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NONMAST-CELL HISTAMINE KINETICS—II

EFFECT OF HISTIDINE DECARBOXYLASE INHIBITORS ON RATES OF DECLINE OF TISSUE ^{3}H-HISTAMINE IN THE FEMALE RAT*

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Abstract—Inhibition of histamine synthesis *in vivo* was examined in terms of effects on the kinetics of decline of exogenous ^{3}H-histamine in various tissues of the female rat. Inhibition of tissue histidine decarboxylase activity by dietary pyridoxine deficiency coupled with semicarbazide administration greatly reduced rates of decline *in vivo* of labeled amine. On the assumption that exogenous ^{3}H-histamine equilibrates with endogenous amine in nonmast-cell pools, estimated rates of turnover of loosely bound histamine were reduced 30–75 per cent. The histidine decarboxylase inhibitor, NSD-1055 (4-bromo-3-hydroxybenzyloxyamine) was ineffective in reducing endogenous histamine levels and did not affect the kinetics of decline of ^{3}H-histamine. It is suggested that the kinetics of ^{3}H-histamine *in vivo* decline and the tissue histidine decarboxylase activity *in vitro* may reflect the same biochemical phenomenon—presumably the synthesis and catabolism of loosely bound nonmast-cell histamine in dynamic endogenous pools.

THE BIOCHEMICAL and pharmacological significance of histamine formed throughout body tissues is largely unknown, just as the physiological importance of the amine remains enigmatic. It is clear that the bulk of stored endogenous histamine is contained in a relatively static state in mast cells and that exogenous amine is not so stored.[1–3] However, in recent years has come the recognition that endogenous tissue histamine is also derived from nonmast-cell sources. The latter sites of synthesis, which are plentiful in certain tissues such as the gastric mucosa of the rat, are largely responsible for the histamine-forming capacity (HFC) *in vitro* of the tissue and do not store the resulting loosely bound or "nascent" histamine for prolonged periods of time.[4] Recent evidence suggests that nonmast-cell histamine pools can be selectively labeled *in vivo* with exogenous isotopic amine and that, in contrast to mast cell histamine, such pools exhibit rapid turnover and account for the bulk of histamine synthesized in body tissues.[5] It is possible that the histamine-forming capacity and the decline *in vivo* of exogenous tissue ^{3}H-histamine are thus directly related in that both reflect the synthesis of endogenous, "nascent" or "nonmast-cell" histamine. Recent evidence from this laboratory supports this viewpoint. Relative synthesis rates *in vivo* of nonmast-cell histamine in various rat tissues were calculated from rate constants for the initial decline of ^{3}H-histamine. Data thus obtained were in good agreement with relative HFC or histidine decarboxylase activities for a number of tissues as determined by methods *in vitro*.[6]

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In the present study the inhibition of histidine decarboxylase is examined in terms of its effects on the kinetics of tissue ^{3}H-histamine decline *in vivo* in comparison with known effects on tissue HFC (*in vitro*).

MATERIALS AND METHODS

^{3}H-histamine (generally labeled, specific activity, 4·2 c/m-mole; obtained from New England Nuclear Corp., Boston, Mass.) diluted with isotonic saline was administered i.v. (tail vein, 2 mc/kg, 54 μg/kg) to female Sprague–Dawley rats (180–200 g). All animals were given a standard pellet diet and were provided food and water *ad libitum*, except for one group which received a commercial pyridoxine-deficient diet for a period of 3 days prior to injection of labeled amine. The latter group also received subcutaneous injections of 50 mg/kg of semicarbazide twice daily during the same 3-day period; the corresponding control group received saline injections. A third group of rats received NSD-1055 (4-bromo-3-hydroxybenzyloxyamine, 100 mg/kg, i.p.) 12 and 2 hr prior to injection of labeled amine; the corresponding control group received saline injections. Animals were sacrificed by cervical dislocation at various times after the injection of ^{3}H-histamine. Tissues were quickly removed, rinsed, blotted and frozen until assayed. In all cases skeletal muscle was obtained from the thigh, and skin from the abdomen. Only the glandular portion of the stomach and the ileal portion of the small intestine were analyzed. Analysis of tissues was accomplished as previously described[5] with fluorometric determination of endogenous histamine and isotope dilution assay of ^{3}H-histamine. The latter procedure involves the conversion of tissue ^{3}H-histamine in perchloric acid extracts plus added carrier histamine to the benzenesulfonyl derivative, recrystallization of the latter to constant specific activity, and determination of ^{3}H-histamine by scintillation counting of the derivative dissolved directly in Bray's solution.[6] Counting efficiency was approximately 30 per cent and all samples were counted for 20 min or to a limit of 10^6 counts. Corrections for quench were determined by the channels ratio method (ext. std.) and applied to all samples. Rate constants (k) for tissue decline of ^{3}H-histamine were calculated from $T_{\frac{1}{2}}$ values obtained from straight lines drawn visually through three time points (e.g. Fig. 1). Each point was the average value from 4 animals except where otherwise noted. In some cases tissues were pooled, while in others samples were assayed individually. Turnover rates were calculated from the derived k values and from estimated endogenous levels of nonmast-cell histamine.[6–8] The latter estimates were based on measured endogenous amine concentrations and the proportion of tissue histamine which is not releasable by compound 48/80 in rats.[9]

RESULTS

Kahlson *et al.*[10] have shown that semicarbazide administered concurrently with a pyridoxine-deficient diet results in a drastic reduction in histamine synthesis (*in vitro*) in a number of tissues. This treatment was examined in order to determine the effects of such reduction in turnover on the rate of decline *in vivo* of ^{3}H-histamine. Figure 1 illustrates results typical of two separate experiments on the rates of ^{3}H-histamine decline in tissues of control animals versus rats pretreated for 3 days prior to injection of ^{3}H-histamine with semicarbazide and simultaneously given a pyridoxine-deficient diet. Results of these experiments, including effects on endogenous histamine levels, are presented in Tables 1 and 2. Treated animals showed a significant reduction of

endogenous amine only in the stomach. These results are in agreement with those reported by Kahlson *et al.*[10] In addition, the present work extends such observations to include the heart and liver as tissues whose endogenous histamine levels are unaffected by this mode of inhibition. In both experiments, treated animals showed a much greater retention of tissue ^{3}H-histamine compared to controls at 3 hr. Rate constants for the decline of ^{3}H-histamine were greatly reduced in most tissues of

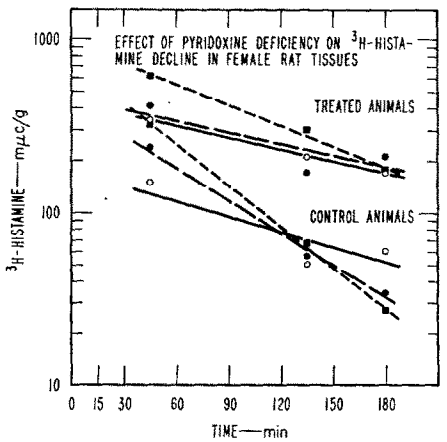

FIG. 1. Decline of ^{3}H-histamine in tissues of control and treated female rats. Treated animals received semicarbazide in conjunction with a pyridoxine-deficient diet. ■ — — — ■, Lung; ○——○, Stomach; ● — — ●, Skin.

TABLE 1. TISSUE ^{3}H-HISTAMINE LEVELS IN CONTROL AND PYRIDOXINE-DEFICIENT RATS AT VARIOUS TIMES AFTER ADMINISTRATION OF THE LABELED AMINE

		^{3}H-histamine (mμc/g tissue ± S.E.)				
Tissue	Group*	¼hr†	¾ hr	2¼ hr	3 hr†	3 hr
Stomach	C	376	148	50	55 ± 6	62 ± 10
	T	578	340	212	218 ± 23‡	173 ± 29§
Skin	C	362	240	56	97 ± 19	34
	T	1075	415	172	1011 ± 168§	215
Ileum	C	712	159	36	35 ± 2	21
	T	499	231	81	127 ± 13‡	83
Lung	C	1147	320	67	70 ± 11	27 ± 6
	T	3517	621	302	264 ± 52‖	174 ± 23‡
Heart	C		500	72		86
	T		1135	660		440
Liver	C		228	59		60
	T		342	124		79
Blood	C	558	137	32	23	34
	T	1481	298	76	13	61

* C = control animals; T = animals pretreated with semicarbazide plus dietary pyridoxine deficiency.

† Results obtained from a separate experiment (¼ hr values are means from tissues of two animals). All other values are from tissues of four animals (pooled, except where standard error data is included).

‡ $P < 0.001$.

§ $P < 0.01$.

‖ $P < 0.02$.

treated animals (Table 2). The decline of labeled amine was virtually unaffected, however, in liver and blood. Turnover rates in control and treated animals were calculated as the product of the rate constant for ^{3}H-histamine decline and the steady state tissue level of endogenous nonmast-cell histamine. The latter was estimated (for both control and treated animals) as a fraction of the measured endogenous level which is

TABLE 2. EFFECT OF PYRIDOXINE DEFICIENCY–SEMICARBAZIDE ON ^{3}H-HISTAMINE DECLINE AND ENDOGENOUS HISTAMINE LEVELS IN TISSUES OF THE FEMALE RAT

Tissue	^{3}H-histamine decline [k(hr^{-1})]		Endogenous histamine			Turnover rate	
			Total (μg/g)		Nonmast-cell (%)*	(μg/g/hr)	(% reduction)
	Control	Treated	Control	Treated		Control	Treated
Stomach	0·43	0·32	19·3 ± 1·6	6·7 ± 0·7†	90	7·5	1·9 (75)
Skin	0·87	0·41	27·4 ± 2·4	30·6 ± 2·8	10	2·4	1·2 (50)
Ileum	0·90	0·55	7·0 ± 0·4	8·7 ± 0·8	95	6·3	4·6 (30)
Lung	1·10	0·53	4·6 ± 1·0	4·3 ± 0·9	100	5·1	2·3 (60)
Heart	0·79	0·43	3·7 ± 0·8	3·5 ± 0·5	50	1·4	0·7 (50)
Liver	0·59	0·69	1·4 ± 0·1	1·4 ± 0·1	75	0·6	0·7 (0)
Blood	0·63	0·69	—	—	—	—	—

* Reference 9.

† $P < 0·01$; rate constants (k, fractional turnover rates) were calculated from the three time point experimental data of Table 1; $k = 0·693/T_{\frac{1}{2}}$; turnover rate = k(μg/g endogenous histamine × percent of nonmast-cell histamine) (see Methods). In contrast to the above data for skin, ileum, lung and heart, normal group variability of k values is similar to the above differences for control vs. treated liver and blood and to the differences between control and treated group tissues shown in Table 3.

not releasable by compound 48/80 in rats.[9] Turnover rates so calculated were reduced 30–75 per cent, with the exception of the liver, which exhibited essentially no change. In the case of the small intestine, the rate constant for ^{3}H-histamine decline was reduced 30 per cent. In the rat, this tissue is the primary site of catabolism of circulating histamine.[11] The reduction in the rate of decline of ^{3}H-histamine in ileum (and in nearly all other tissues) was not accompanied by a reduced rate of decline in blood. This suggests that the decline in tissues is not associated with the catabolism of circulating histamine.

The hydroxylamine derivative, NSD-1055, is reported to be an effective inhibitor of histidine decarboxylase both *in vitro* and *in vivo*.[12] As shown in Table 3, rate constants for the decline of labeled amine in all tissues examined were little affected by treatment with this agent. Similarly, the inhibitor had no significant effect on endogenous histamine levels in stomach, heart and lung. The effectiveness of NSD-1055 as an inhibitor of histidine decarboxylase *in vivo* has been reported as variable in terms of its effects on endogenous histamine levels in rat tissues such as stomach.[13]

DISCUSSION

The female rat was chosen for these studies partly because of the relatively simplified histamine metabolism exhibited by these animals. Histidine decarboxylase appears to be the enzyme largely, if not entirely, responsible for histamine synthesis *in vivo* and the destruction of histamine appears to be largely dependent upon histaminase.[10] Furthermore, previous work by Kahlson *et al.*[10, 14] on relative histamine-forming capacities of tissues and the effects of various treatments thereon pertain largely to the

female rat. Such studies have amply demonstrated that both whole body HFC (urinary excretion of free histamine) and tissue (*in vitro*, isotopic) HFC are lowered to 10–20 per cent of normal in animals given semicarbazide and fed a pyridoxine-deficient diet.[10] This pronounced inhibition of histidine decarboxylase has been demonstrated for skin, tongue, lung, stomach and small intestine. The fact that such

TABLE 3. EFFECT OF NSD-1055 ON ENDOGENOUS HISTAMINE LEVELS AND ON DECLINE OF ^{3}H-HISTAMINE IN TISSUES OF THE FEMALE RAT

Tissue	Group*	^{3}H-histamine (mμc/g)†			^{3}H-histamine	Endogenous histamine
		½ hr	2 hr	3 hr	[k(hr^{-1})]	(μg/g ± S.E.)
Stomach	C	161 ± 13	71 ± 4	44 ± 7	0·52	17·6 ± 0·9
	T	551 ± 52†	186 ± 59	138 ± 21§	0·55	14·5 ± 2·0
Heart	C	2063 ± 119	142 ± 15	96 ± 34	1·22	2·9 ± 0·5
	T	4490 ± 382‡	355 ± 23‡	226 ± 33‖	1·19	3·8 ± 0·7
Lung	C	197 ± 37	23 ± 3	14 ± 4	1·07	4·0
	T	784 ± 229‖	69 ± 20	91 ± 15§	0·85	5·0
Skin	C	114 ± 11	50 ± 9	20 ± 4	0·70	
	T	419 ± 151	66 ± 24	64 ± 27	0·75	
Ileum	C	151 ± 15	62 ± 16	13 ± 1	0·96	
	T	432 ± 75‖	69 ± 40	43 ± 31	0·91	
Liver	C	471	85	24	1·19	
	T	1022	160	68	1·10	

* C = control animals; T = animals given NSD-1055 12 and 2 hr prior to injection of ^{3}H-histamine (12 animals/group). Differences in endogenous levels (means from 10 animals in the case of stomach and heart) are not significant.

† In all cases where a P value is not indicated, the differences between control and treated values are not significant. Blood levels of ^{3}H-histamine (mμc/g) at ½ hr (pooled tissues of 4 animals) were as follows: control, 204; treated, 471.

‡ $P < 0.001$.

§ $P < 0.01$.

‖ $P < 0.05$.

inhibition has no pronounced effect on endogenous histamine levels (with the exception of stomach) is consistent with the concept that the endogenous level in many tissues reflects primarily the tissue content of mast cell or other firmly bound histamine, which is not influenced by the acute inhibition of histidine decarboxylase.[10] In the gastric mucosa, which contains predominantly nonmast-cell histamine, such inhibition does result in a pronounced fall in endogenous histamine levels as substantiated in the present study. Kahlson *et al.*[10] have suggested that the predominantly nonmast-cell histamine of lung and small intestine is firmly bound in a manner distinct from that in mast cells.

The fact that exogenous histamine is not bound in the relatively static histamine stores of mast cells[1–3] eliminates the latter stores as accessible pools for equilibration with injected ^{3}H-histamine. Presumably, turnover in smaller and more dynamic metabolic pools of loosely bound histamine is largely reflected in the tissue HFC and such pools are accessible for equilibration with injected isotopic amine. On this assumption, the decline of exogenous tissue ^{3}H-histamine may reflect the turnover of newly synthesized nascent histamine and the HFC of the tissue. Likewise, changes in

tissue HFC will result in changes in turnover which should be reflected in alterations in tissue ^{3}H-histamine kinetics if the above assumptions are correct. Pretreatments designed to inhibit histidine decarboxylase were intended to establish new steady states with reduced rates of synthesis balanced by reduced rates of catabolism and lowered concentrations of endogenous amine in nonmast-cell pools. After subsequent injection and equilibration of ^{3}H-histamine with the latter, the decline of labeled amine in tissue might thus reflect the altered turnover under the new steady state conditions.

The present results with pyridoxine deficiency–semicarbazide treatment are consistent with the above assumptions. The reported 80–90 per cent reduction in tissue HFC resulting from this treatment compares favorably with the 30–75 per cent reductions in calculated turnover rates *in vivo* based on tissue ^{3}H-histamine decline. Except for the stomach, however, these calculations are based on essentially unchanged endogenous nonmast-cell histamine levels. It is possible, as discussed above, that levels in small, equilibrating nonmast-cell histamine pools are substantially lowered in treated animals in which case the reduction of turnover rates would be even greater than presently calculated. The parallelism between the effects of pyridoxine deficiency–semicarbazide treatment on tissue HFC and on rates of decline of tissue ^{3}H-histamine suggests that these methods *in vitro* and *in vivo*, reflect the same biochemical phenomenon—presumably the decreased synthesis of histamine in endogenous pools. The necessity for caution in interpretation in terms of the nonspecificity of this method of inhibition, however, has been recognized.[15] Of possible significance in this regard is the finding that, in contrast to other tissues examined, the liver shows no reduction in apparent turnover with this treatment. Adult rat liver reportedly contains little or no specific histidine decarboxylase.[16] It is thus possible that the decline of ^{3}H-histamine in liver is not related to turnover of the endogenous amine in a pool associated with specific enzymatic synthesis of the amine. If the decline in this organ is related instead to the catabolism of circulating amine, it appears that such catabolism is not affected by this treatment. Similarly, an inhibition of catabolism of circulating amine reflected in decreased rates of ^{3}H-histamine decline in other tissues would be expected to result in a decreased rate of ^{3}H-histamine loss from blood and such a result was not observed.

Because of the nonspecificity of the foregoing enzyme inhibition method, an alternative establishment of a reduced steady state condition was sought by inhibition of histidine decarboxylase with NSD-1055.[11] Significant reductions in endogenous histamine levels in stomach and heart[12] as well as lung[16] have been reported for experiments *in vivo* with this agent. However, considerable variability in effectiveness *in vivo* has also been reported in rats of different strains and sources.[13] Thus, the finding of little or no effect by this compound on endogenous histamine levels and on rates of ^{3}H-histamine decline is not surprising, but is inconclusive. Johnston and Kahlson[15] have recently reported that NSD-1055 (as well as the α-hydrazino analog of histidine, αHH) is relatively ineffective in reducing whole body HFC (urinary output of free histamine), but somewhat reduced the initial conversion *in vivo* of injected ^{14}C-histidine to urinary ^{14}C-histamine. Furthermore, Reilly and Schayer have concluded that a number of inhibitors such as NSD-1055 apparently do not effectively reach a major site of histamine synthesis *in vivo*.[17] It is also interesting that NSD-1055 can, in certain instances, reduce endogenous histamine levels in stomach, heart and lung, while extensive inhibition of HFC by semicarbazide–pyridoxine deficiency has

such an effect only in stomach. The lack of effect of NSD-1055 on mast cell histamine in the rat has been demonstrated.[12] Interpretation of results obtained with NSD-1055 must await further, more definitive studies on this and similar agents. It is possible, however, that NSD-1055 interferes with the initial catabolism of circulating ^{3}H-histamine prior to uptake of the latter into endogenous pools. Uptake may be enhanced somewhat by preservation of circulating amine. Such a phenomenon (detectable only in terms of radioactivity) might explain the somewhat higher ^{3}H-histamine levels in tissues of treated animals in spite of the lack of effect on endogenous levels and on rates of decline of the labeled amine (Table 3). Consistent with this speculation is the fact that blood levels of ^{3}H-histamine in treated animals were more than double those in controls at ½ hr (Table 3, footnote).

To date, pyridoxine deficiency remains the most potent and reliable method of inhibition *in vivo* of histidine decarboxylase; associated with this technique is a sizeable body of evidence consistently indicating pronounced reductions in both the histidine decarboxylase activity of individual tissues and, to a lesser extent, the whole body histamine-forming capacity as judged by both isotopic and nonisotopic methods. The present work demonstrates a parallelism between the effects of this treatment on the HFC *in vitro* and the decline *in vivo* of exogenous isotopic histamine in several tissues. This fact adds significance to the previously demonstrated parallelism between relative tissue HFC and calculated synthesis rates based on the kinetics of ^{3}H-histamine decline *in vivo*.[6] Furthermore, preliminary studies in this laboratory indicate that the increased HFC induced in some tissues by anaphylaxis is accompanied by an increased rate of decline of ^{3}H-histamine in some tissues, but not in tissues whose HFC is unaltered by anaphylaxis.[18] Together, these results suggest that the present isotopic method may be a useful index *in vivo* of effects on the metabolism of endogenous histamine in dynamic nonmast-cell pools. Such potentially important effects are not generally reflected in changes in endogenous histamine levels and are difficult to study *in vivo*.

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