

From the Department of Dermatology, Hiroshima University School of Medicine,
and the Department of Dermatology, Hiroshima Hospital of Japanese National
Railways, Hiroshima, Japan.

RELEASE OF CHYMOTRYPSIN-LIKE ESTERASE FROM ISOLATED RAT PERITONEAL MAST CELLS BY ARGININE-RICH CALF THYMUS HISTONE

By

SATOSHI DEKIO, HIROSHI ISHIHARA & TAKUSO YAMURA

Mammalian mast cells contain histamine and, in rat and mouse, also serotonin (1-3). Histamine is released from the mast cells, and dilates small blood vessels and enhances vascular permeability in some pathological reactions (4, 5). Serotonin is known to have the same biological significance as histamine (6). A group of substances of cellular origin which release histamine have been referred to as endogenous histamine liberators. In a previous report (7) it has been suggested that arginine-rich calf thymus histone is an important endogenous histamine liberator, which releases histamine from isolated rat peritoneal mast cells. The arginine-rich histone also releases serotonin from the mast cells. Mammalian mast cells contain not only histamine and, in some animal species, serotonin, but also some esterases (8). However, the role of the esterases in biological reactions remains almost unknown. Histochemical and biochemical techniques (9-12) have shown that rat mast cells contain a chymotrypsin-like esterase. As histamine and serotonin are released from the rat mast cells by arginine-rich calf thymus histone (7) it is possible that the chymotrypsin-like esterase is also released from the mast cells

by the arginine-rich histone. In the present study it is indicated that arginine-rich calf thymus histone releases chymotrypsin-like esterase from isolated rat peritoneal mast cells.

MATERIALS AND METHODS

Arginine-rich and lysine-rich calf thymus histone preparations extracted with HCl were kindly supplied by Prof. S. Osawa, Research Institute for Nuclear Medicine and Biology, Hiroshima University.

Preparation of isolated rat peritoneal mast cells was carried out according to the procedure of Archer (13), with slight modifications (7). Twenty ml of Tyrode solution was injected into the peritoneal cavity of a female Wistar rat weighing about 200 g. The abdominal wall was gently massaged, and the peritoneal fluid containing mast cells was withdrawn. The mast cells were washed with Tyrode solution, resuspended in the same solution at a concentration of 5.0×10^4 cells/ml and used as the mast cell suspension.

Esterase was released from the isolated mast cells by the same method as used for the release of histamine and serotonin (7, 13). One ml of Tyrode solution containing 100 $\mu\text{g/ml}$ of arginine-rich and lysine-rich calf thymus histone was mixed with 1 ml of the mast cell suspension, and the mixture incubated at 37°C for 10–60 min and centrifuged at 500 g for 10 min. The supernatant thus obtained was examined for its capacity to hydrolyse synthetic amino acid esters.

Hydrolyses of the synthetic amino acid esters, *p*-tosyl-L-arginine methyl ester (TAME) (Tokyo Kasei, Tokyo) and N-acetyl-L-tyrosine ethyl ester (ATEE) (Tokyo Kasei, Tokyo) were performed according to the modified method of Hestrin (14, 15), with further modifications. 0.6 ml of the sample, 0.9 ml of 0.1 M phosphate buffer (pH 7.4) and 0.15 ml of the same buffer containing TAME or ATEE (40 mM/ml) were mixed, and the mixture sterilized by passing it through a membrane filter (SM 113; Sartorius-Membranfilter, Göttingen). In some experiments a specific inhibitor of chymotrypsin, L-1-tosylamido-2-phenylethyl-chloromethyl ketone (TPCK) (Seikagaku Kogyo, Tokyo) was added to the ATEE solution at a concentration of 20 $\mu\text{g/ml}$ to inhibit a chymotrypsin-like esterase (16). The sterilized mixture was incubated under sterile conditions at 37°C for 14 h, as no hydrolytic activity was observed during a shorter incubation period. After incubation, 1.5 ml of alkali hydroxylamine composed of 1 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ and 1.75 M NaOH was added to 1.0 ml of the incubated mixture and left at room temperature for a further 20 min, after which 1.0 ml of 4 N HCl, 1.0 ml of 18 per cent trichloroacetic acid and 1.0 ml of 10 per cent FeCl_3 were added. The mixture was filtrated and the absorbance of the mixture at 530 nm was measured.

RESULTS

The Capacity of Supernatants from Incubated Mixtures of Arginine-Rich and Lysine-Rich Calf Thymus Histones and Isolated Rat Peritoneal Mast Cells to Hydrolyse TAME and ATEE

The arginine-rich and lysine-rich calf thymus histone solutions and the mast cell suspension were mixed and incubated at 37° C for 10 min, and the capacity of the resultant supernatant to hydrolyse TAME and ATEE was measured (Table 1). The supernatant from the incubated mixture of arginine-rich calf thymus histone solution and the mast cell suspension did not hydrolyse TAME, but hydrolysed ATEE. The supernatant from the mixture of lysine-rich calf thymus histone solution and the mast cell suspension hydrolysed neither TAME nor ATEE. The arginine-rich and lysine-rich histone solutions and the supernatant from the incubated mast cell suspension did not hydrolyse TAME and ATEE either.

TABLE 1

Hydrolytic Activities of Arginine-Rich and Lysine-Rich Calf Thymus Histones and Esterase Released from Rat Mast Cells.

Histones and mast cells	Hydrolytic activities					
	Experiment 1		Experiment 2		Experiment 3	
	TAME	ATEE	TAME	ATEE	TAME	ATEE
Arginine-rich calf thymus histone	0	0	0	0	0	0
Lysine-rich calf thymus histone	0	0	0	0	0	0
Mast cells	0	0	0	0	0	0
Arginine-rich calf thymus histone + mast cells	0	8.4	0	8.6	0	8.0
Lysine-rich calf thymus histone + mast cells	0	0	0	0	0	0
(—)	0	0	0	0	0	0

Hydrolytic activities are expressed as μmol of substrates hydrolysed per min by 50 μg of calf thymus histones or esterase preparations from 2.5×10^4 rat mast cells.

These results suggest that arginine-rich calf thymus histone released an esterase, which did not hydrolyse TAME but hydrolysed ATEE. Lysine-rich calf thymus histone did not release such an esterase from isolated rat peritoneal mast cells.

Release of Esterase, Which Hydrolysed ATEE, from Isolated Rat Peritoneal Mast Cells, by Arginine-Rich Calf Thymus Histone under Various Incubation Times

The arginine-rich calf thymus histone solution and the mast cell suspension were mixed and incubated at 37° C for 10, 20, 40 and 60 min. The capacity of the esterase released from the mast cells to the supernatant to hydrolyse ATEE was measured at the time intervals mentioned above (Table 2). The arginine-rich histone released the esterase within 10 min and, after that, the histone did not release the esterase from the mast cells. After 20–60 min the mast cells spontaneously began to release the esterase gradually.

Effect of TPCK on Hydrolysis of ATEE by Esterase Released from Isolated Rat Peritoneal Mast Cells

The arginine-rich calf thymus histone solution and the mast cell suspension were mixed and incubated at 37° C for 10 min. The effect of TPCK on the hydrolysis of ATEE by the esterase released from the mast cells to the supernatant was measured (Table 3). ATEE in the phosphate buffer *without* TPCK was hydrolysed by the released esterase as in Table 1, but ATEE in the buffer *with* TPCK was not hydrolysed by the esterase. TPCK is suggested to inhibit the hydrolysis of ATEE by the esterase released from the mast cells.

DISCUSSION

The data described above show that arginine-rich calf thymus histone releases an esterase which hydrolyses ATEE and is inhibited by TPCK, but does not hydrolyse TAME under the present experimental conditions. Lysine-rich calf thymus histone does not release such an esterase. As TAME is hydro-

TABLE 2

Hydrolytic Activities of Arginine-Rich Calf Thymus Histone and Esterase Released from Rat Mast cells After Incubation at Various Times.

Histone and mast cells (incubation time)	Capacity to hydrolyse ATEE		
	Experiment 1	Experiment 2	Experiment 3
Arginine-rich calf thymus histone			
10 min	0	0	0
20 min	0	0	0
40 min	0	0	0
60 min	0	0	0
Mast cells			
10 min	0	0	0
20 min	1.2	2.0	1.4
40 min	2.2	3.0	2.0
60 min	4.2	5.4	4.6
Arginine-rich calf thymus histone + mast cells			
10 min	8.2	8.4	7.6
20 min	8.0	8.0	7.8
40 min	7.6	8.0	8.0
60 min	7.6	8.2	8.0
(—)			
10 min	0	0	0
20 min	0	0	0
40 min	0	0	0
60 min	0	0	0

Hydrolytic activities are expressed as μmol of substrates hydrolysed per min by $50\text{ }\mu\text{g}$ of arginine-rich calf thymus histone or esterase preparations from 2.5×10^4 rat mast cells.

lysed by trypsin (17) and ATEE is hydrolysed by chymotrypsin (18), the released esterase is chymotrypsin-like. The inhibition by a specific chymotrypsin inhibitor, TPCK, also supports the idea that the released esterase is chymotrypsin-like. But, it is nevertheless unclear whether or not a trypsin-like esterase is released from the mast cells by arginine-rich and lysine-rich calf thymus histones; the possibility remains

TABLE 3

Effect of TPCK on Esterase Released from Rat Mast Cells.

Histone, mast cells and TPCK	Capacity to hydrolyse ATEE		
	Experiment 1	Experiment 2	Experiment 3
Arginine-rich calf thymus histone + mast cells	8.0	7.7	8.1
Arginine-rich calf thymus histone + mast cells + TPCK	0	0	0
TPCK	0	0	0
(—)	0	0	0

TPCK was added to ATEE solution at a concentration of 20 $\mu\text{g/ml}$. Hydrolytic activities are expressed as μmol of substrates hydrolysed per min by esterase preparations from 2.5×10^4 rat mast cells.

that the arginine-rich and lysine-rich histones form a complex with a trypsin-like esterase at the arginyl and lysyl sites of the histone molecules (19). Since there is little difference in the amino acid composition and protein structure of arginine-rich and lysine-rich histones in different species or organs (20), the chymotrypsin-like esterase might also be released from the mast cells even if histones of animal species or organs, other than calf thymus histone were studied.

It is a well-known fact that many mast cells are seen in tissues undergoing chronic destruction, such as chronically inflamed tissue and malignant tumours (21). But the role of the mast cells in these tissues is almost unknown. The data from the present experiments suggest a possible role for the mast cells. In the tissues undergoing chronic destruction, arginine-rich histone might be released from the nuclei of the destroyed cells. The histone acts on the mast cells and releases esterase(s), which digest the destroyed tissues. Histamine is also released from the mast cells by arginine-rich histone (7). Histamine dilates small blood vessels and enhances vascular permeability (4-5). The released histamine might enhance the transportation of the materials digested by the released esterase(s) from the destroyed tissues to the small blood

vessels. Serotonin is also released from the mast cells by arginine-rich histone (7), and it has the same biological role as histamine in some animal species (6).

SUMMARY

Release of chymotrypsin-like esterase from isolated rat peritoneal mast cells by arginine-rich and lysine-rich calf thymus histones was investigated. The arginine-rich histone released the esterase, but the lysine-rich histone did not release the esterase from the mast cells. The isolated mast cells spontaneously released the esterase gradually. The chymotrypsin-like esterase released from the mast cells is considered to digest the destructed material in tissues in a state of chronic destruction, such as chronically inflamed tissues and malignant tumours.

ACKNOWLEDGEMENT

The authors wish to thank Prof. S. Osawa, Research Institute for Nuclear Medicine and Biology, Hiroshima University, who kindly supplied us with arginine-rich and lysine-rich calf thymus histone preparations.

REFERENCES

1. Riley, J. F. & West, G. B. (1953): The presence of histamine in mast cells. *J. Physiol.* *120*, 528-537.
2. Benditt, E. P., Wong, R. L., Arase, M. & Poeper, E. (1955): 5-Hydroxytryptamine in mast cells. *Proc. Soc. exp. Biol. (N.Y.)* *90*, 303-304.
3. Sjoerdsma, A., Waakes, T. P. & Weissbach, H. (1957): Serotonin and histamine in mast cells. *Science* *125*, 1202-1203.
4. Lewis, T. & Grant, R. T. (1924): Vascular reactions of the skin injury. II. The liberation of a histamine-like substance in injured skin. *Heart* *11*, 209-265.
5. Calnan, C. D. (1964): Urticarial reactions. *Brit. med. J.* *ii*, 649.
6. Herxheimer, H. (1955): 5-Hydroxytryptamine shock in guinea pig. *J. Physiol.* *128*, 435-445.
7. Dekio, S., Kinoshita, M., Ishihara, H. & Yamura, T. (1974): Histamine and serotonin release from isolated rat peritoneal mast cells by arginine-rich nuclear proteins. *Hiroshima J. med. Sci.* *23*, 97-104.

8. Selye, H. (1965): The mast cells. pp. 53, 325-326. Butterworth, Washington.
9. Lagunoff, D. & Benditt, E. P. (1961): Histochemical examinations of chymotrypsin-like esterase. *Nature (Lond.)* 192, 1198-1199.
10. Lagunoff, D., Phillips, M. T., Iseri, O. A. & Benditt, E. P. (1964): Isolation and preliminary characterization of rat mast cell granules. *Lab. Invest.* 13, 1331-1334.
11. Budd, G. C., Darzynkiewicz, Z. & Barnard, E. A. (1967): Intracellular localization of specific proteases in rat mast cells. *Nature (Lond.)* 213, 1202-1203.
12. Kwaan, H. C. & Astrup, T. (1967): Demonstration of cellular fibrinolytic activity by the histochemical fibrin slide technique. *Lab. Invest.* 17, 140-145.
13. Archer, G. T. (1967): The release of histamine from the mast cells of the rat. *Aust. J. exp. Biol.* 37, 383-390.
14. Hestrin, S. (1949): The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *J. biol. Chem.* 180, 249-261.
15. Muramatsu, M., Onishi, T., Makino, S., Hayakumo, Y. & Fujii, S. (1965): Inhibition of tryptic activity by various synthetic inhibitors. *J. Biochem.* 58, 214-226.
16. Schoellmann, G. & Show, E. (1963): Direct evidence for the presence of histidine in the active center of chymotrypsin. *Biochemistry* 2, 252-255.
17. Schwert, G. W., Neurath, H., Kaufmann, S. & Snoke, J. E. (1948): The specific esterase activity of trypsin. *J. biol. Chem.* 172, 221-230.
18. Kaufmann, S., Schwert, G. W. & Neurath, H. (1948): The specific peptidase and esterase activities of chymotrypsin. *Arch. Biochem.* 17, 203-205.
19. Bergmann, M. & Fruton, J. S. (1941): The specificity of proteases. *Adv. Enzymol.* 1, 63-98.
20. Hnilica, L. S. (1967): Proteins of the cell nucleus. *Progr. Nucl. Acid Res. mol. Biol.* 7, 25-106.
21. Janes, J. & McDonald, J. R. (1948): Mast cells; their distribution in various human tissues. *Arch. Pathol.* 45, 622-634.

Authors' address:

Satoshi Dekio, M.D.
 Department of Dermatology
 Hiroshima Hospital of
 Japanese National Railways
 Futabanosato 3-1-36
 Hiroshima
 Japan

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.