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SEX DIFFERENCES IN HISTIDINE DECARBOXYLASES IN RAT STOMACH*

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Abstract—The stomach of the male rat contains both a soluble and an insoluble histidine decarboxylase. The soluble enzyme in the glandular stomach is the one which is generally assumed to be associated with gastric secretion. The insoluble enzyme is located in the debris fraction of the forestomach and the activity varies widely in different animals. In female rats the activity of the insoluble enzyme is very low, whereas the activity of the soluble enzyme in the glandular stomach is similar to that found in male rats.

In male rats starvation causes a more rapid depletion of the insoluble enzyme than of the soluble histidine decarboxylase. Refeeding or an injection of insulin causes a sharp rise in histidine decarboxylase activity in the glandular stomach, but does not affect the insoluble enzyme in the forestomach. At the present time there is little evidence to suggest that the insoluble enzyme is of major importance in studying the role of histamine in gastric secretion.

INTRODUCTION

THE "SPECIFIC" histidine decarboxylase in the glandular stomach of the rat is generally assumed to be the enzyme primarily involved in the formation of histamine in this tissue and it has been closely studied in attempts to unravel the role of histamine in gastric functioning. Recently, however, attention has been drawn to another enzyme in the stomach which is also capable of decarboxylating histidine.[1] This enzyme differs in its anatomical location, being found mainly in the thin-walled forestomach or rumen, and it also exhibits different characteristics *in vitro* from that found in the glandular stomach. The observation that the activity in the forestomach was often very much higher than that in the glandular stomach led Radwan and West[1] to speculate that this enzyme may have important implications in the formation of histamine in gastric tissue. There is at least one other laboratory, however, which has expressed doubt as to the significance of the enzyme in the forestomach.[2] In view of the potential confusion that could arise from the conflicting data, the present study was concerned with elucidating more clearly the differences between the two enzymes.

METHODS

Male and female Sprague–Dawley rats (Charles River) weighing 175–225 g were used throughout. Animals were killed by decapitation and the stomachs were removed, cleaned and divided into two portions, the thin-walled forestomach or rumen and the thicker glandular stomach. The intermediate area, about 1 mm on either side of the

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demarcation line, was discarded. Tissues from four animals were pooled. The separate portions of the stomach were finely minced with scissors, homogenized in 4 vol. of 0·25 M sucrose in a Sorvall Omnimixer and centrifuged at 10,000 *g* for 30 min. The supernatant fraction was decanted and the debris washed and then reconstituted to the original volume. Aliquots of both debris and supernatant fractions were tested for enzyme activity. Histidine decarboxylase was assayed as described previously.[3] Briefly, the reaction was carried out in a Warburg flask and the incubation mixture consisted of carboxyl-^{14}C-L-histidine (0·1μc in 0·1 ml containing 5 μg L-histidine), 10 μg pyridoxal phosphate, 1 ml of enzyme extract and 0·1 M phosphate buffer to a final volume of 2 ml. Under the conditions of substrate concentration used, the optimal pH value of the forestomach is about 5·5 and that of the glandular stomach is about 7·0. Incubation was allowed to proceed for 2 hr at 37° in a shaking incubator during which time the evolved $^{14}CO_2$ was absorbed on filter paper impregnated with Hyamine hydroxide. The reaction was stopped by the addition of 0·2 ml of 1 M citric acid and shaking was continued for a further hour to allow complete absorption of $^{14}CO_2$. The filter strips were then removed, added to a toluene counting solution and counted in a liquid scintillation counter as previously described.[3]

RESULTS

When male rat whole stomachs were tested for histidine decarboxylase, a substantial amount of enzyme activity was often found in the debris fraction. The amount of activity in this fraction, however, was highly variable and much more variable than that found in the supernatant fraction. Similar experiments carried out in female rats showed that the histidine decarboxylase activity in the debris fraction was almost always low (Table 1).

TABLE 1. DISTRIBUTION OF HISTIDINE DECARBOXYLASE ACTIVITY (cpm/2 ml) IN THE WHOLE STOMACH OF MALE AND FEMALE RATS*

Exp. No.	Male stomach		Female stomach	
	Supernatant	Debris	Supernatant	Debris
1	680	150	730	125
2	870	1105	1300	20
3	800	2115	720	110

* Each value is obtained from pooling tissues from four rats.

In male rats the activity in the insoluble fraction is located almost exclusively in the thin-walled forestomach or rumen (Table 2). In contrast, the soluble enzyme is located entirely in the glandular stomach. The enzyme activities in the supernatant fraction of the forestomach and the debris fraction of the glandular stomach are consistently low irrespective of the amount of activity found in the active fractions. In female rats the activity in the debris portion of the forestomach was very low, whereas the activity in the soluble fraction of the glandular stomach was similar in magnitude to that found in male rats. At the present time there is no explanation for this apparent sex difference in the insoluble histidine decarboxylase and, since this

investigation was concerned primarily with the insoluble enzyme, male rats were used throughout subsequent experiments.

TABLE 2. DISTRIBUTION OF HISTIDINE DECARBOXYLASE ACTIVITY (cpm/2 ml) IN THE STOMACH OF MALE AND FEMALE RATS*

Exp. No.	Male				Female			
	GS	GD	FS	FD	GS	GD	FS	FD
1	1000	70	120	5860	690	90	0	70
2	1920	150	130	7740	1190	5	10	20
3	1150	150	70	700	1660	90	0	60

* Each value is obtained from pooling tissues from four rats. G = glandular stomach; F = forestomach; S = supernatant fraction; D = debris.

The characteristics of the two enzymes *in vitro* have been delineated by Radwan and West[1] and we confirmed their observation that the insoluble enzyme has an optimal activity at pH around 5·5, whereas the soluble enzyme in the glandular stomach activity at around 7·4. On starvation, the insoluble enzyme in the forestomach declines sharply in activity within 24 hr of removal of food. The glandular histidine decarboxylase also declines on starvation, but more slowly (Fig. 1). Refeeding produces one of the most striking differences between the two enzymes. The enzyme in the glandular stomach responds rapidly to food and within a few hours is within the normal range of activity. The insoluble enzyme, on the other hand, remains low and may take 2, 3 or even 4 days to return to high levels of activity. The results of a typical experiment involving starvation for 48 hr and then restoration of food are shown in Fig. 1.

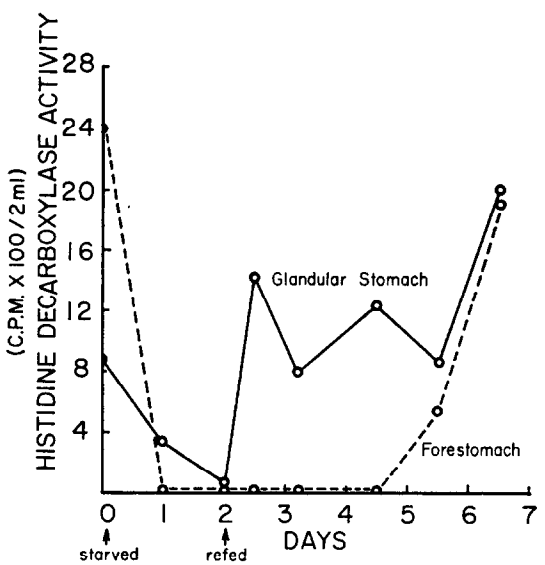

FIG. 1. Effect of starvation and refeeding on histidine decarboxylase activity in the male rat stomach. Each value is obtained from pooling the tissues from four rats.

This marked difference between the two enzymes was extended further by observing the response to insulin. This compound has been shown by several different laboratories to be a potent stimulant of the soluble enzyme in the glandular stomach.[4-6] Rats were starved for 24 hr, injected with insulin (5 units/kg) subcutaneously and the enzyme activity was determined 3 hr later. The histidine decarboxylase activity of the glandular stomach was markedly increased by insulin, whereas the insoluble enzyme activity in the forestomach was not altered (Table 3).

TABLE 3. EFFECT OF A SUBCUTANEOUS INJECTION OF INSULIN (5 units/kg) ON HISTIDINE DECARBOXYLASE ACTIVITY (cpm/2 ml) IN FORESTOMACH AND GLANDULAR STOMACH OF MALE RATS STARVED FOR 24 hr*

Exp. No.	Forestomach		Glandular stomach	
	Control	Insulin	Control	Insulin
1	20	5	200	885
2	30	10	75	930
3	15	20	230	800

* Each value is obtained from pooling tissues from four rats.

DISCUSSION

During the past few years there has been an impressive accumulation of evidence to indicate that the histidine decarboxylase in the glandular stomach is related to gastric secretion in the rat. Changes in activity of this enzyme have been broadly correlated with changes in gastric secretion and, while there is no unanimity on the precise role of histamine in this tissue, there has been no reason to suspect that we may all have been looking at the wrong enzyme. The work of Radwan and West,[1] however, drew attention to another enzyme found in the forestomach which was also capable of decarboxylating histidine *in vitro* and undoubtedly this finding has caused some concern among those investigators involved in trying to unravel the role of histamine in gastric secretion.[2, 7]

The present study has confirmed the existence of an insoluble histidine decarboxylase in the forestomach of male rats without being able to demonstrate a similar finding in female rats. Only male rats were used by Radwan and West.[1]

We must confess at the outset that we have no explanation for this apparent sex difference. In the original experiments demonstrating the presence of a decarboxylase in the insoluble fraction of rat stomach, both male and female rats were used and no sex difference was observed.[3] During the past 2 years, however, we have failed continuously to find activity in female rats under conditions where it was easily demonstrable in male rats. Whether the sex difference reported here is, therefore, a real phenomenon or one peculiar to this institution is a problem that is best resolved by an independent laboratory. Our main object, however, was to determine whether the enzyme in the forestomach, when it could be found, was deserving of special attention. It is unlikely that the high enzyme activity *in vitro* is an artifact of the $^{14}CO_2$ method since similar results have been obtained with the pipsyl method, which measures the histamine directly.[8] The enzyme in the forestomach appears suspiciously like the bacterial histidine decarboxylase, but addition of streptomycin to the incuba-

ion mixture was without effect. Streptomycin has been shown to inhibit bacterial decarboxylation occurring during the incubation.[9] Pretreatment of the animals with antibiotics prior to removal of the stomach gave inconclusive results, for a practical difficulty in studying the enzyme in the forestomach is the wide range of values encountered. These values in freely fed animals may range from 200 to 10,000 cpm and interpretation of the effect of drugs on the enzyme is obviously difficult.

With respect to the role of histamine in gastric functioning, our results, thus far, do not imply that the insoluble histidine decarboxylase is of major importance *in vivo* when compared to the soluble enzyme found in the glandular stomach. The reasons are briefly as follows: (1) The insoluble enzyme is located in the non-acid-secreting forestomach, whereas the soluble histidine decarboxylase is found in the glandular stomach. (2) In contrast to the glandular histidine decarboxylase, the enzyme in the forestomach is not increased in activity by gastric stimulants such as insulin or pentagastrin. (3) Starvation reduces the activity of the insoluble enzyme even more rapidly than the glandular histidine decarboxylase but, in marked contrast to the latter enzyme, it does not return immediately on refeeding. (4) From our own results we would have to conclude that female rats do not have the insoluble enzyme in any significant amounts while appearing indistinguishable from males with respect to the glandular enzyme and gastric secretory responses. (5) The histamine content of the forestomach is about 10 per cent of that in the glandular portion and is not reduced on starvation.[1]

If the enzyme in the forestomach were in some way obligatory in supplying histamine for either triggering or maintaining some aspect of gastric secretion, then it would be difficult to reconcile the fact that the enzyme activity is very low during periods of active secretion such as occur during refeeding or after an injection of insulin into starved animals. On the other hand, it is conceivable that in the freely fed male rat, when the enzyme activity is high, the histamine formed may contribute to the secretory process. Experiments exploring this possibility are in progress.

The changes in histamine metabolism which correlate with changes in gastric secretion occur in the glandular stomach. The stimulation of histidine decarboxylase by insulin is now well established and we have previously suggested that vagal stimulants such as insulin may stimulate histidine decarboxylase through the release of endogenous gastrin.[4] If this should be shown to be the case, then it would establish further the relationship between gastrin and histamine in this region of the stomach. The present study on the enzyme in the forestomach provides little justification for detracting attention from the histidine decarboxylase in the glandular stomach.

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