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Presence and measurement of *tele*-methylhistamine in mast cells

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Abstract

The histamine metabolite *tele*-methylhistamine (*t*-MH) was identified and measured in crude and purified peritoneal mast cells (MCs). Peritoneal dialysates, peritoneal cells, and purified MCs all contained *t*-MH in concentrations representing about 0.2% of the corresponding histamine (HA) levels. *T*-MH levels in crude cells represented about 70% of the total dialysate levels, indicating the presence of extracellular as well as intracellular *t*-MH. *T*-MH levels per MC in purified fractions were similar to those of crude fractions, indicating a MC origin for the intracellular *t*-MH. Histamine methyltransferase activity was not detected in crude or purified MC fractions, and incubations with the monoamine oxidase inhibitor pargyline failed to increase the content or release of *t*-MH in either fraction, suggesting a very slow or non-existent histamine methylation in MCs. Compound 48/80 produced a temperature-dependent release of HA and *t*-MH in crude and purified preparations, and Triton X-100 also released both amines. In all cases, the degree of release of both amines was correlated, consistent with a granular origin for *t*-MH in MCs. The low concentrations of *t*-MH in MCs do not necessarily indicate a role for MCs in HA metabolism, but suggest that *t*-MH may be a valuable marker for non-MC HA.

Numerous studies have shown the importance of mast cells (MCs) in immunologic as well as inflammatory processes. The functions of these cells, although far from completely established, include the synthesis, sequestration, storage and release of substances with vasoactive, chemotactic or enzymatic properties [1].

Perhaps the best known MC mediator is histamine (HA), stored in high concentrations within MC granules that are discharged upon antigenic challenge or tissue damage. HA released from MCs acts through both H_1- and H_2-receptors to alter vascular permeability, vascular resistance as well as chemotaxis [1, 2].

Histamine metabolism occurs by two different pathways [3]: oxidative deamination by diamine oxidase (DAO), and methylation by histamine methyltransferase (HMT). However, the extent of HA metabolism after its release from MCs is not clear. FURANO and GREEN [4] showed that rat MCs do not metabolize endogenous or exogenous HA. The presence of DAO in granulocytes [5] suggests that these cells may inactivate HA released from MCs. Thus, MCs release HA, but may not metabolize it. Presently, we have identified and measured very small amounts of the HA metabolite *tele*-methylhistamine (*t*-MH) in rat peritoneal MCs.

Materials and methods

Peritoneal dialysates were prepared from male Sprague–Dawley rats (350–450 g) according to the method of LAGUNOFF [6], cooled in ice and centrifuged (110 × *g*, 5 min), yielding a crude MC pellet. Crude MC fractions contained 4.1 ± 1.1% MCs. In a few experiments, crude MCs were purified by centrifugation through 30% albumin gradients (Pathocyte IV, Miles Laboratories) as described [6]. Results are shown for preparations whose MC purity was at least 84%. MCs were identified and counted by staining with toluidine blue.

HA was measured by a single isotope radioenzymatic method [7], essentially the same as described earlier [8], with rat kidney HMT [9]. *T*-MH was measured by our recent modification [10] of the gas chromatographic–mass spectrometric (GC–MS) method of HOUGH et al. [11]. The method is based on the extraction and derivatization of tissue homogenates, with *pros*-methylhistamine as internal standard; this substance was not found in any MC preparation. Routine measurements of *t*-MH levels were performed by selected ion monitoring of *m/e* 304, a major ion fragment of both the *t*-MH and *p*-MH derivative [11]. Other ions (see below) were also monitored to confirm the identification of

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t-MH. HMT was measured according to TAYLOR and SNYDER [12]. As the HA and *t*-MH content of dialysate preparations varied considerably from day to day, differences in the amine contents of crude pellet and dialysate fractions were determined by paired *t*-tests.

For release studies, MC fractions (1.1×10^6 MCs/tube) were incubated in 14 ml polypropylene centrifuge tubes in a total volume of 1 ml of balanced salt solution [6] for 30 min. The samples were separated by centrifugation in a Beckmann Microfuge B, and pellets and supernatant fractions were assayed as described.

Results

Analyses of crude and purified MC fractions showed the presence of *t*-MH. The identification of *t*-MH in these fractions was confirmed by multiple selected ion monitoring (Fig. 1). Endogenous *t*-MH exhibited a retention time identical to that of the standard, and ratios of the areas under each ion curve (Table 1) were the same for the standard and biological sample.

Rat peritoneal dialysates contained *t*-MH (42.9 fg/MC, Table 2). MC pellets also contained *t*-MH, but the levels (28.5 fg/MC, Table 2) were lower than in dialysates (paired *t*-test, $p < 0.05$). When expressed as amine per fraction, the crude pellet consistently contained about 70% of the total dialysate *t*-MH, the other 30% remaining in the cell-free supernatant. Purified MCs also contained *t*-MH (Table 2).

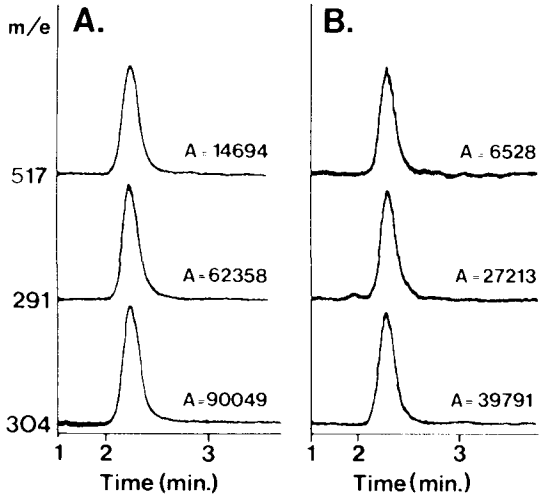

Figure 1
Selected ion monitoring of samples assayed for *tele*-methylhistamine (*t*-MH). Standard *t*-MH (A) and unpurified mast cells (B) were extracted and derivatized as described. To authenticate the identification of *t*-MH in MCs, three ions prominent in the electron impact mass spectrum of the *t*-MH derivative were simultaneously monitored after injection of the samples. Symmetrical peaks in these ion scans with a retention time of 2.2 min are shown from both samples. As each scan is normalized by computer, the relative peak size is not evident except as indicated by the area measurements. Ratios of these areas are shown in Table 1.

Table 1
Verification of the identity of *tele*-methylhistamine in mast cells.

Sample	Ion ratio		
	304/517	304/291	291/517
Standard	6.13	1.44	4.24
Mast cells	6.10	1.46	4.17

The ratios of the areas of the selected ion scans of Fig. 1 are shown. The finding that mast cell extracts exhibit ion ratios nearly identical to those of the standard confirms the presence of *t*-MH in these cells.

Crude dialysates contained HA levels of 19 pg/MC, not different from the HA content of crude MC pellets (19.2 pg/MC, Table 2). Crude dialysate supernatant fractions contained less than 1% of the HA content of the dialysate. As expected, purified MCs retained their HA (Table 2).

To characterize the possible release or metabolism of *t*-MH in MCs, samples were incubated in the presence and absence of pargyline, an inhibitor of monoamine oxidase. Incubation of crude MCs for 30 min in the absence of pargyline at 0 or 37° did not increase supernatant or total *t*-MH levels, and pargyline (10^{-5} *M*) had no effect on the content or release of *t*-MH (not shown). Similar results were obtained with purified MC fractions.

HA releasing agents were used to study the localization of *t*-MH within the MC. Compound 48–80 (5 μg/ml), a non-destructive degranulating agent, caused a temperature-dependent release of 78% of *t*-MH and 91% of HA in purified fractions (Table 3). Similar effects were observed with crude MCs (Table 3). Triton X-100 (0.5 mg/ml), a non-specific agent, released 95–100% of *t*-MH and HA in both preparations (Table 3). In all circumstances, the degree of histamine release correlated significantly with the release of *t*-MH (Fig. 2).

HMT activity was not detected in either crude or purified MC fractions (not shown).

Table 2
Histamine and *tele*-methylhistamine content of rat peritoneal mast cells.

Preparation	*t*-MH (fg/MC)	HA (fg/MC)	Molar Ratio *t*-MH/*p*-MH
Dialysate	42.9 ± 12.9	19,000 ± 3800	0.00159 ± 0.0003
Pellet	28.5 ± 10.2[a]	19,200 ± 3500	0.00101 ± 0.0002[a]
Purified	52.1, 49.8	43,400, 28,000	0.00107, 0.00158

The mean ± SEM are shown for groups of four experiments (dialysate and pellet) or individual results (purified). Fractions were prepared by centrifugation of dialysates and, in some cases, purification with albumin gradients. *T*-MH levels in mast cells are about 1/600 of those of histamine.
[a] Significantly different from dialysate values by *paired t*-test.

Table 3
Effect of drugs on the release of histamine and *tele*-methylhistamine from mast cells.

Drug	Temperature (°C)	Amine release (% of total)			
		Crude MC		Purified MC	
		t-MH	HA	*t*-MH	HA
Buffer	0	2.2	0.5	18.6	17.1
48–80 (5 μg/ml)	0	4.9	2.4	0	2.4
Buffer	37	2.5	1.5	40.9	26.8
48–80 (5 μg/ml)	37	64.6	41.8	77.7	90.9
Triton X-100 (500 μg/ml)	37	95.7	96.4	100.0	98.5

Crude and purified mast cells (MCs) were incubated 30 min in the presence and absence of 48–80 and Triton at the temperatures shown. Samples were centrifuged, and the pellet and supernatant fractions were assayed for histamine (HA) and *tele*-methylhistamine (*t*-MH). Release was calculated as percent of total amine found in the supernatant. Values are mean of triplicates for HA, and single or duplicate determinations for *t*-MH. Total (i.e. pellet + supernatant) amine levels were (mean ± SEM): HA, 23.3 ± 3.0 pg/MC; *t*-MH, 35.5 ± 4.9 fg/MC ($n = 15$). Neither total HA nor total *t*-MH levels were changed by any treatment.

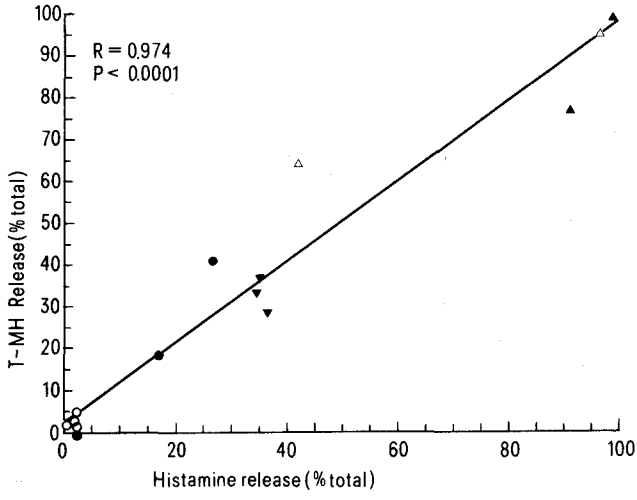

Figure 2
Correlation of the release of histamine (HA) and *tele*-methylhistamine (*t*-MH) from mast cells (MCs). The results of Table 3, as well as other studies are plotted. Open symbols represent crude MCs, solid symbols are purified preparations. Circles are incubations with buffer at 0 and 37°, with pargyline (10^{-5} *M*, 37°) and 48–80 (5 μg/ml, 0°), all giving 0–4% release. Triangles show the release at 37° after the same concentration of 48–80 and after Triton X-100 (0.5 mg/ml). The solid inverted triangles are from a preparation probably damaged during purification. In all cases, the similarity in the release of the amines is evident.

Discussion

Analysis of MCs by sensitive and specific GC–MS methods has permitted us to detect very small amounts of *t*-MH in crude and purified cells. This identification is confirmed by the selected ion monitoring of standards and extracts (Fig. 1, Table 1). Overall, MC *t*-MH levels are about 1/600 of the HA levels (Table 2). However, not all of the *t*-MH of crude dialysates was in MCs, since about 30% of the total dialysate *t*-MH remained in the cell-free supernatant after centrifugation. The presence of *t*-MH in supernatant fractions was not the result of cellular lysis, since less than 1% of the total HA was in this fraction (Table 2). If significant amounts of peritoneal *t*-MH were in a cellular element other than MCs, then purification of crude fractions (containing 4% MCs) would have resulted in a drastically lower *t*-MH content, as expressed per MC. That *t*-MH levels do not change after purification (Table 2) indicates that this cellular *t*-MH is of MC origin. The HA content of the MC fractions is in agreement with others [13, 14].

T-MH is oxidized predominently by monoamine oxidase (MAO) in tissues such as brain and liver [15–17], and treatment with MAO inhibitors increases brain *t*-MH levels [15]. If MCs actively form and metabolize *t*-MH, then inhibition of MAO by pargyline should lead to *t*-MH accumulation and/or release. Incubations with pargyline did not increase total or supernatant *t*-MH levels in crude or purified MCs. These results, taken with the lack of HMT activity in these cells, suggest a very slow or absent methylation of HA in MCs. These findings are also consistent with earlier studies [4] reporting no metabolism of labelled HA by MCs. Even under conditions of MC degranulation, we were unable to increase *t*-MH levels in MCs (Table 3).

We also used cytolytic and non-cytolytic releasing agents to determine whether MC *t*-MH was in cytoplasm (and thus be released only after cell disruption, e.g. Triton), or in HA-containing granules (and thus be released after cell disruption as well as after specific degranulation). Our findings (Table 3, Fig. 2) that *t*-MH was always released to the same extent as HA (independent of the stimulus) support the conclusion that MC *t*-MH is of granular origin.

Abundant evidence indicates that methylation of HA produces *t*-MH, but this amine can also be formed by decarboxylation of *tele*-methylhistidine (*t*-MHis), an endogenous amino acid [18]. Although *t*-MHis is mostly excreted unchanged [19], SCHWARTZ et al. [20] showed that peritoneal MCs synthesize and store labelled *t*-MH after *in vivo* administration of labelled *t*-MHis. As with our measurements of endogenous *t*-MH, this labelled *t*-MH was released by 48/80, suggesting a granular localization. This mechanism for *t*-MH biogenesis in MCs is also consistent with our inability to detect HMT activity in MCs, but requires further study. Whatever the biogenic mechanism, our inability to increase MC *t*-MH release or content with pargyline indicates that *t*-MH in MCs is not rapidly metabolized by MAO. This conclusion is supported by earlier workers [21] who could not detect MAO in MCs.

The consequences of the existence of endogenous *t*-MH in MCs are not clear. As *t*-MH is virtually inactive on HA receptors [22], the presence of small amounts of *t*-MH in MCs may not be important for MC function. Our findings also do not clearly indicate a role for MCs in HA metabolism. The presence of *t*-MH in MCs may be due to the relatively non-selective ion exchange properties of sulfomucopolysaccharides in MC granules [21, 23]. Since MCs contain so little *t*-MH relative to HA, *t*-MH measurements may be useful in characterizing non-MC stores of HA in tissues like brain. In contrast to MCs, brain contains similar amounts of *t*-MH and HA [24]; in such organs and tissues, *t*-MH levels may be indicative of non-MC HA metabolism. This hypothesis is being further pursued.

Acknowledgments

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