by administering pyridoxine to the intact animal or by adding pyridoxal phosphate to isolated enzyme systems *in vitro*. There is a preliminary report in the literature suggesting that the latter occurs.[7] Furthermore, since it has been reported recently[6] that a highly purified histaminase enzyme from hog kidney contains flavine-adenine-dinucleotide (FAD) and pyridoxal as prosthetic groups, the action of both these substances on the histaminase activity of intestine from pyridoxine-deficient rats was considered of interest.

EXPERIMENTAL

Pyridoxine-deficient diet. Four pairs of litter-mate, male, Sprague-Dawley rats (45–60 g) were used. One rat of each pair was fed a pyridoxine-deficient diet *ad libitum*, while its litter mate served as the control, being pair-fed an identical diet with the addition of 80 μg pyridoxine HCl daily. The diet used was based on that described previously,[9] with the exception that pyridoxine HCl was omitted for the deficient rats, and thiamine HCl included. Both deficient and control animals received 1% phthalylsuphathiazole (Thalazole, May and Baker) in their diets, which were given for 30–43 days.

Preparation of histaminase. The histaminase activity of the small intestine, excluding the duodenum, was measured in four paired experiments, with tissues from one pyridoxine-deficient rat and one pair-fed, litter-mate control, both tissues being subjected to identical procedures. Histaminase determination was made as follows. After death by stunning and exsanguination, the small intestine was removed, washed through with 0·9% saline and dried gently between filter papers. The tissue was finely minced with scissors and an extract prepared by grinding with sand in a mortar with 4 vol. saline. The extract was centrifuged (at room temperature) at 8,500 rev/min (7,500 *g*) for 20 min. The resulting supernatant was used as a crude source of histaminase.

Estimation of histaminase activity. Histaminase activity was estimated in 50-ml Erlenmeyer flasks, with the following incubation mixture: histaminase extract, 2·0 ml (equivalent to 0·5 g tissue); 0·2 M sodium phosphate buffer, pH 7·4, 3·0 ml; 0·9% saline, 1·0 ml. In experiments 1–3 where additions were made, pyridoxal-5′-phosphate (100 μg) or FAD (50 μg), or both, were dissolved in 0·2 ml of the 1·0-ml saline and added to aliquots of the histaminase extract, prepared from the same animal. Incubation of the mixture was for 1 hr at 37° in pure oxygen in a metabolic shaker. This was preceded by a 10-min preincubation period before 200 μg of the substrate, histamine acid phosphate, was added. The reaction was stopped by addition of sufficient 2 N HCl to reduce the pH to approximately 5, and by boiling for 1 min. The incubation mixture was then cooled, filtered, and made up to 100 ml. An aliquot was assayed for histamine on the atropinized guinea-pig ileum in an automatic apparatus.

Histaminase activity is expressed as micrograms histamine base inactivated per gram tissue per hour.

RESULTS AND DISCUSSION

Before considering the effect of pyridoxine deficiency on intestinal histaminase, it is necessary to offer some explanation for the rather large reduction in activity that occurerd in the control group with increasing time on the diet (Table 1) This reduction probably reflected the diminution in overall food

TABLE 1. INTESTINAL HISTAMINASE ACTIVITY OF PYRIDOXINE-DEFICIENT AND CONTROL RATS WITH AND WITHOUT ADDITION OF PYRIDOXAL AND FAD

Expt.*	Time on diet (days)	Deficient				Control			
		No additions	+ Vit. B_6	+ FAD	+ Vit.B_6 + FAD	No additions	+ Vit. B_6	+ FAD	+ Vit.B_6 + FAD
		(μg histamine base inactivated/g tissue per hr)							
1	30	72·5	101·3	88·5	99·7	140·0	133·2	134·5	140·0
2	32	45·6	62·8	45·3	51·8	109·0	107·5	104·6	107·0
3	33	66·9	78·0	89·3	94·9	77·6	94·7	103·2	98·7
4	43	37·0			39·9	96·1			96·7
	Mean	55·5†	80·7	74·4	71·6	105·6†	111·8	114·1	110·6
	S.E.	±8·46	±11·20	±14·53	±15·08	±13·13	±11·32	±10·21	±10·05
	Increase over no additions		45·4%	34·1%	29·0%		5·9%	8·0%	4·7%

* Results given are those obtained in a single deficient animal and its pair-fed, litter-mate control, subjected to identical procedures.

† Values significantly different at 2% level.

intake imposed by the pair-feeding technique, with a consequent fall in the intake of dietary histidine and histamine.

The results given in Table 1 show a significant reduction in histaminase activity in the deficient group, compared with the controls. Although it is not possible to draw definite conclusions from the small number of experiments made here, the data nevertheless provide further evidence that pyridoxine is the coenzyme of histaminase.

The experiments with the addition of cofactors provide no convincing evidence for the restoration of enzyme activity in the deficient preparations Although there was a trend toward increased activity in all three deficiency experiments, this was also evident to a lesser degree in experiment 3 of the control series. The inability of cofactors to restore diminished histaminase activity in the deficient experiments may have been due to the fact that the deficiency state affected the apoenzyme as well as the coenzyme. It is possible that apoenzyme synthesis was impaired in pyridoxine-deficient rats. It has been noted previously that the impaired activity of the pyridoxine-dependent enzymes liver DOPA and cysteic acid decarboxylase from pyridoxine-deficient rats, was not restored by pyridoxal *in vitro*.[10] Positive evidence has been produced recently[11] for loss of apoenzyme in pyridoxine deficiency. The livers of pyridoxine-deficient rats showed greatly diminished activity for the pyridoxine-dependent enzyme cysteine sulphinic acid decarboxylase, which was not restored by pyridoxal *in vitro*. However, when the deficient rats were given injections of pyridoxine, apoenzyme levels were restored to normal in 6 hr.

Since FAD also failed to produce any significant reactivation it is suggested that the present experiments are further evidence that FAD and pyridoxal are prosthetic groups of histaminase, and their failure here was possibly due to an effect of the deficiency state on the apoenzyme.

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A sensitive assay for 5-hydroxytryptophan decarboxylase

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5-HYDROXYTROPTOPHAN decarboxylase (5-HTPD) activity is conventionally measured by manometric[1] or fluorometric[2, 3] techniques. The most sensitive of these methods[3] is capable of detecting the formation of 200 mμg of serotonin during the incubation period. This report describes a simple, sensitive, and specific radiometric assay for 5-HTPD activity which involves the measurement of ^{14}C-serotonin formed from DL-5-hydroxytryptophan-^{14}C. 5-HTPD activity can be routinely determined in tissues which enzymatically produce only 5 mμg of the radioactive amine during 1 hr incubation. Fifty or more assays can be performed in 3 hr. 5-HTPD activity has been measured in some tissues for which no quantitative estimates have been previously available.

METHODS

Tissues were homogenized in ice-cold 0·05 M phosphate buffer, pH 7·4. Enzyme preparation (0·1 to 0·6 ml), 0·1 ml of 0·5 M phosphate buffer (pH 7·4), 0·1 ml of a 1 mg/ml solution of iproniazid, and 0·1 ml of a 100 μg/ml pyridoxal phosphate solution and water to make a final volume of 0·9 ml were incubated in a 15-ml glass-stoppered centrifuge tube in air at 37°. After 3 min prior incubation, 11·4 mμmoles of DL-5-hydroxytryptophan-^{14}C (36,000 cpm, 2·64 mc/mm) was added, and the incubation was continued for 30 min. The reaction was stopped by the addition of 0·5 ml of 0·5 M borate buffer, pH 10. The mixture was saturated with sodium chloride, and the ^{14}C-serotonin formed was extracted by shaking into 6 ml of a 3:2 mixture of 1-butanol and chloroform. After centrifugation, the aqueous layer was aspirated and the organic phase washed with 1 ml of 0·05 M borate buffer, pH 10. Two ml of the resultant organic phase was transferred to a counting vial and evaporated to dryness in a stream of not air. The radioactivity was measured in a liquid scintillation spectrometer after the addition of 3 ml of ethanol and 10 ml of phosphor. Alternatively, the radioactivity in 1 ml of the organic phase was counted directly after the addition of 3 ml of ethanol and 10 ml of phosphor. In this case a correction was made for about 20% quenching. Under these conditions, recovery of ^{14}C-serotonin added to tissues was 90% (±4%), while less than 0·5% of added DL-5-hydroxytryptophan-^{14}C was extracted. All values were corrected for heated enzyme blank.

RESULTS AND DISCUSSION

The reaction was linear with time for at least 2 hr and proportional to tissue concentration in liver over a 5-fold concentration range (Table 1) and in all other tissues examined. At high tissue concentrations the enzymatic activity was not linear.

The product of the enzymatic activity was identified as ^{14}C-serotonin by co-chromatography with the authentic compound in 1-propanol–1 N NH_4OH (75:25) and 1-propanol–1 N acetic acid (75:25).

Specificity was determined by measurement of hepatic 5-HTPD in the presence of dihydroxyphenylalanine, a competitive inhibitor of 5-HTPD. Ninety per cent inhibition was obtained at 10^{-3} M and 63% inhibition at 10^{-4} M concentrations of the inhibitor.