CEREBROSIDE SULFATE-AMINE INTERACTION IN NEOPLASTIC MAST CELLS

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Received 20 July 1970

1. Introduction

Cerebroside sulfate (CS) is an acidic lipid that has been identified in amine containing tumor mast cells [1, 2]. The ability of acidic lipids to form complexes *in vitro* with positively charged organic compounds such as 5-hydroxytryptamine (5-HT) or histamine (H) has been noted [3, 4]. The present study describes the formation *in vivo* of cerebroside sulfate-amine complexes in the HC line of tumor mast cells.

2. Materials and methods

HC cells derived from the Furth-Hagen-Hirsch mastocytoma [5] were carried in LAF_1, female mice (Jackson Labs, Bar Harbor, Me.). Seven days after the injection of the ascites cells, the tumor bearing mice received an intraperitoneal injection of 0.05 μCi of ^{14}C-5-HT (sp. act. 56 mCi/mmole) or ^{14}C-histamine (sp. act. 58 mCi/mmole). Thirty minutes later the cells were harvested, suspended in 0.34 M sucrose, and sonicated (at 2 A, for 2 min) at 0°. The sonicate was spun at 1,500 *g* for 10 min to remove whole cells. A large particle fraction was collected by centrifugation at 5,500 *g* for 10 min. Further purification of the fraction was performed on a discontinuous sucrose density gradient (0.6–1.5 M, see fig. 1) by centrifugation at 204,000 *g* for 60 min at 0°. Five fractions were collected from the top of the tube and aliquots of each fraction were assayed for endogenous 5-HT and histamine [6–8], and fumarase activity [9]. Corresponding fractions from several gradients were pooled, extracted with a lipid solvent [10], chromatographed on a magnesium silicate column [11], and assayed for cerebroside sulfate content [1, 12, 13]. The ^{14}C-amines were extracted by the same method as that used for the endogenous amines, and the radioactivity was measured in a liquid scintillation counter.

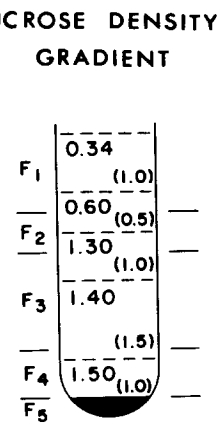

Fig. 1. The fractions collected from the top of the tube were designated F_1–F_5 and each corresponds to a visible band between two layers (bands occur between 0.6 M and 1.3 M, 1.3 M and 1.4 M, and 1.4 M and 1.5 M sucrose). The large numbers (in the tube) indicate the sucrose molarities of the gradient layers. The numbers in parentheses (1.0) etc., are the volumes of the layers.

To determine the presence of CS-amine complexes, the fractions from several gradients were pooled and extracted with chloroform/methanol [10]. The chloroform phase was chromatographed by the thin-layer method, on silica gel G (Merck), in chloroform/metha-

Table 1
The density gradient distribution of cerebroside sulfate, amines and fumarase from the 5,500 *g* fraction of HC cells *.

Fraction	% CS	RSC †	% 5-HT	RSC †	% Histamine	RSC †	% Fumarase	RSC †
F_1	26.1 ± 2.0	0.98	14.6 ± 0.5	0.52	14.8 ± 1.2	0.53	34.8 ± 1.5	1.26
F_2	58.7 ± 5.1	2.70	14.1 ± 0.62	0.62	17.4 ± 0.5	0.77	37.2 ± 1.8	1.64
F_3	2.1 ± 0.2	0.17	8.8 ± 0.3	0.71	4.9 ± 0.3	0.40	17.3 ± 1.8	1.42
F_4	5.6 ± 0.8	0.55	6.5 ± 0.4	0.54	5.4 ± 0.5	0.44	8.0 ± 1.5	0.66
F_5	7.5 ± 0.7	0.29	55.8 ± 1.9	2.16	57.5 ± 1.3	2.23	2.7 ± 0.1	0.10

* Values represent the mean of 3 experiments (± standard error of the mean) expressed as percent distribution of the total.
† Relative specific concentration (RSC) = % of lipid, amines, or fumase/% of total protein.

nol/water solvent systems [14]. The cerebroside sulfate-amine complexes and other lipid extractable compounds were detected by exposure to iodine vapor, and after elution the presence of CS components and ^{14}C-amines were determined. The identity of the ^{14}C-amines was determined by thin layer chromatography in butanol/acetic acid/water (12:3:5) [15]. The 5-hydroxytryptamine and histamine standards were visualized on the plates by spraying with *p*-dimethylaminobenzaldehyde [17] and diazotized sulfanilic acid [16] reagents, respectively.

3. Results

Chemical analysis of the density gradient fractions showed almost complete separation of the amine containing particles (F_5), from CS and fumarase (F_2). The bulk of the CS appeared in a fraction that showed high fumarase activity, characteristic of mitochondria. The endogenous amines were predominantly localized in the multivesicular granule fraction (F_5) (table 1). A significant portion of the exogenous ^{14}C-amines, on the other hand, were incorporated into the CS, fumarase containing fraction (F_2). The chloroform/methanol (2:1), extract prepared from pooled F_2 fractions from several gradients, showed that about 37% of ^{14}C-5-HT and 52% of ^{14}C-histamine contained in this fraction were extracted into the organic solvent (table 2). Thin-layer chromatography of the chloroform phase showed that about 45% of the radioactivity of 5-HT and 52% of the radioactivity of histamine were located in a band shown to contain CS by chemical methods. When the CS-amine complexes were prepared by mixing their components *in vitro* and extracting them into the organic solvent, the complexes were almost completely dissociated during chromatography (fig. 2). Hydrolysis of the cerebroside sulfate-amine

Table 2
The density gradient distribution and the organic solvent extraction of ^{14}C-amines from the 5,500 *g* fraction of HC cells *.

Fraction	% ^{14}C-5-HT	RSC †	% ^{14}C-Histamine	RSC †	% of amine extracted into organic solvent	
					^{14}C-5-HT	^{14}C-Histamine
F_1	18.1 ± 1.9	0.69	13.5 ± 1.4	0.49	10.3 ± 2.7	18.4 ± 3.6
F_2	31.7 ± 1.7	1.38	36.5 ± 1.1	1.59	36.8 ± 2.0	51.6 ± 3.9
F_3	7.9 ± 2.3	0.49	6.9 ± 1.9	0.43	8.4 ± 0.3	10.1 ± 0.6
F_4	4.3 ± 1.0	0.29	4.4 ± 0.8	0.31	4.9 ± 0.4	9.3 ± 0.7
F_5	38.0 ± 1.5	1.82	38.7 ± 0.5	1.85	16.9 ± 3.9	24.1 ± 1.8

* Values represent the mean of 3 experiments (± standard error of the mean) expressed as percent distribution of the total or percent of amine extracted.
† Relative specific concentration (RSC) = % of ^{14}C-amines/% of total protein.

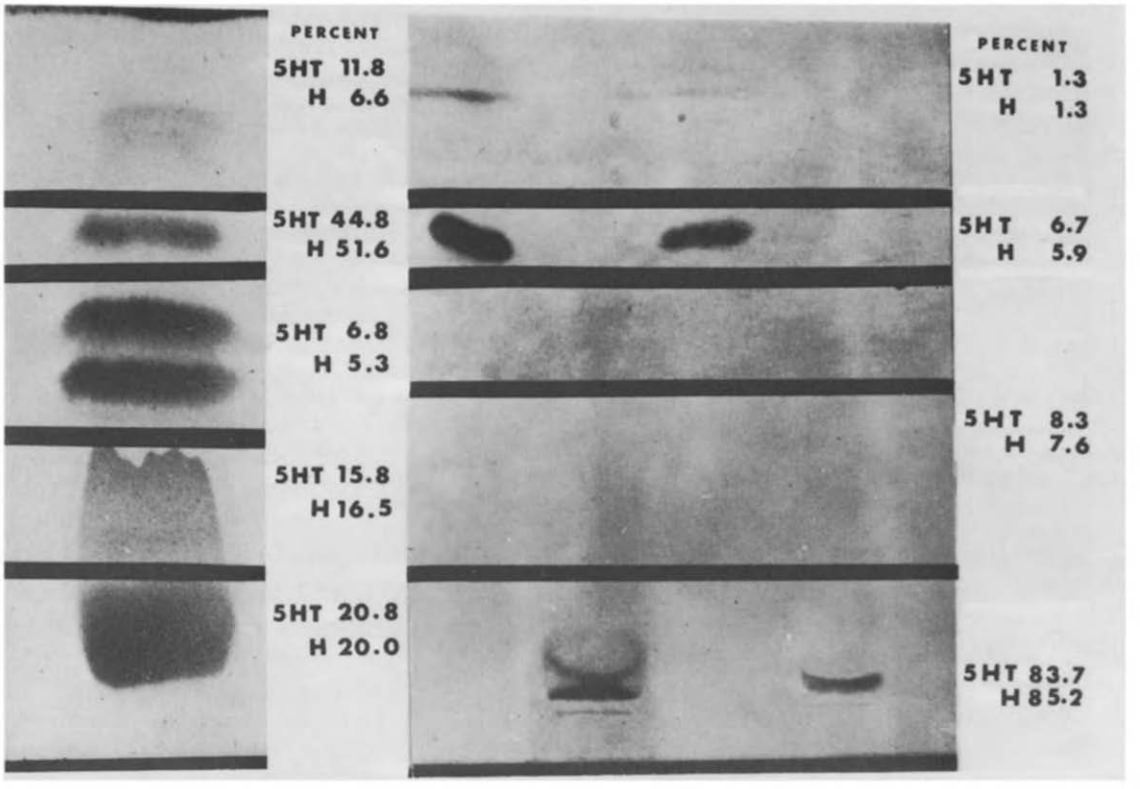

Fig. 2. Thin-layer chromatograms. The left-hand portion of the figure is a chromatogram of the total lipid extract prepared from gradient fraction F_2. A significant amount of radioactivity (44.8% of 5-HT and 51.6% of H) appeared in the cerebroside sulfate band. The right hand portion of the figure shows a chromatogram of the CS-amine complexes prepared *in vitro*. The complexes appear to be almost completely dissociated with 83.7% of the 5-HT and 85.2% of the histamine remaining at the origin. CS is the only spot on the plate, beyond the origin, and contains a small percentage of the radioactivity.

complexes, formed *in vivo*, with 0.1 N HCl and subsequent analysis, demonstrated that 85–87% of the ^{14}C-radioactivity of the complexes cochromatographed with standard histamine or 5-HT.

4. Discussion

We have shown that CS is located in a particulate fraction of mast cells that is separable from the multivesicular, amine-containing granules, and therefore this compound is probably not involved in the binding of biogenic amines in the mast cells storage granules. A significant portion of the exogenous amines that are taken up by mast cells, however, is incorporated into a cell fraction which has a high CS content and is rich in the mitochondrial enzyme fumarase. This fraction may correspond to the extragranular amine pool in tumor mast cells, reported by Green and Furano [18]. Thin-layer chromatography of the chloroform/methanol extract of this fraction demonstrated the presence of cerebroside sulfate-amine complexes differing from those formed *in vitro*, since the latter dissociate completely upon thin-layer chromatography. Cerebroside sulfate-amine complexes also occur in X-162 cells derived from the Dunn-Potter mastocytoma [19]. It should be noted that these lines of mast cells have no demonstrable monoamine oxidase activity [20, 21]. It is possible, therefore, that the formation of the lipid-amine complexes in these cell lines may represent an alternate amine metabolic step.

Acknowledgements

The authors are grateful to Miss Lynn Corrado for skilled technical assistance and to Dr. J.M.Ritchie (Yale University) for provision of animal care facilities. This work was supported by the University of Connecticut Research Foundation (30307-35-002).

References

[1] J.P.Green and J.D.Robinson, J. Biol. Chem. 235 (1960) 1621.
[2] K.Masek and H.Felsenfeld, Pharmacologist 11 (1969) 239.
[3] J.P.Green, J.D.Robinson, jr., and M.Day, J. Pharmacol. Exptl. Therap. 131 (1961) 12.
[4] R.W.Albers and G.J.Koval, Biochim. Biophys. Acta 60 (1962) 359.
[5] J.Furth, P.Hagen and E.L.Hirsch, Proc. Soc. Exptl. Biol. Med. 95 (1957) 824.
[6] E.P.Shore, A.Burkhalter and V.Cohn, jr., J. Pharmacol. Exptl. Therap. 127 (1959) 82.
[7] G.R.S. Carlini, G.A.Fisher and N.J.Giarman, J. Pharmacol. Exptl. Therap. 146 (1964) 74.
[8] D.F.Bogdanski, H.Weissbach and S.Udenfriend, J. Pharmacol. Exptl. Therap. 122 (1958) 182.
[9] E.Racker, Biochim. Biophys. Acta 4 (1950) 211.
[10] J.Folch, M.Lees and G.H.Sloane Stanley, J. Biol. Chem. 226 (1957) 497.
[11] G.Rouser, A.J.Bauman, G.Kritchevsky, D.Heller and J.D.O'Brien, J. Am. Oil Chemists Soc. 38 (1961) 544.
[12] N.S.Radin, F.B.Lavin and J.R.Brown, J. Biol. Chem. 217 (1955) 789.
[13] C.J.Lanter and E.G.Trams, J. Lipid Res. 3 (1962) 136.
[14] H.Wagner, L.Horhammer and P.Wolff, Biochem. J. 334 (1961) 175.
[15] H.Tanimukai, J. Chromatogr. 30 (1967) 155.
[16] G.Hunter, Can. J. Res. Sec. E. 27 (1949) 230.
[17] K.K.Reddi, E.Kodicek, Biochem. J. 53 (1953) 286.
[18] J.P.Green and A.V.Furano, Biochem. Pharmacol. 11 (1962) 1049.
[19] T.B.Dunn and M.Potter, J. National Cancer Inst. 18 (1957) 587.
[20] J.P.Green, European J. Pharmacol. 3 (1968) 68.
[21] K.Masek and H.Felsenfeld, unpublished observation.