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# TURNOVER AND SYNTHESIS OF DIAMINE OXIDASE (DAO) IN RAT TISSUES. STUDIES WITH HEPARIN AND CYCLOHEXIMIDE

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**Abstract**—High diamine oxidase (DAO) and histaminase activity are present in rat intestine, thymus and adrenals, and a close correlation exists between the two activities in these tissues (*Biochem. Pharmac.* **24**, 979 (1975)). The distribution of histaminase in normal and germ-free rat tissues and the release of this enzyme from intestine, thymus and adrenals was investigated in further detail with a tritium-release assay. Contrary to previous reports, histaminase activity was detected in brain, in the hypothalamus, thalamus and medulla but not in cortex and cerebellum. The enzyme was released by heparin into blood from intestine and adrenals but not from thymus. In high doses, heparin produced almost complete (>80 per cent) depletion of the enzyme in intestine within 1 hr. The enzyme activity reappeared and returned to normal levels by 24 hr. Prior administration of cycloheximide prevented the repletion of enzyme activity. The time course of the responses to the drugs suggested that DAO is synthesized continuously at a relatively rapid rate ($t_{\frac{1}{2}} \sim 10$ hr). Studies *in vitro* indicated that DAO unlike monoamine oxidase diffuses from the mucosal surface into the lumen of the gut. DAO may therefore have a role in deaminating diamines of bacterial origin in the intestinal contents.

The enzyme diamine oxidase (diamine, $O_2$ oxidoreductase [deaminating], E.C.1.4.3.6) catalyzes the deamination of both histamine and diamines, such as putrescine and cadaverine [1]. Along with histamine-*N*-methyl transferase, it is responsible for the metabolism of histamine in the body, and in some species including the rat [2–4], diamine oxidase plays the major role in the degradation of histamine. The highest levels of enzyme are found in the intestine [5], thymus [6] and, in some species, kidney [5]. The enzyme is also produced by the placenta [7, 8], and increasing levels of the enzyme appear in plasma during the course of pregnancy [9–11].

An interesting property of the enzyme is that it is released into circulation by the administration of heparin in man [12, 13], rat [14], rabbit [14], guinea pig [14, 15] and other vertebrates [16]. In man, the plasma enzyme levels increase after relatively small doses (10 units/kg) of heparin [17]. The enzyme appears within minutes and then disappears from plasma in an exponential fashion [13, 17]. The kinetics of this response suggests that the release of enzyme is immediate and short-lived. Kobayashi and Maudsley have shown there is no response to heparin in rat once the intestine is removed. This indicates that the major source of the enzyme appearing in plasma is the intestine [18].

The purpose of the present study was to identify tissues with high diamine oxidase activity in rat and to determine which of these tissues are depleted of enzyme activity by administration of heparin, and secondly, to study the turnover and release of the enzyme in one organ, the intestine. The turnover rate was determined by measurement of the rate of reappearance of enzyme after depletion with heparin and the rate of disappearance after inhibition of protein synthesis with cycloheximide. The release of intestinal DAO was also investigated *in vitro* and compared with that of monoamine oxidase, which coexists with DAO in the gut.

## MATERIALS AND METHODS

Male, Sprague–Dawley rats (Taconic Farms), 180–220 g, were fed Purina rat chow and distilled water *ad lib*. Germ-free rats (Sprague–Dawley) were obtained from the Division of Research Resources, N.I.H., and were used immediately.

Heparin sodium solution, 1000 and 10,000 units/ml (Upjohn Co., Kalamazoo, Mich.), was given intravenously into the tail vein. Powdered sodium heparin USP (Hynson, Westcott, Dunning, Inc., Baltimore, Md.) was used to prepare solutions of higher strength. Cycloheximide (Calbiochem Corp., San Diego, Ca.) was administered in saline by intraperitoneal injection. The animals were killed by dislocation of the neck. Blood was obtained by cardiac puncture using 5-ml plastic disposable syringes which contained 5 units of heparin. Plasma was obtained by centrifugation of the blood at 2000 *g* for 20 min. The intestine was cut open and briefly rinsed by dipping into water. The mucosal surface was not washed vigorously to avoid loss of enzyme activity (see below). Tissues were washed in distilled water, blotted dry, then frozen on dry ice for storage at −20°. Brain sections were prepared for us by Dr. R. M. Kobayashi according to the procedure of Glowinski and Iversen [19, 20].

The dose of cycloheximide used in these studies, 2 mg/kg, was one that produced no apparent signs of toxicity over the course of 24 hr but produced maximum reduction in intestinal histaminase activity. Preliminary experiments had indicated that smaller doses, 0.5 mg/kg, produced only partial depletion, 35

Table 1. Distribution of histaminase activity in rat tissues

| Tissue | n | Histaminase activity (units/g) | |
|---|---|---|---|
| Placenta* | 6 | 13,426 ± 1,127 | (9,024 16,500) |
| Small intestine (mid-ileum)† | 35 | 5,384 ± 370 | (2,583 7,535) |
| germ free rats | 5 | 8,130 ± 475 | (6,970 9,750) |
| Thymus | 15 | 852 ± 86 | (384 1,400) |
| germ free rats | 5 | 726 ± 105 | (467 975) |
| Adrenals | 11 | 364 ± 63 | (108 820) |
| Stomach | | | |
| Membranous | 2 | 167 | (166,168) |
| Glandular | 2 | 250 | (242,276) |
| Pancreas | 2 | 41 | (39,43) |
| Lung | 5 | 36 ± 7 | (25 57) |
| Spleen | 5 | 28 ± 3 | (26 36) |
| Bone marrow (femur) | 2 | 28 | (26,30) |
| Liver | 8 | 10 ± 2 | (3 16) |
| Kidney | 5 | 8 ± 1 | (5 11) |
| Heart | 5 | 6 ± 3 | (2 14) |
| Plasma | 13 | 3.5 ± 1.1 | (1.6 7.5) |
| Skin (paw) | 3 | < 2 | |
| Submaxillary gland | 1 | < 2 | |
| Testicles | 3 | < 2 | |
| Whole brain | 8 | < 2 | |
| Cerebral cortex | 3 | < 2 | |
| Cerebellum | 3 | < 2 | |
| Medulla pons | 3 | 3.5 ± 0.5 | (2.4 4.5) |
| Thalamus | 3 | 4.3 ± 0.5 | (3.0–4.4) |
| Hypothalamus | 4 | 5.5 ± 1.6 | (4.2 6.3) |

* Placentas were obtained from female Sprague–Dawley rats on the 20th to 23rd day of pregnancy.

† The following values were obtained along the intestinal tract in one rat: duodenum 3,160; jejunum 4,570; upper, middle and lower third of the ileum 5,220, 5,460 and 6,260 respectively; cecum 7,800; and upper part of large intestine 5,400 units/g. In a second rat, values of 5,400, 8,200 and 7,420 units/g were obtained for the jejunum, middle and lower part of the ileum respectively.

Values are means ± S.E.M. Range of values are shown in parentheses. 2 units/g is the minimal detectable activity in tissues as discussed under Materials and Methods.

per cent ($n = 10$, $P < 0.025$) of enzyme activity, and that doses of 5 mg/kg or greater produced death in some animals within 24 hr.

For the assay of enzyme activities, tissues were homogenized freshly in 9 vol of ice-cold sodium phosphate buffer, 0.1 M (pH 6.8). Plasma was used undiluted.

Histaminase activity* was measured by the tritium release assay of Beaven and Jacobsen [21] in which [$\beta$-$^3$H]histamine is deaminated with quantitative release of the $\beta$-tritium to form tritiated water. In this procedure, the sample is incubated with [$\beta$-$^3$H]-histamine (0.1 $\mu$Ci, 14 pmoles) in phosphate buffer, pH 6.8 (total vol 0.2 ml) and water is collected by sublimation in glass Thunberg tubes. Assay blanks were prepared by incubation of tissue homogenate or plasma in the presence of aminoguanidine, $2 \times 10^{-5}$ M, a specific inhibitor of diamine oxidase [22]. Enzyme activity is expressed as units/ml or g of tissue where 1 unit equals 1 pmole of [$\beta$-$^3$H]histamine deaminated/hr of incubation. In the usual assay, an activity of 2 units/g tissue or 0.2 units/ml plasma yielded about 70 dpm of $^3$H above a blank value of 300 dpm $^3$H and was the minimal detectable activity.

*In this present work, histaminase activity is considered the same as diamine oxidase activity and the two terms are used interchangeably. See Discussion.

Monoamine oxidase activity was assayed by a modification of the method of Robinson *et al.* [23]. The concentration of substrate, [$\alpha$-$^{14}$C]benzylamine, was $10^{-9}$ M (100 nCi/ml). Activity is expressed as units/ml or g where 1 unit equals 1 nmole [$\alpha$-$^{14}$C]-benzylamine deaminated/hr of incubation.

For the studies *in vitro*, 2- to 3-cm segments of ileum (wt about 1 g) were used. These were either inverted or left uninverted and were made into closed sacs by tying both ends of the segments with cotton thread. The sacs were incubated at 37 for varying periods of time in a shaking incubator which was set at 40 oscillations/min. At the end of the incubation the bath fluid and tissue were assayed for enzyme activity.

## RESULTS

*Distribution of histaminase activity in rat tissues.* Apart from placenta, the highest levels of histaminase activity as measured by the tritium release assay were found in small intestine, thymus and adrenals (Table 1). High activity was found in intestine and thymus in both normal and germ-free animals. Stomach contained moderate amounts of activity. There was also some activity in pancreas, lung, spleen and bone marrow, low activity in liver, kidney, heart and plasma,

and no activity could be detected in skin, submaxillary gland, testicles and whole brain. When enzyme activity was assayed in various parts of brain, no activity was found in cerebral cortex and cerebellum. Traces of activity were present, however, in the hypothalamus, thalamus and medulla pons with the highest levels being present in the hypothalamus. In all cases, tritium release was suppressed by aminoguanidine, which specifically inhibits DAO activity but not monomaine oxidase. Along the gastrointestinal tract, enzyme activity appeared to increase and was highest in the terminal portion of the ileum and in the cecum. Beyond the cecum, activity was lower (Table 1).

*Effect of heparin on tissue histaminase activity.* Histaminase activity increased in plasma, decreased in ileum and adrenals and remained unchanged in thymus after heparin. These changes were dose-dependent (Fig. 1). After large doses, 40,000 units/kg of heparin, the ileum was depleted almost completely of enzyme activity. In adrenals, enzyme activity decreased markedly (by 76 per cent) with small doses of heparin, but the decrease was less marked (35 and 50 per cent) with larger doses of heparin, due possibly to the higher circulating levels of enzyme in plasma with these doses (Fig. 1).

Additional doses of heparin did not further deplete the intestine. For example, after a single dose of 4000 units of heparin, i.v., enzyme activity was decreased by 57 per cent in one experiment and 51 per cent in another. The injection of up to four additional doses of heparin, 30 min apart, did not deplete intestine of activity by more than 62 per cent ($n = 4$), whereas the same amount of heparin given in a single dose of 20,000 units/kg depleted the intestine by up to 86 per cent.

Kinetic studies showed that after the injection of heparin the decline in intestinal enzyme activity was rapid and complete by 30 min to 2 hr. At this time, plasma levels of enzyme activity were at their highest (Fig. 2). Thereafter, enzyme activity reappeared slowly in the intestine and declined in plasma (Fig. 2). Plasma enzyme levels, in fact, fell to below their original levels and did not return to normal levels until 24 hr later (Fig. 2, insert), at which time enzyme activity had also returned to normal high levels in intestine. The period of low activity in plasma appeared to correspond with the period of low activity in intestine.

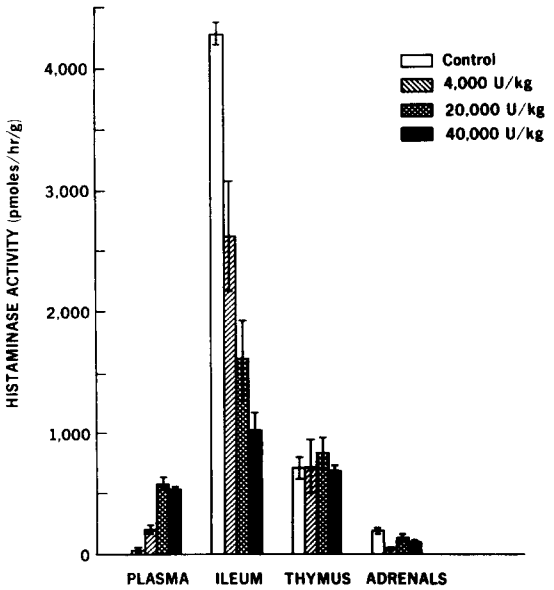


Fig. 1. Effect of heparin on histaminase activity in various rat tissues. Rats received 4000, 20000, or 40000 units/kg heparin i.v. as indicated and were killed 30 min later. Values are the mean ± S.E.M. for tissues from 6 animals.

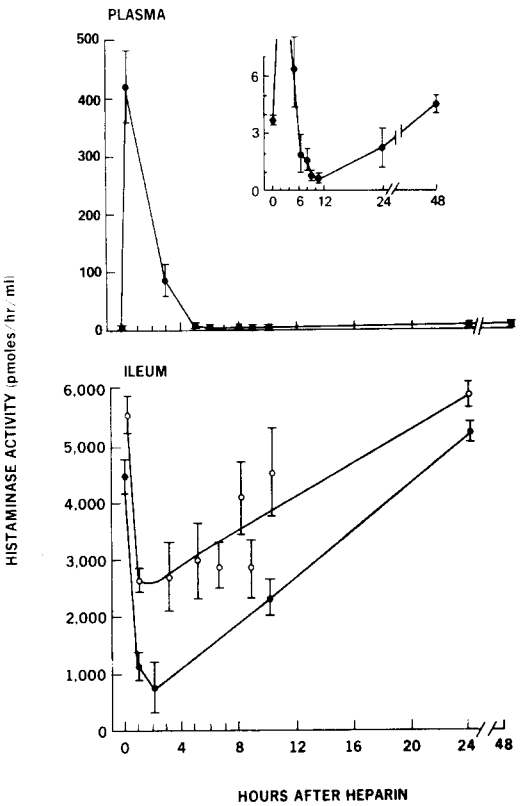


Fig. 2. Time course of changes of histaminase activity in rat small intestine and plasma following the injection of a single dose of heparin. Rats received 4000 units/kg heparin i.v. and were killed at various times after the injection. Values are mean ± S.E.M. for groups of 5–7 animals. The values are from 2 experiments as indicated by the open and closed circles.

Prior administration of cycloheximide prevented repletion of enzyme activity after depletion with heparin (Fig. 3). Alone, cycloheximide in doses of 2 mg/kg, i.p., depleted the intestine of DAO activity within 24 hr (Fig. 3).

*Release of histaminase and monoamine oxidase activities* in vitro. Histaminase activity readily diffuses from ileum *in vitro*. Studies with inverted and noninverted ileal sacs indicated that the enzyme diffused from the mucosal but not the serosal surface (Table 2). The addition of heparin, 40 units/ml, to the bath fluid did not further release or enhance the diffusion of the diamine oxidase activity into the bath fluid (Table 2). In contrast monoamine oxidase, which is present in high levels in the rat intestine, did not diffuse from the intestinal sacs (Table 2). Other studies

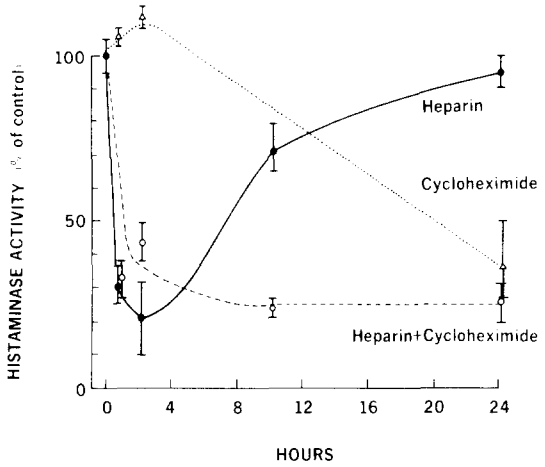


Fig. 3. Effect of cycloheximide on repletion of histaminase activity in rat small intestine after depletion by heparin. Rats were injected with cycloheximide (2 mg/kg) or saline i.p., and 1 hr later were given heparin, 4000 units/kg, i.v., or saline. The animals were killed at various time intervals. Values for the individual time points were determined by separate experiments, each with its own control group. Values are expressed as per cent (mean ± S.E.M) of the control values (average 4468 ± 417 pmoles/hr/g, n = 15).

had shown that the soluble monoamine oxidase activity in ileum, 522 (range 400–663, n = 4) units/g, was the highest in the body except for stomach, 876 units/g, adrenals, 896 units/g tissue.

## DISCUSSION

The present work provides more extensive data on the distribution of histaminase activity in rat tissue than is given by our earlier publications [6, 21, 24]. The work also shows that the enzyme is released by heparin from intestine and adrenals but not from thymus. In intestine, the release is rapid and almost complete with high doses of heparin. This rapid release is in contrast to a slow decline in histaminase activity after inhibition of protein synthesis with cycloheximide. The time course of these responses indicates that the enzyme is synthesized continuously and at a relatively rapid rate in intestine. We shall return to these points later in the discussion.

In the past, there has been controversy as to whether histaminase and diamine oxidase are the same enzyme (for example, see discussion by Zeller [1]). The term histaminase was first used by Best and McHenry [25] to describe a histamine-destroying principle in dog kidney. The term was subsequently adopted by other workers to describe a similar principle in hog kidney [26], human placenta [7] and plasma of pregnant women [27]. Zeller and his associates showed in a series of studies that these same tissues deaminated aliphatic diamines and that histamine and the diamines competed for deamination. This work has been reviewed by Zeller [28, 29]. He proposed that a single enzyme was responsible for the deamination of histamine and diamines, and he introduced the term diamine oxidase to describe this enzyme. Subsequent work has supported Zeller's contention. Highly purified preparations of enzyme from hog kidney [30–33] and, more recently, placenta [34, 35] have been shown to deaminate both histamine and diamines. An exact correlation between histaminase and diamine oxidase activities has been observed also in plasma during the course of pregnancy [10]. In the latter study, histaminase activity was assayed by biological assay by measurement of the rate of histamine destruction, and diamine oxidase activity was measured by a modification of the procedure of Okuyama and Kobayashi [36] in which [$^{14}$C]putrescine was used as substrate.

In contrast to the above studies, Kapeller-Adler and McFarlane [37, 38], using different analytical procedures, obtained purified preparations of enzyme from hog kidney and human placenta which appeared to deaminate histamine but not diamines. These authors concluded that histaminase and diamine oxidase were separate enzymes. Zeller later attributed the inability of these authors to demonstrate deamination of diamines to an artefact in their assay procedure [1]. Although we have shown that there is a close correlation between histaminase activity, as measured by the deamination of [$\beta$-$^{3}$H]histamine, and diamine oxidase, as measured by the deamination of [$^{14}$C]putrescine [24], we have continued to use the term histaminase in our studies [6, 24, 39–44] in

Table 2. Diffusion of histaminase and soluble monoamine oxidase activity from inverted and noninverted segments of rat ileum

| Preparation* | n | Enzyme activity: Tissue (units/g) | Enzyme activity: Bath fluid, without heparin (units 10 ml bath fluid) | Enzyme activity: Bath fluid, with heparin (units 10 ml bath fluid) |
|---|---|---|---|---|
| | | Histaminase activity | | |
| Noninverted sac | 5 | 5822 (3374–9072) | 104 (40–160) | 112 (43–290) |
| Inverted sac | 5 | 5175 (3951–6314) | 834 (410–1030) | 740 (686–900) |
| | | Soluble monoamine oxidase activity | | |
| Noninverted sac | 2 | 537 (541,533) | 0 (< 1) | |
| Inverted sac | 2 | 657 (655,658) | 0 (< 1) | |

* The preparations consisted of small noninverted or inverted ileal segments, tied at each end. These segments were incubated in 10 ml Tyrode ringer with or without added heparin (2,000 units/incubation) for 30 min as described in Materials and Methods. Monoamine oxidase was assayed in the supernatant fraction, (500 *g* for 10 min), of the tissue homogenate.

reference to activity measured by the tritium release assay. Other enzymes are capable of deaminating histamine, for example, pig plasma benzylamine oxidase [45] and human serum monoamine oxidase [46], and these could contribute to the histaminase activity in certain tissues. There are differences also in the deamination of histamine and diamines as far as the effects of substrate concentration and pH are concerned, both with purified enzyme and crude tissue extracts. These differences and the mechanism of tritium release have been discussed in detail in an earlier paper [24] and in the publications of Bardsley and co-workers [35, 47].

The present studies confirm our earlier findings in rat that high enzyme activity exists in placenta, intestine, thymus and adrenals and lesser amounts in other tissues. Unlike in other species, there is little activity in rat kidney. In intestine and thymus, high activity was found in both normal and germ-free rats. Although these two organs are important components of the immune system, exposure to bacteria does not appear to be an important factor in determining the levels of enzyme activity.

No activity could be detected in whole brain, as was reported previously [21]; however, when discrete areas of the brain were analyzed, histaminase activity was observed in hypothalamus, thalamus and pons. This distribution appears to be similar to that observed for histamine *N*-methyltransferase, histidine decarboxylase and histamine in brain [48]. There has been discussion on whether histamine has a role in the CNS and whether deamination is an important route of metabolism for the amine in brain (for example, see article by Schwartz *et al.* [49]). Although DAO activity has been demonstrated previously in fish brain [50], the present finding is the first demonstration that histaminase activity exists in mammalian brain. Earlier studies had not been conclusive about this point. Studies by Zeller and coworkers [51] and Burkard and associates [50] had indicated that DAO activity in mammalian brain was low or less than the sensitivity of the procedures used. It is difficult to assess the importance of the enzyme in brain. Its activity is low, but it is possible that the enzyme is present in high levels in highly localized areas. The fact that the distribution of diamine oxidase appears to parallel that of the other histamine-metabolizing enzymes may be significant. The inability to detect the diamine oxidase in whole brain is probably due to dilution of midbrain with cortex and cerebellum, which are devoid of this enzyme.

The studies with heparin indicate that the enzyme is not released from all tissues. The intestine, because of its size, is likely to be the major source of the enzyme appearing in plasma after the injection of heparin, as was suggested earlier by the experiments of Kobayashi and Maudsley [18]. The intestine may also be the major source of enzyme in plasma. This is suggested by the finding that plasma histaminase activity is low when the intestine is depleted of enzyme and that the plasma levels return to normal when the levels of enzyme in intestine are restored 24–48 hr after the heparin injection. Studies in this laboratory have shown that plasma histaminase levels are low in patients with certain disorders of lipid metabolism [17]. These patients also give an abnormally small rise in plasma histaminase activity in response to heparin. Whether the small response in these patients was a reflection of low levels of enzyme in intestine could not be determined [17].

The mechanism of release of histaminase by heparin is unknown. The highly ionic nature of heparin might suggest that the mechanism is by ionic displacement, although simple displacement could not be demonstrated *in vitro* with heparin. The fact that the extent of release is dependent on initial dose rather than cumulative dose of heparin suggests that the phenomenon is dependent on blood levels of heparin. The release is also immediate and short-lived, and kinetic studies in humans suggest that histaminase is released directly into the blood stream, perhaps from vessel walls [17]. A rapid release of DAO activity has been observed previously in guinea pig. In this species, the major source of the enzyme is liver, and an 88 per cent release of liver DAO activity was noted within 5 min after a large dose of heparin [52].

The ability to release the enzyme from rat intestine has allowed us to study the turnover of the enzyme in this organ. Histaminase appears to be continually produced with a turnover time of about 10 hr. A continuous production and release of diamine oxidase has also been observed with perfused human placenta [53]. The studies *in vitro* indicate that a substantial part of the release is into the gut lumen. These results suggest that the enzyme has a role in deaminating diamines of bacterial origin in the lumen of the gut. However, monoamine oxidase, which is also presumed to play a role in inactivating amines of bacterial origin, is not released into the gut lumen.

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