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THE EFFECT OF AMINOGUANIDINE, HISTAMINE, CHLORPROMAZINE AND ANTIBACTERIAL AGENTS ON HISTIDINE DECARBOXYLASE IN THE STOMACH OF THE RAT

BY

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Lovenberg, Weissbach & Udenfriend (1962) showed that at least two enzymes capable of forming histamine are present in many mammalian tissues ; one is specific for L-histidine whereas the other is non-specific, for it decarboxylates a large number of natural and synthetic aromatic amino-acids as well as L-histidine. The enzyme present in rat stomach has generally been considered to be of the specific type (Waton, 1956 ; Schayer, 1957a; Telford & West, 1961a), but recently Radwan & West (1967a) identified two types of histidine decarboxylase in this tissue. The enzyme present in the thin fundic portion has a high activity and possesses many properties like those of the specific histidine decarboxylase found, for example, in rat foetal liver (Telford & West, 1961b ; Håkanson, 1963) and in rat hepatoma (Kameswaran & West, 1961 ; Mackay, Riley & Shepherd, 1961), whereas that in the less active thick muscular pyloric portion resembles more the non-specific enzyme of guinea-pig kidney (Mackay *et al.*, 1961) and of rabbit kidney (Ganrot, Rosengren & Rosengren, 1961).

The present work compares the effects of different drugs on the reactivity of the fundic and pyloric enzymes. Where possible, comparisons have also been made between the properties of these enzymes and those of the specific histidine decarboxylase of bacterial origin and of the rat foetus.

METHODS

Groups of four or more male Sprague-Dawley rats (obtained from Fisons Pharmaceuticals Ltd., Holmes Chapel), weighing 120–150 g were used in most experiments. They were fed on a cube diet (No. 41B, Associated London Flour Millers Ltd.), allowed unrestricted drinking water, and housed at 21° ± 0.5° C. Pregnant female rats of the same strain, killed at 17–19 days of gestation, provided the foetal material. Animals were killed by a blow on the head, and tissues were dissected out, cleaned and weighed. The stomachs were opened along the line of the lesser curvature and

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divided into two portions; the thin fundic part was separated from the thick muscular pyloric part, an intermediate area about 2 mm wide on either side of the demarcation line being discarded. Each portion was then weighed separately.

Preparation of tissue extract

Pooled tissues from freshly killed animals were cut into small pieces and homogenized in 0.9% w/v saline (5 ml./g) in a glass homogenizer; the homogenates were then centrifuged at 5,000 g for 15 min in a refrigerated centrifuge, and aliquots of the supernatant fluid were removed for the incubation experiments.

Bacterial histidine decarboxylase

The bacterial enzyme, obtained as a dry powder from Nutritional Biochemicals Corporation (Cleveland, Ohio) was dissolved in isotonic saline (0.9% w/v sodium chloride) to provide a solution containing 10 μg/ml. Aliquots of 0.5 ml. were used for the incubation experiments.

Histidine decarboxylase activity

The $^{14}CO_2$ method originally devised by Kobayashi (1963) and described in detail by Radwan & West (1967a) was used in all experiments. Briefly, the tissue extract (equivalent to 50–100 mg of tissue) was incubated with carboxyl-labelled ^{14}C-histidine in a closed system and the $^{14}CO_2$ liberated during the decarboxylation process was estimated in a Packard Tri-Carb liquid scintillation counter. The pH of the incubation mixture was adjusted to the optimum value for the enzyme being assayed; that is, 5.6 and 7.6 were used for the fundic and pyloric portions of rat stomach, respectively (Radwan & West, 1967a), 6.6 for the rat foetus (Burkhalter, 1962) and 4.6 for the bacterial enzyme (Radwan, unpublished observations). The pH of the solution of the test drug was always adjusted to the optimum value before being added (in aliquots of 0.1 ml.) to the incubation mixture. Blank incubations containing no enzyme and control incubations containing no drugs were also carried out under similar conditions. Results were corrected for the blank values and are expressed as percentages of control incubations. Each value in the figures and table represents the mean of at least two separate estimations, and differences of 20% or more are significant ($P=0.05$).

The BSH method devised by Schayer, Rothschild & Bizony (1959) and modified by Kahlson, Rosengren & Thunberg (1963) was used in some experiments. It involves incubation of the tissue extract with ring-2-^{14}C-L-histidine and estimation of the ^{14}C-histamine formed after its conversion to the derivative, dibenzene-sulphonylhistamine. Details of the method have been described elsewhere (Radwan & West, 1967b). It has recently been reported that results obtained with the $^{14}CO_2$ method compare favourably with the BSH method (Maudsley, Radwan & West, 1967).

RESULTS

Effect of aminoguanidine

When aminoguanidine, an inhibitor of histaminase, was included in the incubation mixture, marked inhibition of the histidine decarboxylase activity in the fundic stomach extract was obtained, although the enzyme activity in the pyloric extract was little affected (Fig. 1). For example, a dose of 500 μg aminoguanidine completely inhibited the fundic activity whereas 1,000 μg had no effect on the pyloric enzyme. The results obtained with the $^{14}CO_2$ method agreed well with those using the BSH method when the action of aminoguanidine on the fundic enzyme was investigated (Fig. 1). Fig. 2 shows the effects of aminoguanidine on rat foetal and the bacterial histidine decarboxylases. The bacterial enzyme was particularly sensitive to the inhibitor, doses as low as 25 μg of aminoguanidine producing as much inhibition of activity as that produced by 500 μg or more on the rat foetal enzyme; furthermore, 50 μg aminoguanidine completely inhibited the bacterial enzyme (Fig. 2).

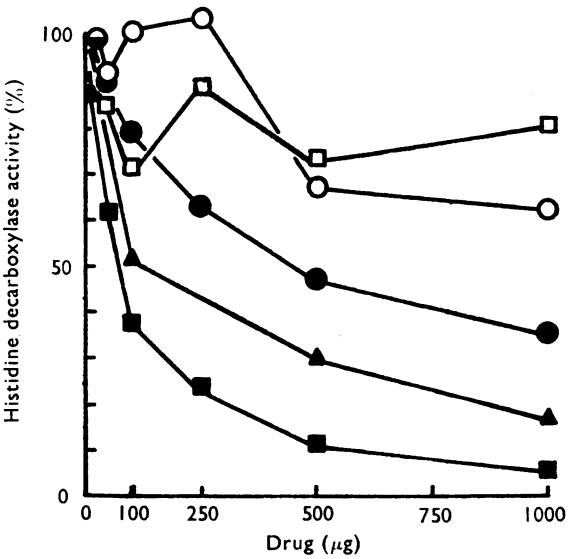

Fig. 1. Effect of including aminoguanidine in the incubation mixtures on the histidine decarboxylase activity of the fundic (■——■) and pyloric (□——□) portions of rat stomach. Effect of histamine on the fundic (●——●) and pyloric (○——○) enzymes is also shown. Enzyme activities were determined by the $^{14}CO_2$ method and are expressed as percentages of the values of control incubations containing no inhibitor. Effect of aminoguanidine on the fundic enzyme (▲——▲) using the BSH method has been included for comparison purposes.

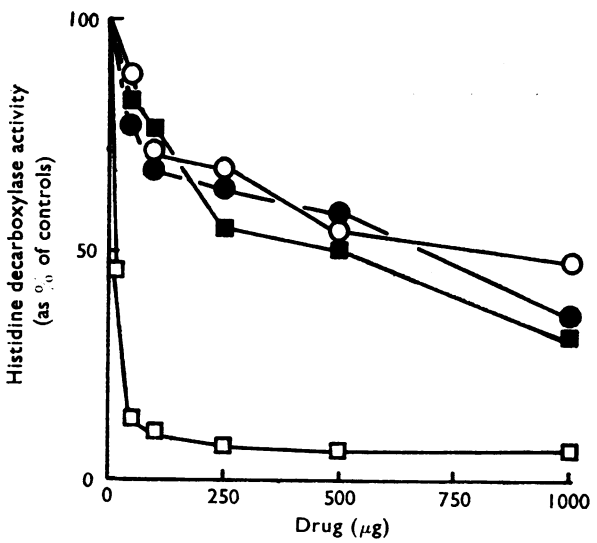

Fig. 2. Effect of including aminoguanidine in the incubation mixtures on the histidine decarboxylase activity of the rat foetal (■——■) and bacterial (□——□) enzymes. Effect of histamine on the rat foetal (●——●) and bacterial (○——○) enzymes is also shown. Enzyme activities were determined by the $^{14}CO_2$ method and are expressed as percentages of the values of control incubations containing no inhibitor.

Effect of histamine

Histamine in doses up to 1,000 μg partially inhibited the enzymes of rat stomach although it was slightly more active against the fundic histidine decarboxylase (Fig. 1). Histamine also only partially inhibited the rat foetal and the bacterial enzymes, doses of 1,000 μg producing little more than 50% inhibition (Fig. 2).

Effect of chlorpromazine

Chlorpromazine, an inhibitor of imidazole-*N*-methyltransferase (Brown, Tomchick & Axelrod, 1959; Merril, Snyder & Bradley, 1966), significantly inhibited the histidine

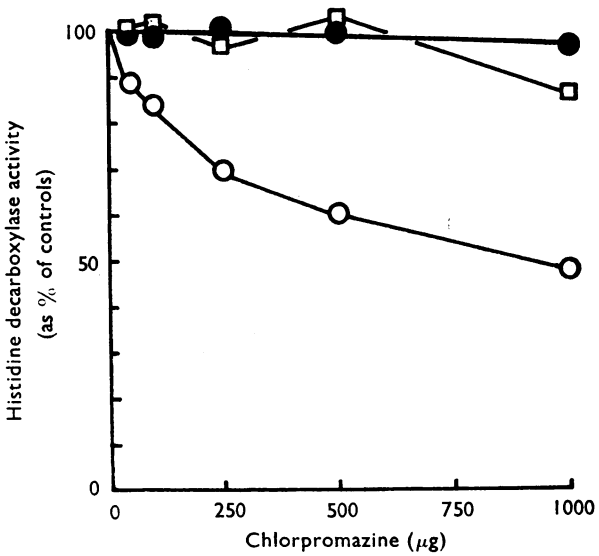

Fig. 3. Effect of including chlorpromazine in the incubation mixtures on the histidine decarboxylase activity of the fundic (●——●) and pyloric (○——○) portions of rat stomach and of the rat foetus (□——□). Enzyme activities were determined by the $^{14}CO_2$ method and are expressed as percentages of the values of control incubations containing no inhibitor.

decarboxylase activity of the pyloric portion of the stomach but had no effect on the fundic or rat foetal enzymes (Fig. 3). Doses of 1,000 μg of chlorpromazine, for example, produced about 50% inhibition of the pyloric enzyme.

Effect of antibacterial agents

Table 1 shows that the histidine decarboxylase activity of the fundic portion of rat stomach was not affected by chloramphenicol, sulphadimidine or neomycin but large doses of chlortetracycline completely inhibited it. The pyloric enzyme, on the other

TABLE 1

EFFECT OF ANTIBACTERIAL AGENTS ON THE HISTIDINE DECARBOXYLASE ACTIVITIES OF THE FUNDIC AND PYLORIC PORTIONS OF RAT STOMACH AND OF THE BACTERIAL ENZYME

Activities were determined by the $^{14}CO_2$ method and are expressed as percentages of control values.

Agent	Dose (mg/3 ml. of incubation mixture)	Histidine decarboxylase (% of control)		
		Rat stomach		
		Fundic	Pyloric	Bacterial
Chloramphenicol	6·25	—	—	101
	12·50	113	40	99
	25·00	111	15	—
Chlortetracycline	1·25	106	—	—
	2·50	105	—	—
	6·25	106	—	97
	12·50	74	0	92
	25·00	10	0	—
Sulphadimidine	6·25	—	—	100
	12·50	109	80	99
	25·00	107	81	—
Neomycin	6·25	—	—	95
	12·50	84	2	92
	25·00	81	0	—

hand, was completely inhibited by large doses of chloramphenicol, neomycin or chlortetracycline. These agents had no effect on the bacterial enzyme although large doses were not always tested because of shortage of enzyme (Table 1).

DISCUSSION

The results of the present study confirm the findings of Radwan & West (1967a) that the enzyme present in the thin fundic portion of rat stomach which is capable of forming histamine *in vitro* differs in reactivity from that located in the thick pyloric portion. The fundic enzyme activity is not altered by chlorpromazine or the antibacterial agents but is markedly reduced by aminoguanidine and by histamine ; the pyloric enzyme, on the other hand, is reduced by chlorpromazine and by antibacterial agents but is not markedly altered by aminoguanidine or histamine. The similarity between the actions of aminoguanidine and histamine in these experiments indicates that the former drug may be acting through the protection of the latter amine from destruction by the enzyme histaminase. Kobayashi & Ivy (1959) reported that the fundic portion of rat stomach contains more histaminase than does the pyloric portion and the effect of aminoguanidine is therefore much less evident in the pyloric portion.

The rat stomach is devoid of imidazole-*N*-methyltransferase (Brown *et al.*, 1959) and hence the effect of chlorpromazine on the pyloric enzyme probably does not involve the accumulation of histamine and is more likely the result of a direct action on the histidine decarboxylase. West (1958) reported that low doses of chlorpromazine markedly inhibited the 5-hydroxytryptophan decarboxylase activity of rat kidney. In the present work, chlorpromazine had no effect on the fundic enzyme or on the specific enzyme found in rat foetal extracts. This finding supports the view that the enzyme in the fundic portion of rat stomach is of the specific type whereas that in the pyloric portion is non-specific (Radwan & West, 1967a).

Gastric acid secretion in the rat has been reported to be slightly inhibited by chlorpromazine (Amure & Ginsburg, 1964) but potentiated by aminoguanidine (Schayer, 1957b). These two actions are similar to those on the histidine decarboxylase activity of the acid-secreting portion (the pyloric portion) of the rat stomach. Aminoguanidine, having little effect on the pyloric enzyme, probably exerts its action by preventing the destruction of histamine by other tissues, whereas the inhibitory effect of chlorpromazine on this enzyme is probably greater than its histamine-protective action, and so inhibition of acid secretion occurs. These findings support the view of Code (1965) that histamine is the local chemostimulator of gastric acid secretion.

Wilson (1954) and Schayer, Wu & Smiley (1954) showed that inhibition of the bacterial flora in the intestine is followed by a decrease in the urinary excretion of histamine, and Schayer, Davis & Smiley (1955) therefore suggested that a protein-free diet containing antibacterial agents be used when the histamine content of urine is being used as a measure of histamine metabolism. In the present experiments, the pyloric enzyme was easily inhibited by these agents, but it should be emphasized that the doses used were large. These results show that there are different enzymes in the two portions of the rat stomach and these respond differently to drugs. The antibacterial agents inhibit the

intestinal flora and thus affect the level of urinary histamine but they have no inhibitory action on the isolated bacterial enzyme, even in large concentrations. It has already been reported by Callingham, Kobayashi, Maudsley & West (1965) and confirmed by Levine & Watts (1966) that streptomycin does not affect histidine decarboxylase activity of rat and guinea-pig tissues.

SUMMARY

1. Using an isotopic method for the estimation *in vitro* of histidine decarboxylase activity, different enzymes have been identified in the fundic and pyloric portions of the rat stomach, and these react differently to drugs.

2. The activity of the enzyme located in the fundic portion is not altered by chlorpromazine or antibacterial agents but is markedly inhibited by aminoguanidine and by histamine.

3. The activity of the enzyme present in the pyloric portion is inhibited by chlorpromazine and by antibacterial agents but is not markedly altered by aminoguanidine or histamine.

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