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PRESENCE OF HISTAMINASE AND ORNITHINE DECARBOXYLASE ACTIVITIES IN RAT THYMUS

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Abstract—Rat thymus was found to contain high levels of histaminase (diamine oxidase), an enzyme that deaminates histamine, putrescine and other diamines. The histaminase activity was high in thymus of a variety of rat strains, including germ-free rats and the spontaneously hypertensive rat. The enzyme activity remained high after involution of the thymus in older rats. Depletion of thymus lymphocytes by administration of a single dose of dexamethasone (100–2000 μg/kg s.c.) produced a 40–60 per cent decrease in thymus weight but no decrease in histaminase activity per thymus. These results indicated that histaminase is located in thymic tissue other than lymphocytes. Thymus also contained high ornithine decarboxylase activity but no histidine decarboxylase activity, which suggested that putrescine, rather than histamine, is a major substrate for the thymus histaminase. Since putrescine is the precursor of the polyamines, spermidine and spermine, the rat thymus may provide a useful model to study the role of these amines in tissues.

IN A PRELIMINARY study, the rat thymus was found to contain high histaminase (diamine oxidase)† activity.[1] This finding was of interest, since thymus is an organ in which there is rapid production and turnover of lymphocytes,[2] and two substrates of histaminase, histamine and putrescine,[3] are thought to have a function in tissue[4–7] and cell growth.[8]

The present work was undertaken to assess the role of histaminase in thymus. The level of histaminase activity in thymus was compared with that in other rat tissues. Histaminase activity was also assayed after depletion of the thymus lymphocytes with dexamethasone to determine the location of the enzyme. In addition, ornithine decarboxylase and histidine decarboxylase, the enzymes responsible for the synthesis of putrescine[9] and histamine,[10] were measured to obtain information on the possible substrates of thymus histaminase.

MATERIALS AND METHODS

Animals. Rats were housed at 22–24° and were fed *ad libitum* a diet of Purina laboratory chow and distilled water. Sprague–Dawley and Osborne–Mendel rats were obtained 1 week before use from the Division of Research Services, National Institutes of Health, or from Zivic Miller Laboratories, Inc. (Allison Park, Pa.) as indicated in text. Pathogen-free spontaneously hypertensive Wistar (SH/NIH) rats,[11,12] F_{22-27}

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† The terms histaminase and diamine oxidase [diamine: O_2 oxidoreductase (deaminating), EC 1.4.3.6] are used synonymously throughout this paper.

generations, and normotensive Wistar (Wistar/NIH) rats were obtained at 4 weeks of age from the Division of Research Services, NIH. Normotensive Wistar (Wistar/Kyoto) rats were bred in our own laboratories from animals supplied by Dr. Y. Yamori, Department of Pathology, Faculty of Medicine, University of Kyoto, Japan; the SH/NIH rats were originally derived from rats of the normotensive Wistar/Kyoto strain.[11] Germ-free Sprague–Dawley rats were obtained from the Division of Research Services, NIH, and were used immediately.

Blood pressure was determined on unanesthetized rats using a tail sphygmographic method.[13] Adrenalectomy was performed through bilateral incisions in rats anesthetized with ether. Dexamethasone sodium phosphate was administered s.c. in 0·2 ml of 0·9% sodium chloride. Doses were calculated in terms of dexamethasone free alcohol. Control animals received 0·2 ml sodium chloride. Animals were killed by decapitation. The thymus and other tissues were removed, rinsed briefly in water, blotted and weighed. Tissues were frozen and stored at −15°, except for the assays of histidine or ornithine decarboxylase activities for which freshly excised and homogenized tissues were used.

Assay of histaminase activity. The thymus was homogenized in 19 vol. of 0·1 M sodium phosphate buffer, pH 6·8. Histaminase activity was determined by the procedure of Beaven and Jacobsen[1] in which tritium is released from β-^{3}H-histamine upon deamination by histaminase and is measured as tritiated water. The incubation was carried out in the bottom of a Thunberg tube and contained 20 μl of the thymus homogenate, β-^{3}H-histamine (0·1 μCi, 15 pmoles), and 0·1 M sodium phosphate buffer, pH 6·8, to a volume of 200 μl. Samples were incubated for 60 min at 37°. At the end of the incubation, 200 μl of a solution of unlabeled histamine (10^{-2} M) in 0·01 N HCl was added; the contents of the Thunberg tube were frozen. Water was collected by sublimation and assayed for tritium by liquid scintillation counting. Histaminase activity was calculated as picomoles of histamine deaminated per hour of incubation per gram of tissue.

Assay of histidine and ornithine decarboxylase activities. Histidine and ornithine decarboxylase activities were assayed by measurement of the release of $^{14}CO_2$ from DL-histidine-carboxyl-^{14}C and DL-ornithine-carboxyl-^{14}C respectively. The procedure used was similar to that described for the assay of histidine decarboxylase,[14] except that for the assay of ornithine decarboxylase DL-ornithine-carboxyl-^{14}C and L-ornithine were used as the substrate. The incubation contained tissue homogenate (100 mg tissue/ml of sodium phosphate buffer, 0·1 M, pH 6·8), 1 ml; carboxyl-labeled DL-amino acid, 0·1 μCi, and sufficient unlabeled L-amino acid to make 500 nmoles of the L-isomer; pyridoxal phosphate, 20 nmoles; and sodium phosphate buffer, 0·1 M, pH 6·8, to a total volume of 2·0 ml. Incubations were for 90 min, after which time perchloric acid was added and $^{14}CO_2$ collected, as described previously.[14] In a few experiments, where insufficient thymus tissue was available, the assay was performed with 0·5 ml of homogenate instead of 1·0 ml. All assays were performed in duplicate. Control incubations (blanks) were run in two ways. In one, the tissue homogenate was omitted and replaced with the phosphate buffer (buffer blank). In the other, tissue homogenate was added but the decarboxylase activity was inhibited by the addition of 4-bromo-3-hydroxybenzyloxyamine (NSD-1055), 2 μmoles (NSD-1055 blank). Decarboxylase activity was calculated as nanomoles of $^{14}CO_2$ formed per hour per gram of tissue; the assumption was made that the $^{14}CO_2$ was derived

exclusively from the L-isomer of the carboxyl-labeled amino acid. Values of 2–3 nmoles $^{14}CO_2$ formed/hr/g were obtained for the NSD-1055 blank and 4–5 nmoles $^{14}CO_2$ formed/hr/g for the buffer blank. Values were corrected for the NSD-1055 blank rather than the buffer blank.

Histidine decarboxylase activity was also assayed by the isotope-dilution procedure of Schayer *et al.*[15] as modified in this laboratory (M. A. Beaven and F. L. Atkins, unpublished). The thymus homogenate, 0·1 ml (10 mg tissue), was incubated for 3 hr at 37° with L-histidine-2(ring label)-^{14}C, 0·1 μCi; unlabeled L-histidine to make 50 nmoles of the L-isomer; pyridoxal phosphate, 2 nmoles; aminoguanidine, 50 nmoles; and sodium phosphate buffer, 0·1 M, pH 6·8, in a total volume of 0·2 ml. Unlabeled histamine (40 mg) was added at the end of the incubation. The histamine was isolated from the incubation mixture, converted to the dibenzene–sulfonyl derivative and assayed for ^{14}C. Values of 0·3 nmole/hr/g were obtained for the NSD-1055 and buffer blanks by this procedure.

Materials. Dexamethasone sodium phosphate and NSD-1055 were gifts from Merck, Sharp & Dohme (Rahway, N.J.) and Smith & Nephew Research Ltd. (Harlow, Essex, England). Radioactive amino acids were purchased from New England Nuclear Corp., Boston, Mass., and unlabeled amino acids from Mann Research Laboratories, Orangeburg, N.Y. Pyridoxal phosphate was obtained from CalBiochem, Los Angeles, Calif., and Hyamine hydroxide from Packard Instrument Corp., Downers Grove, Ill.

RESULTS

Histaminase activity in thymus and other rat tissues. Histaminase activity was high in thymus and intestine, and moderately high in adrenals and stomach. There was some activity in lung, pancreas and spleen, and low activity in muscle, kidney, liver, heart and plasma. No histaminase activity was detectable in brain (Table 1).

TABLE 1. HISTAMINASE ACTIVITY IN RAT TISSUES*

Tissue	n	Histaminase activity (pmoles/hr/g)
Small intestine	8	4863 ± 480
Thymus	9	825 ± 93
Adrenal	6	205 ± 15
Stomach (glandular)	4	139 ± 40
Lung	5	38 ± 7
Pancreas	4	32 ± 3
Spleen	5	25 ± 3
Muscle	4	12 ± 1
Kidney	4	7 ± 1
Liver	4	6 ± 1
Heart	4	6 ± 1
Plasma	10	3·2 ± 0·2
Brain	5	0 ± 0

* Tissues are from male Sprague–Dawley (NIH) rats, 8–10 weeks old. Values are the means ±S.E.M. The activities in kidney, liver and heart have been reported by Beaven and Jacobsen.[1]

Thymus weight and histaminase activity in various rat strains, germ-free and adrenalectomized rats. Thymus weight and thymus histaminase activity varied with the strain of rat (Table 2). Thymus histaminase activity was high in all strains, but was particularly high in the SH/NIH rat (Table 2). Germ-free and adrenalectomized rats had thymus enzyme activity similar to that of control rats of the same strain (Table 2).

TABLE 2. THYMUS WEIGHT AND HISTAMINASE ACTIVITY IN VARIOUS STRAINS OF RATS*

Animals	n	Age (wk)	Thymus wt (mg)	Thymus histaminase activity (pmoles/hr/g)
Wistar/NIH	5	7†	257 ± 6	696 ± 35
Wistar/Kyoto	6	7†	202 ± 24	939 ± 172
Wistar/Kyoto	4	9	223 ± 28	1138 ± 165
SH/NIH, F_{27}	8	7†	297 ± 11	1839 ± 65
SH/NIH, F_{26}	4	12	242 ± 8	1760 ± 94
Osborne–Mendel (NIH)	10	7	463 ± 20	1195 ± 114
Sprague–Dawley (NIH)	5	8	576 ± 20	726 ± 104
Sprague–Dawley (NIH), germ-free	5	8	405 ± 26	827 ± 59
Wistar/NIH, adrenalectomized	3	10	250 (216, 249, 289)	571 (487, 658, 570)
Wistar/NIH, sham operated	2	10	229 (216, 242)	480 (440, 519)
SH/NIH adrenalectomized	3	10	314 (299, 352, 292)	1039 (1157, 1035, 926)
SH/NIH, sham operated	2	10	285 (260, 311)	1069 (1152, 986)

* Female rats were used in these studies, except for the germ-free rats where both male and female rats were used. Adrenalectomy was performed 2 days before the experiment. Results are expressed as means ±S.E.M., except where means and individual values are shown.

† These animals were born on the same day and maintained under identical conditions.

Influence of age on thymus weight and thymus histaminase activity. The influence of age was studied in the SH/NIH and Wistar/NIH rat. The body weights of both strains of rats were similar at all ages (Table 3). Hypertension developed in the SH rat as reported previously.[12] The thymus weight was significantly greater in the SH/NIH rats than in the Wistar/NIH rats at 6–10 weeks of age. In both groups of rats, thymus weight decreased after 10 weeks of age. Histaminase activity was higher in the thymus of SH/NIH rats and diminished with age to a small extent in these rats but not in Wistar/NIH rats (Table 3).

Effect of dexamethasone treatment on thymus weight and histaminase activity. Dexamethasone in doses of 100–2000 μg/kg s.c. produced a 40–60 per cent decrease in thymus weight and a 60–190 per cent increase in histaminase activity per gram of thymus within 24 hr (Fig. 1). However, there was no significant change in histaminase activity per thymus (Fig. 1). When dexamethasone was administered daily for 4 days in doses of 500 μg/kg, thymus weight declined by 75 per cent during the course of

TABLE 3. CHANGES IN BLOOD PRESSURE, BODY WEIGHT, THYMUS WEIGHT AND HISTAMINASE ACTIVITY WITH AGE IN NORMOTENSIVE (WISTAR/NIH) AND SPONTANEOUSLY HYPERTENSIVE (SH/NIH) WISTAR RATS*

Animals	n	Age (wk)	Blood pressure (mm Hg)	Body wt (g)	Thymus wt (mg)	Thymus histaminase activity (pmoles/hr/g)
Wistar/NIH	5	6	121 ± 3	98 ± 2	181 ± 7	497 ± 26
Wistar/NIH	11	8	120 ± 2	135 ± 3	206 ± 2	456 ± 38
Wistar/NIH	7	10	127 ± 3	162 ± 2	254 ± 7	415 ± 30
Wistar/NIH	7	16	123 ± 3	194 ± 5	194 ± 22	494 ± 41
Wistar/NIH	5	25	125 ± 3	204 ± 6	103 ± 4	452 ± 38
SH/NIH	8	6	125 ± 3	94 ± 2	247 ± 9	1260 ± 89
SH/NIH	16	8	161 ± 3	141 ± 4	304 ± 9	1100 ± 54
SH/NIH	7	10	187 ± 3	163 ± 3	320 ± 11	1156 ± 86
SH/NIH	8	16	193 ± 3	188 ± 2	215 ± 11	1002 ± 83
SH/NIH	7	25	199 ± 4	209 ± 3	136 ± 13	808 ± 95

* Female rats were used in these studies. All values for the SH/NIH rats are significantly ($P < 0.01$) higher than those for the normotensive Wistar/NIH rats, except for the thymus weights at 16 and 25 weeks. Results are expressed as means ±S.E.M.

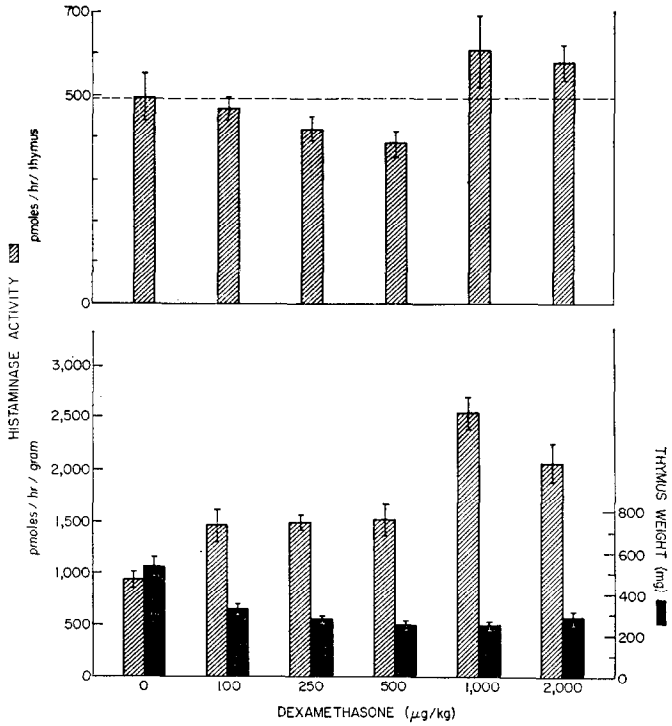

FIG. 1. Effect of dexamethasone on thymus weight and histaminase activity. Male Sprague–Dawley rats (NIH), 8 weeks old, received dexamethasone s.c. and were killed 24 hr later. The upper graph depicts histaminase activity per thymus; the dashed line indicates the values in control animals (0 μg/kg of dexamethasone). The lower graph depicts histaminase activity per gram of thymus. Results are expressed as the mean (±S.E.M.) of values from six rats.

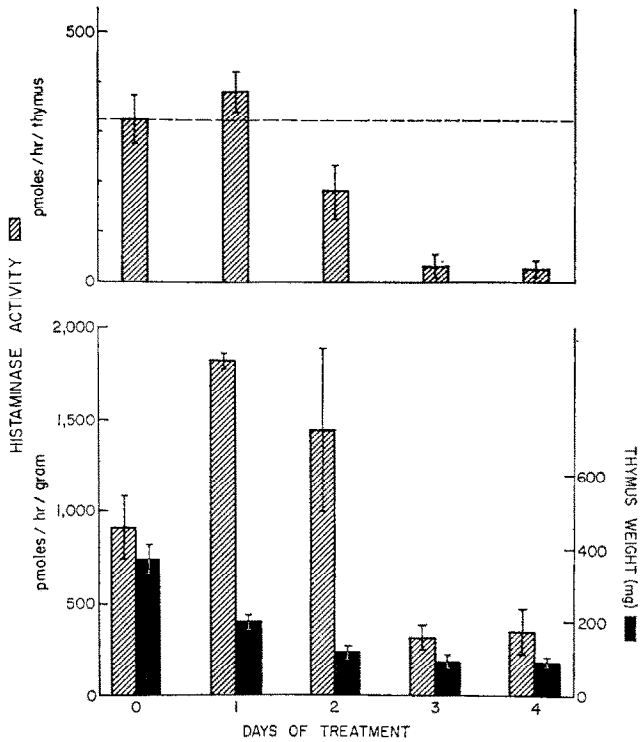

FIG. 2. Effect of daily doses of dexamethasone on thymus weight and histaminase activity. Male Sprague–Dawley rats (Zivic Miller), 7 weeks old, received dexamethasone (500 μg/kg, s.c.) each day and were killed 1, 2, 3 and 4 days after the first dose. The upper graph depicts histaminase activity per thymus; the dashed line indicates the values in control animals (0 days). The lower graph depicts histaminase activity per gram of thymus. Results are expressed as the mean (±S.E.M.) of values from five rats.

treatment (Fig. 2). The level of histaminase activity increased on the first day, but then decreased to low levels by the third and fourth days of treatment (Fig. 2). In terms of histaminase activity per thymus, there was no increase on the first day; on subsequent days, activity decreased and by the third and fourth day the activity per thymus was less than 10 per cent of the controls.

Histidine and ornithine decarboxylase activities in rat thymus. No histidine decarboxylase activity could be detected in thymus of SH/NIH or Wistar/NIH rats by the $^{14}CO_2$ release assay or by the more sensitive isotope-dilution assay procedure (Table 4). Appreciable ornithine decarboxylase activity was found in thymus of Wistar/NIH and SH/NIH rats. Unlike the thymus histaminase activity, the thymus ornithine decarboxylase activity was similar in both strains of rats (Table 4).

DISCUSSION

In recent years evidence has accumulated that the thymus is necessary for the development of the immune system and the maintenance of an adequate pool of immunologically competent cells in the body.[16] The thymus is an active producer of lymphocytes,[17] which turn over at a rapid rate and have a mitotic activity that is

TABLE 4. HISTIDINE DECARBOXYLASE, ORNITHINE DECARBOXYLASE AND HISTAMINASE ACTIVITIES IN THYMUS OF NORMOTENSIVE (WISTAR/NIH) AND SPONTANEOUSLY HYPERTENSIVE (SH/NIH) WISTAR RATS*

Animals	n	Thymus wt (mg)	Thymus histidine decarboxylase activity (nmoles/hr/g)	Thymus ornithine decarboxylase activity (nmoles/hr/g)	Thymus histaminase activity (pmoles/hr/g)
Wistar/NIH	5	198 ± 6	< 0·3	42 ± 7	563 ± 79
SH/NIH, F_{26}	5	244 ± 14	< 0·3	43 ± 5	1381 ± 78

* Female rats, 6 weeks old, were used in these studies. Histidine decarboxylase activity was determined by the isotope-dilution procedure,[15] in which the values for the NSD-1055 and buffer blanks were 0·3 nmole/hr/g. Ornithine decarboxylase activities were corrected for NSD-1055 blank values of 3 nmole/hr/g. Results are expressed as means ±S.E.M.

five to ten times higher than that of lymphocytes elsewhere.[2] The turnover of lymphocytes in thymus is not enhanced by antigenic stimulation, as in spleen and lymph nodes, and is the same in normal and in germ-free mice.[18] There are indications that the rapid proliferation of lymphocytes in thymus is promoted by the epithelial–reticular structure which surrounds the lymphocytes, possibly through the release of a humoral agent.[2,16]

The thymus is an organ that can be influenced by a number of factors. It diminishes in size with age, during stress, or after the administration of adrenal corticosteroids.[19] The decrease in thymus size is due to a decline in the number of lymphocytes,[20] which constitute the bulk of the thymus. The thymus is particularly sensitive to the action of glucocorticoids. After the administration of cortisone, there is a rapid and selective destruction of thymus lymphocytes, and mitosis of lymphocytes is inhibited.[20] In contrast, in mice 2 days after adrenalectomy, thymus weight and lymphocyte mitotic activity increase.[21]

The present studies show that the rat thymus contains high histaminase and ornithine decarboxylase activities. Since the histaminase activity does not decrease after depletion of thymus lymphocytes with a single dose of dexamethasone and remains at high levels in older rats, the histaminase activity is probably located not in the lymphocyte but in some other thymus cell. The observed increase in histaminase activity per gram of thymus after dexamethasone may have been due to concentration of the enzyme within a smaller thymus. Histaminase activity does disappear after more prolonged treatment with dexamethasone. The histaminase activity varies according to the strain and source of rat. At present, we have no explanation for this variation or for the high activity in the SH/NIH rat.

The high ornithine decarboxylase activity in thymus suggests that putrescine is synthesized in this organ. Recent studies in this laboratory* have shown that the ornithine decarboxylase activity is located in the thymus lymphocyte and that when the lymphocytes are incubated with DL-ornithine-2-^{14}C and the radioactive products separated by paper electrophoresis, two radioactive peaks, ornithine and putrescine, are obtained. The labeled putrescine accumulates in appreciable amounts in the lymphocytes, but it does not appear in the incubation medium. Putrescine is

* F. L. Atkins and M. A. Beaven, submitted for publication.

the precursor of the polyamines, spermidine and spermine, in rat tissues,[22] and the finding of high ornithine decarboxylase activity in thymus is consistent with the earlier observation of Jänne *et al.*[23] that thymus contains the highest levels of spermidine and spermine of all rat tissues that they examined.

In addition to the polyamines, rat thymus contains histamine.[24] The presence of histidine decarboxylase activity in thymus has also been reported.[25] However, this enzyme was not detected in the present study, which suggests that histamine may be a less important substrate for histaminase than putrescine. The situation in thymus may be analogous to that in the developing placenta. The placenta, like thymus, contains high diamine oxidase and ornithine decarboxylase activities and low histidine decarboxylase activity.[26]

It has been suggested that histamine and polyamines have a role in the regulation of growth and repair of tissues because both histidine decarboxylase and ornithine decarboxylase activities are found in high levels in the developing fetus, regenerating liver, and certain rapidly growing tumors.[4] Other workers have suggested that polyamines may play a part in the regulation of RNA synthesis, since polyamine synthesis increases under conditions in which RNA synthesis is stimulated, as in the developing chick embryo, regenerating liver, and in liver after the injection of growth hormone.[5,6] The high levels of polyamines, as well as ornithine decarboxylase and histaminase activities, in rat thymus may be related to the rapid cell growth and proliferation of lymphocytes in this tissue.

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