Biochemical Pharmacology, Vol 26, pp 197–202 Pergamon Press, 1977 Printed in Great Britain

RELEASE OF SEROTONIN FROM MAST CELLS INDUCED BY *N*-(2-ETHYLHEXYL)-3-HYDROXYBUTYRAMIDE AND CATECHOLAMINE

ATSUSHI ICHIKAWA, HIDEO KANEKO, YURIKO MORI and KENKICHI TOMITA
Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto, Japan 606

(*Received* 13 *September* 1975; *accepted* 6 *July* 1976)

Abstract—A release of serotonin (5-HT), but not that of histamine, from rat peritoneal mast cells, mouse mastocytoma P-815 cells, and rabbit platelets induced by *N*-(2-ethylhexyl)-3-hydroxybutyramide (butoctamide) *in vitro* was synergistically augmented by epinephrine or norepinephrine. In the presence of 2.3 mM butoctamide and 270 μM epinephrine, the 5-HT release was optimal at pH 7.4 and 37°, and was suppressed by various enzymic and metabolic inhibitors. A viability test and microscopy indicated that, on exposure to butoctamide and epinephrine, normal mast cells were degranulated, but most of the cells still retained viability. In contrast, neoplastic mast cells showed more severe morphological alterations, and became non-viable.

Although the concentration of serotonin (5-HT) in rat and mouse mast cells is considerably lower than that of histamine [1–4], 5-HT is released, often simultaneously with histamine, from mast cells in response to various histamine liberators, such as compound 48/80 [1], polymyxin B [1], antigen (to sensitized cells) [2], nicotin [3], and heat stable factors in alkaline tissue extracts [5], or by a surface active agent like *n*-decylamine [1].

If both biogenic amines are bound similarly in the mast cell granules as proposed by Uvnäs *et al.* [4,6,7], these two amines are expected to be released in direct proportion to their relative concentrations in the granules. However, the per cent release of one amine induced by some agents under certain conditions is somewhat higher than that of the other [2,3]. Therefore, the search for a selective releaser for either amine might be of value in elucidating the overall releasing mechanism of these two amines.

In this report, we describe the stimulatory effect of *N*-(2-ethylhexyl)-3-hydroxybutyramide (butoctamide), which was reported to be an inducer of paradoxical sleep in cats [8], on the release of 5-HT from normal and neoplastic mast cells, and from platelets in the presence of catecholamines. Butoctamide is a synthetic analogue of 1-methylheptyl-γ-bromoacetoacetate, which was isolated from human cerebrospinal fluid by Yanagisawa and Yoshikawa [9], and also reported to induce paradoxical sleep in cats [10].

METHODS AND MATERIALS

Isolation and incubation of mast cells and platelets. Mast cells were collected from the peritoneal fluids of six or more normal Wistar rats (250–350 g) by a modification of the method of Chakravarty and Zeuthen [11]. Mixed peritoneal cells were suspended in the phosphate-buffered saline [PBS: 137 mM NaCl, 2.6 mM KCl, 8.1 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.9 mM $CaCl_2$, and 0.5 mM $MgCl_2$ (pH 7.4)] of Dulbecco and Vogt [12] containing heparin (300 μg/ml), and isolated, according to Thon and Uvnäs [13], by Ficoll density gradient (30 and 40% in PBS) centrifugation at 0–4°. The cells were washed three times with PBS, and resuspended in the same medium (2–5 $\times$ 10^5 cells/ml). The cell population consisted of 90 per cent or more viable mast cells.

Neoplastic mast cells (mouse mastocytoma line P-815) [14] were kindly supplied by Dr. M. Potter, National Cancer Institute, NIH, Bethesda, Maryland, and were maintained in the ascitic form in (BALB/c $\times$ DBA/2)F_1 hybrid mice. The cells were harvested in the ascitic fluids of the mice inoculated with 10^6 cells 1 week earlier. The cells were washed and resuspended in PBS as above (3–10 $\times$ 10^6 cells/ml).

Platelets were isolated from the blood of a heparinized rabbit by the method of Dillard *et al* [15]. The washed platelets were suspended in PBS as above (5 $\times$ 10^6 platelets/ml).

Aliquots (1 ml) of the mast cell or platelet suspension containing the compounds to be tested were placed in plastic (10 $\times$ 50 mm) or siliconized glass (9 $\times$ 10 mm) tubes, and incubated at 37° usually for 30 min with gentle shaking.

The reaction was terminated by cooling the incubation mixture to ice-cold. After centrifuging at 350 *g* for 3 min at 0–4°, histamine in the supernatant fluids was estimated by the method of Shore *et al.* [16], and 5-HT by that of Snyder *et al.* [17]. Lactic dehydrogenase (LDH) was assayed by measuring the decrease in absorbancy at 340 nm of NADH in the presence of pyruvate [18]. One unit of the enzyme activity catalyzed the oxidation of 1 nmole NADH/min at 25°.

The total contents of histamine and 5-HT, and the total activity of LDH in intact cells and platelets were estimated in the extracts of the cells or platelets which had been freeze-thawed at least three times.

The total LDH activity was much higher in neoplastic mast cells (395 units/10^6 cells) than in normal mast cells (21.2 units/10^6 cells). The values calculated from the data reported by Diamant and Glick [19] and by Ellis *et al.* [20] were 270 units/10^6 mouse mastocytoma cells and 53.5 units/10^6 rat mast cells.

To study the effect of pH, the cells were washed once with a 10 mM barbital buffer of desired pH (6.2 to 9.0) containing 0.15 M NaCl, 0.1 mM KCl and 1 mM $CaCl_2$, and resuspended in that buffer.

When the effects of inhibitors were examined, the cells were preincubated with inhibitor for 10 min at 37° prior to the addition of the amine releaser.

Cell viability. The nigrosin (0.2% in Hank's solution) staining test of Kaltenbach *et al.* [21] was used to determine the cell viability. In addition, the discharge of the cellular protein and of LDH into the medium during incubation was also estimated according to Diamant [22] and Ellis *et al.* [20]. Protein was determined colorimetrically by the method of Lowry *et al.* as described by Layne [23].

Materials. Butoctamide and its related *N*-(alkyl)-3-hydroxybutyramides [24], and alkylamines were kindly supplied by Dr. A. Sakuma, Research Laboratory of Lion Dentifrice Co. Ltd., Tokyo, Japan. Butoctamide derivatives were dissolved in a small volume of 0.25% Tween 80 in 0.9% NaCl and diluted properly with PBS.

Solutions of L-epinephrine-HCl and DL-norepinephrine (1 mg/ml) were obtained from Sankyo Pharmaceutical Co., Tokyo, Japan. Compound 48/80 was purchased from Burroughs Wellcome & Co., Tuckahoe, N.Y. Cyclic adenosine 3',5'-monophosphate (cyclic AMP) was obtained from Seishin Pharmaceutical Co., Tokyo, Japan. Other reagents were obtained commercially.

RESULTS

Effect of N-(*alkyl*)-3-*hydroxybutyramide and alkylamine on the release of histamine and serotonin from normal mast cells.* As shown in Table 1, of the various alkylamines tested, *n*-hexylamine showed some activity to release both histamine and 5-HT from normal mast cells, while its lower analogues showed only slight activities. The releasing activity was markedly increased with *n*-octyl- [25] and *n*-decyl- [1] amines.

In contrast, the activities of their corresponding 3-hydroxybutyramide derivatives were generally much lower than those of the alkylamines.

Of these analogues, the *N*-(2-ethylhexyl)-derivative (butoctamide) was unique in that its releasing activity for 5-HT, but not that for histamine, was tremendously augmented by epinephrine, resulting in the release of about 90 per cent of the total 5-HT. Butoctamide alone, like most other analogues, showed only a weak releasing activity for both amines.

The amine releases by these agents were accompanied with similar per cent discharges of LDH from the cells. However, the addition of epinephrine, which increased 4-fold the 5-HT release by butoctamide (19.8 → 89.7 per cent), did not significantly enhance the LDH discharge by this amine (18.1 → 22.9 per cent).

N-(*n*-decyl)-3-hydroxybutyramide was very effective in releasing 5-HT and LDH without added epinephrine, but its releasing activity for histamine was much lower than that for 5-HT or LDH. Furthermore, its histamine releasing activity was not significantly augmented by the catecholamine (39.6 → 45.7 per cent).

2-Ethylhexylamine, the amino moiety of butoctamide, also showed some releasing activity for both amines, but it was not augmented by epinephrine.

Epinephrine or norepinephrine had practically no effect on the basal release of either amine from mast cells. Histamine retained by the cells which had been exposed to butoctamide with epinephrine was almost completely liberated by a subsequent treatment with compound 48/80 (10 μg/ml) after washing the cells once with PBS.

Of two adrenergic blocking agents tested, phenoxybenzamine (POB) and dichloroisoproterenol (DCI), at a concentration of 700 μM, caused less than 10 per cent release of 5-HT from mast cells. POB did not affect the 5-HT release induced by 2.3 mM butoctamide with 270 μM epinephrine (82 → 78 per cent), while DCI slightly enhanced it (82 → 95 per cent). The

Table 1. Release of histamine and serotonin from normal rat mast cells by alkylamine or *N*-(alkyl)-3-hydroxybutyramide with or without epinephrine*

Alkylamine or *N*-(alkyl)-3-hydroxybutyramide	Per cent release					
	Histamine		Serotonin		Lactic dehydrogenase	
	Without epinephrine	With epinephrine	Without epinephrine	With epinephrine	Without epinephrine	With epinephrine
n-Butylamine	5.3 ± 0.1		4.9 ± 0.2			
n-Pentyl-	9.8 ± 0.4		5.9 ± 0.3			
n-Hexyl-	32.5 ± 0.5		30.3 ± 0.4		16.0 ± 0.8	16.2 ± 0.6
n-Heptyl-	40.3 ± 1.2		42.2 ± 1.5		25.1 ± 1.8	26.8 ± 0.3
2-Ethylhexyl-	45.0 ± 0.3	47.6 ± 0.5	42.9 ± 0.3	45.0 ± 0.5	42.5 ± 1.5	53.8 ± 3.2
n-Octyl-	85.3 ± 1.7		88.0 ± 1.9		63.2 ± 1.6	65.9 ± 1.5
n-Decyl-	92.5 ± 1.4		95.0 ± 1.0		75.2 ± 1.3	76.8 ± 1.5
None		5.8 ± 0.2		3.9 ± 0.2	3.2 ± 0.3	2.6 ± 0.2
N-(*n*-butyl)-3-hydroxybutyramide	8.4 ± 0.3	8.0 ± 0.2	2.7 ± 0.1	2.2 ± 0.2	6.8 ± 0.6	6.9 ± 0.5
N-(*n*-pentyl)	10.7 ± 0.3	10.9 ± 0.5	1.0 ± 0.1	3.6 ± 0.3	15.1 ± 0.9	16.8 ± 0.5
N-(2-methylpentyl)-	10.8 ± 0.4	11.5 ± 0.4	8.7 ± 0.3	8.5 ± 0.4		
N-(*n*-hexyl)-	17.9 ± 0.3	19.5 ± 0.4	23.5 ± 0.3	22.0 ± 0.4		
N-(2-ethylhexyl)-(butoctamide)	20.5 ± 0.3	25.2 ± 0.4	19.8 ± 0.3	89.7 ± 0.8	18.1 ± 0.3	22.9 ± 0.4
N-(1-methylheptyl)-	29.0 ± 0.4	34.0 ± 0.3	32.3 ± 0.2	36.7 ± 0.5	32.3 ± 1.2	45.3 ± 1.3
N-(*n*-decyl)-	39.6 ± 0.3	45.7 ± 0.6	90.7 ± 0.5	96.7 ± 0.5	78.2 ± 2.1	72.5 ± 1.4

* Rat peritoneal mast cells were incubated at 37° for 30 min in 1.0 ml of PBS containing 1 mM alkylamine or 2.3 mM *N*-(alkyl)-3-hydroxybutyramide (previously dissolved in 0.1 ml of 0.25% Tween 80 in 0.9% NaCl) and with or without 270 μM epinephrine. The values are the mean ± S. E. of three incubations with duplicate samples. The total content of amines was: 20.5 ± 0.42 μg histamine and 0.73 ± 0.12 μg 5-HT/10^6 cells. The total activity of lactic dehydrogenase was: 21.2 ± 0.5 units/10^6 cells.

Table 2. Release of histamine and serotonin from normal and neoplastic mast cells, and from platelets by butoctamide and catecholamine*

	Per cent release					
	Histamine		Serotonin		Lactic dehydrogenase	
Cell	Butoctamide	Butoctamide + epinephrine (norepinephrine)	Butoctamide	Butoctamide + epinephrine (norepinephrine)	Butoctamide	Butoctamide + epinephrine
Mast cells (rat)	20.5 ± 0.2	25.2 ± 0.3 (23.8 ± 0.6)	19.8 ± 0.2	89.7 ± 0.8 (80.3 ± 0.3)	11.5 ± 0.3	24.2 ± 1.5
Mastocytoma P-815 cells (mouse)	10.1 ± 0.1	12.8 ± 0.2 (10.9 ± 0.6)	20.7 ± 0.3	79.5 ± 0.5 (65.3 ± 0.8)	20.8 ± 2.5	45.3 ± 1.8
Platelets (rabbit)			40.5 ± 0.4	92.5 ± 0.5 (95.2 ± 0.2)	5.8 ± 0.2	29.2 ± 2.5

* Mase cells and platelets were incubated in the presence of 2.3 mM butoctamide and 270 μM epinephrine or norepinephrine at 37° for 30 min. The total content of histamine and 5-HT (μg/10^6 cells or platelets) was: 10.5 and 0.86 in mast cells; 0.21 and 0.12 in mastocytoma cells; and 80 (5-HT) in platelets. The total activity of LDH (units/10^6 cells or platelets) was: 28.5 in mast cells; 395 in mastocytoma cells; and 20.8 in platelets. The values are the mean ± S. E. of three incubations with duplicate samples.

basal release of 5-HT by butoctamide alone was also not affected by POB, and slightly elevated by DCI (20 → 37 per cent).

Effect of butoctamide and catecholamine on the release of amines from neoplastic mast cells and platelets. The augmenting effect of epinephrine or norepinephrine on 5-HT release induced by butoctamide was also observed with neoplastic mast cells and platelets (Table 2). Platelets seemed to be more sensitive than mast cells to the action of butoctamide with or without epinephrine. On the other hand, the per cent discharge of LDH was higher in mastocytoma cells (45 per cent) than in normal mast cells (24 per cent) or platelets (29 per cent) when exposed to butoctamide with epinephrine at 37° for 30 min.

Influence of the dose of butoctamide and epinephrine upon the release of 5-HT and histamine. In the presence of 270 μM epinephrine, the magnitude of 5-HT release from normal mast cells was dependent upon the concentration of butoctamide. A near maximal response (at 37° and pH 7.4, and for 30 min) was obtained with 2.3 mM butoctamide (Fig. 1A). Conversely, in the presence of 2.3 mM butoctamide, a near maximal release of 5-HT was obtained with 270 μM epinephrine, and its minimum effective dose was about 54 μM (Fig. 1B).

Under these optimal conditions for 5-HT release, the per cent release of histamine was much lower, and about $\frac{1}{4}$ to $\frac{1}{3}$ of that of 5-HT. Similarly, the per cent discharge of LDH was lower than that of 5-HT, and close to that of histamine.

The response of neoplastic mast cells to the amine-releasing action of butoctamide with epinephrine was similar to that of normal mast cells. However, the LDH discharge induced by these two agents was higher in neoplastic mast cells than in normal ones (40 per cent vs 20 per cent, 30 min).

Time course of 5-HT release. In contrast to the rapid amine release from mast cells induced by compound 48/80 [1,7,26–28] and antigen [29], 5-HT release by butoctamide with epinephrine proceeded rather slowly. At the optimal pH (7.4) and temperature (37°), the per cent release of 5-HT from both normal and neoplastic mast cells was linear for at least 30 min in the presence of 2.3 mM butoctamide and 270 μM epinephrine (Fig. 2).

Under these conditions, the discharge of LDH and protein from the cells was also linear for about

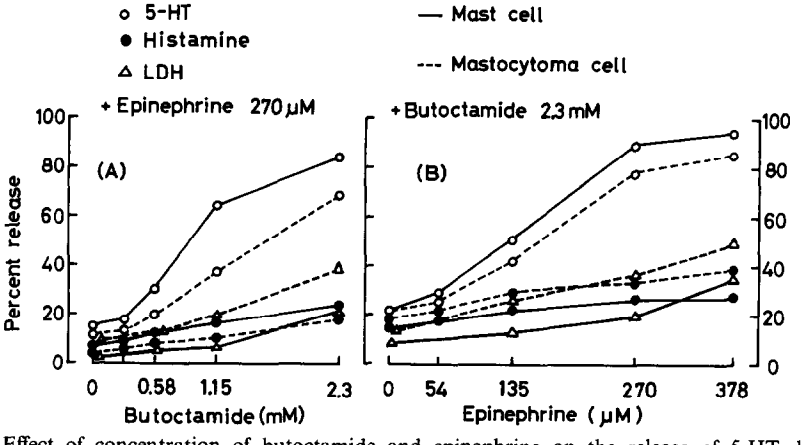

Fig. 1. Effect of concentration of butoctamide and epinephrine on the release of 5-HT, histamine and LDH from normal and neoplastic mast cells. Cells were incubated at pH 7.4 and 37° for 30 min. The total contents of histamine and 5-HT (μg/10^6 cells) were: 8.7 and 0.78 in mast cells, and 0.18 and 0.15 in mastocytoma cells. The total activity of LDH (units/10^6 cells) were: 25.2 in mast cells, and 375 in mastocytoma cells. Each point is the mean of percentual release of 5-HT (○), histamine (●), and LDH (△) in two experiments.

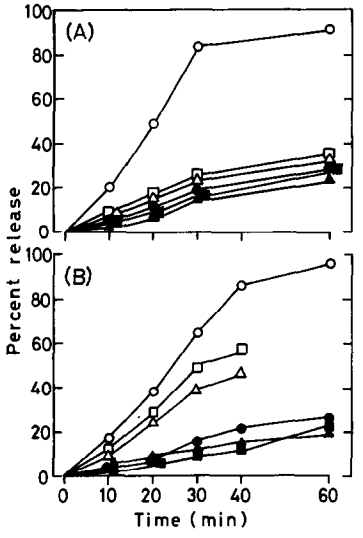

Fig. 2. Time course of the release of 5-HT, LDH and proteins from mast cells and mastocytoma P-815 cells induced by butoctamide and epinephrine. Normal rat mast cells (A) and mouse mastocytoma P-815 cells (B) were incubated at 37° and pH 7.4 for up to 60 min in the presence of 2.3 mM butoctamide with (open symbols) or without (closed symbols) 270 μM epinephrine. Key: ○, ● 5-HT; △, ▲ LDH; and □, ■ protein.

30 min. However, in contrast to neoplastic mast cells, the per cent discharge of both LDH and protein from normal mast cells was much lower than that of 5-HT and was close to that of histamine.

Effect of pH upon 5-HT release. As in the case of histamine release induced by compound 48/80 [1,30, 31], 5-HT release from both normal and neoplastic mast cells induced by butoctamide with or without epinephrine was optimal around pH 7.4. Figure 3 shows the results with neoplastic mast cells.

In this respect, the action of butoctamide with or without epinephrine was different from that of *n*-decylamine, a non-specific surface active agent. The amine-releasing action of *n*-decylamine with cell disruption was known to be weak in acidic media, and steadily elevated with increasing alkaline pH, without showing any distinct pH optimum [30,31].

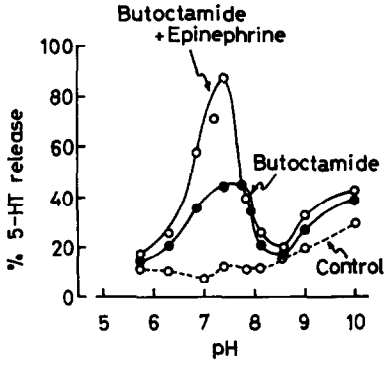

Fig. 3. Effect of pH on 5-HT release from mastocytoma P-815 cells induced by butoctamide and epinephrine. Neoplastic mast cells were incubated with 2.3 mM butoctamide with or without 270 μM epinephrine, or with the vehicle at 37° for 30 min in 10 mM barbital buffers (pH 6.2 to 9.0). To produce pH 5.8 or 10.0, 0.2 N HCl or NaOH was added to the medium.

Effect of temperature. The 5-HT release induced by butoctamide with epinephrine from both types of mast cells was enhanced with increasing temperature from 20° up to 40°, but it was abruptly decreased above 40°. Pre-incubation below 40° for 10 min did not inhibit 5-HT release by the subsequent incubation of the cells with butoctamide and epinephrine at 37°. However, pre-heating of the cells above 45° irreversibly inhibited the action of butoctamide and epinephrine. Figure 4 shows the results with mastocytoma P-815 cells.

Therefore, so far as the effect of temperature was concerned, the action of butoctamide with epinephrine was similar to that of compound 48/80 [1,26] or of antigen [30], but was different from that of *n*-decylamine, which was not affected by pre-heating the cells up to 50° [1,30].

Effect of inhibitors. The action of butoctamide with epinephrine was not Ca^{2+} dependent [30]. Omission of Ca^{2+} from the incubation medium or addition of 2 mM EDTA to the PBS- or barbital-medium did not affect 5-HT release from mast cells.

In contrast, the preincubation (10 min, 37°) of the cells with 5 mM Zn^{2+}, Cu^{2+} or Ni^{2+}, or 1 mM NaF resulted in a strong inhibition of 5-HT release by butoctamide with epinephrine (Table 3).

Zn^{2+} and Cu^{2+} are known to inhibit phospholipase A, which disrupts mast cells *in vitro* [30–32], and NaF is an inhibitor for phospholipase C, another lytic enzyme conceivably involved in the amine release [30].

The 5-HT releasing action of butoctamide with epinephrine was also suppressed by preincubation of the cells with 0.5 mM ninhydrin, an NH_2-blocking agent [26,30,31], or *N*-ethylmaleimide, an alkylating agent [31].

Preincubation with 1 mM 2,4-dinitrophenol or NaCN [30] was also inhibitory for 5-HT release, and this inhibition was not overcome by the addition of glucose to the incubation medium. However, anoxia [30] (N_2 atmosphere) did not affect the action of butoctamide with epinephrine.

In addition, cyclic AMP, a potent inhibitor of histamine release from mast cells induced by inorganic pyrophosphate or ATP (about 50 per cent inhibition

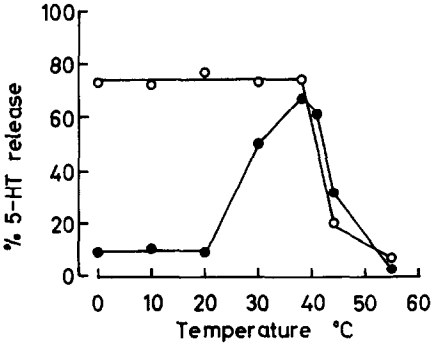

Fig. 4. Effect of temperature on 5-HT release from mast cells induced by butoctamide and epinephrine. Neoplastic mast cells were incubated at different temperatures for 30 min with 2.3 mM butoctamide and 270 μM epinephrine (●—●), or preincubated at different temperatures for 10 min prior to the incubation with the releasers at 37° (○—○).

Table 3. Effect of inhibitors on 5-HT release induced by butoctamide and epinephrine*

Inhibitors	Concen (mM)	Per cent release of 5-HT: Mast cell	Per cent release of 5-HT: Mastocytoma cell
None (butoctamide + epinephrine)		100	100
$Pb[NO_3]_2$	5.0	90	85
$ZnCl_2$	5.0	49	30
$CuCl_2$	5.0	13	15
$NiCl_2$	5.0	10	19
NaF	0.1	55	53
	1.0	32	43
Ninhydrin	0.5	60	79
N-ethylmaleimide	0.5	35	44
2,4-Dinitrophenol	1.0	40	40
NaCN	0.1	58	67
	1.0	40	38
Cyclic AMP	10.0	70	

* Cell suspensions (1.0 ml) were incubated with or without inhibitors for 10 min at 37°, and then for another 20 min after the addition of 2.3 mM butoctamide and 270 μM epinephrine. The values are the mean of two incubations.

by 0.1 to 1.0 mM cyclic AMP) [33], was less effective in suppressing 5-HT release by butoctamide and epinephrine (about 30 per cent inhibition after preincubation with 10 mM cyclic AMP). This moderate suppressive effect of cyclic AMP on 5-HT release by butoctamide with epinephrine was accompanied with the decrease in discharge of LDH (about 68 per cent inhibition). However, cyclic AMP did not suppress at all the release of 5-HT or LDH induced by *n*-decylamine.

Morphological alteration. Light microscopy revealed that the surface of normal mast cells became somewhat irregular when exposed to 2.3 mM butoctamide at 37°C for 30 min, but most of the cells were neither degranulated (Fig. 5b) nor stainable with nigrosin.

On exposure to 2.3 mM butoctamide and 270 μM epinephrine, many cells were swollen and degranulated (Fig. 5c). In spite of a nearly 90 per cent release of 5-HT under these conditions (Table 1), the per cent of cells stained with nigrosin was 30–40 per cent, roughly in accord with the 20 per cent discharge of LDH (Tables 1 and 2, and Fig. 2).

No detectable morphological changes were observed when the cells were incubated with epinephrine or with the vehicles.

In contrast, on exposure to butoctamide, mastocytoma cells were swollen (Fig. 5e), and about 50 per cent of them became stainable with nigrosin. When incubated with butoctamide and epinephrine, a considerable number of cells were disrupted (Fig. 5f). About 60–70 per cent of the cell population became stainable with nigrosin, roughly in parallel to the 40–45 per cent discharge of LDH (Table 2 and Fig. 2).

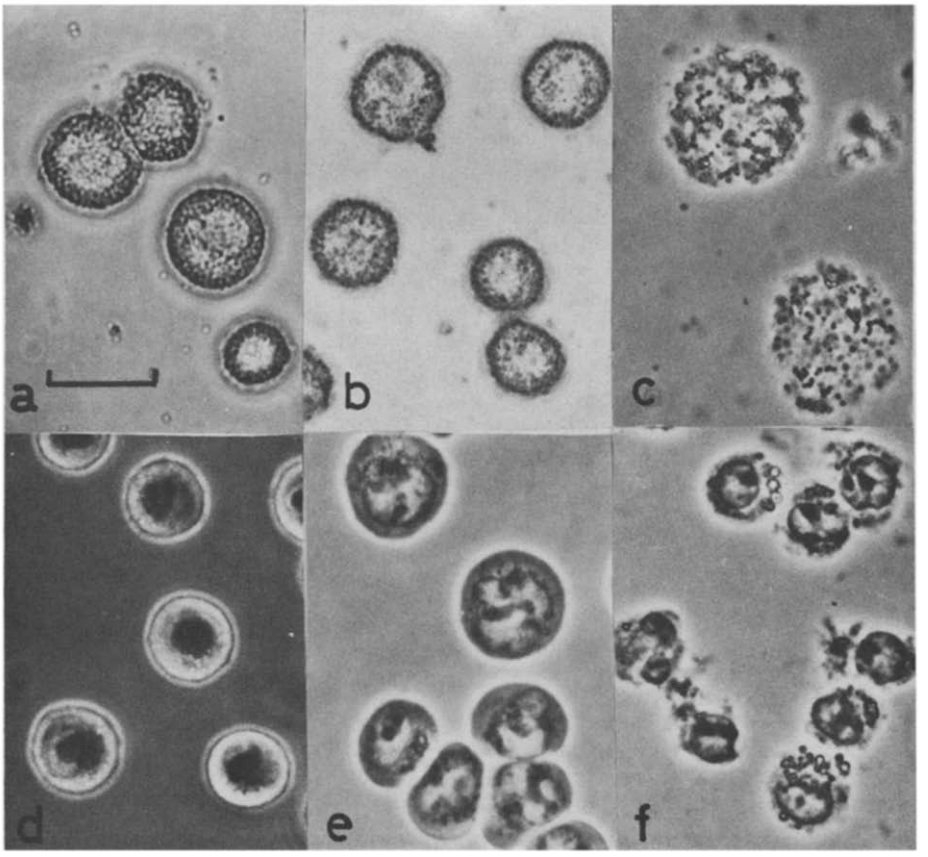

Fig. 5. Light micrographs of mast cells. (a) Normal, untreated rat peritoneal mast cells; (b) mast cells treated with 2.3 mM butoctamide; and (c) mast cells treated with 2.3 mM butoctamide and 270 μM epinephrine at 37° for 30 min. (d) Mouse mastocytoma P-815 cells, untreated; (e) treated with 2.3 mM butoctamide; and (f) treated with 2.3 mM butoctamide and 270 μM epinephrine at 37° for 30 min. The marker (1.94 cm) represents approximately 10 μm.

DISCUSSION

Our observations indicate that *N*-(2-ethylhexyl)-3-hydroxybutyramide (butoctamide), an inducer of paradoxical sleep in cats [8], acts as a releaser for 5-HT rather than for histamine from mast cells *in vitro* in the presence of epinephrine or norepinephrine.

Although butoctamide is a derivative of a C_8-alkylamine, the releasing action of this amide with epinephrine was much different, in several respects, from that of *n*-octylamine or *n*-decylamine, a surface active agent able to disrupt mast cells in a non-enzymatic way [30].

The activity of butoctamide with epinephrine was: (1) fairly specific for 5-HT release; (2) optimal around pH 7.4; (3) irreversibly inhibited by pre-heating the cells above 45°; and (4) suppressed by various enzymic and metabolic inhibitors (Table 3).

It is known that the disruptive amine release from mast cells by *n*-decylamine was steadily elevated with increasing alkaline pH, but showed no definite pH optimum [30,31]. Also its action was not inhibited by pre-heating the cells up to 50° [30], and practically not influenced by most of inhibitors of the typical amine releasers [30,31].

Specificity of butoctamide was further emphasized by the fact that epinephrine did not augment the 5-HT releasing action of *N*-(1-methylheptyl)-3-hydroxybutyramide, a closely related analogue of butoctamide (Table 1).

Epinephrine and norepinephrine are known to be not only ineffective in releasing histamine from mast cells, but also inhibitory to histamine release induced by compound 48/80 or antigen [34]. Therefore, the mechanism of the enhancing effect of catecholamine on 5-HT release induced by butoctamide is not clear yet.

As in the case of compound 48/80, a selective histamine-releasing agent [35], the release of 5-HT from mast cells induced by butoctamide with epinephrine coincided with degranulation (Fig. 5c), but it was also accompanied by some discharges of LDH and protein (Figs. 1 and 2).

About 20 per cent of both LDH and protein was discharged from normal mast cells for 30 min under the optimal conditions for 5-HT release (Fig. 2), and during this period 30–40 per cent of the cell population became non-viable as judged by the nigrosin staining test.

Since degranulation and histamine release induced by compound 48/80 (1 μg/ml) did not affect the basal discharge of LDH and the stainability with trypan blue of mast cells [20,35], our results indicated a certain loss of the integrity of cellular membrane on exposure to butoctamide with epinephrine.

So far as dose–response relationship, pH optimum, temperature sensitivity and effects of various inhibitors are concerned, responses of neoplastic mast cells to the 5-HT releasing action of butoctamide with epinephrine were similar to those of normal mast cells. However, on exposure to these two agents, neoplastic mast cells showed more severe morphological alterations (Figs. 5e and 5f) and gave higher discharges of LDH and protein than normal cells (Fig. 2).

Since our results indicated that 5-HT and histamine could be released selectively from mast cells under certain conditions, it seems to be necessary to know more about the detailed structure of granular matrix and cellular membrane with regard to the storage and discharge of the two amines.

REFERENCES

1. N. C. Moran, B. Uvnäs and B. Westerholm, *Acta physiol. scand.* **56**, 26 (1962).
2. A. Prouvost-Danon, J. M. Peixoto and M. Q. Javierre, *Life Sci.* **5**, 1867 (1966).
3. H. Felsenfeld and L. A. Corrado, *Biochem. Pharmac.* **22**, 2381 (1973).
4. A. Bergendorff and B. Uvnäs, *Acta physiol. scand.* **84**, 320 (1972).
5. N. J. Giarman, L. T. Potter and M. Day, *Experientia* **16**, 492 (1960).
6. C. H. Åborg, J. Novotny and B. Uvnäs, *Acta physiol. scand.* **69**, 276 (1967).
7. B. Uvnäs, *Fedn Proc.* **33**, 2172 (1974).
8. S. Torii, I. Yanagisawa, S. Inubushi and A. Sakuma, *Oyo Yakuri* (*Pharmacometrics*) **3**, 381 (1969).
9. I. Yanagisawa and H. Yoshikawa, *Biochim. biophys. Acta* **329**, 283 (1973).
10. S. Torii, K. Mitsumori, S. Inubushi and I. Yanagisawa, *Psychopharmacologia* **29**, 65 (1973).
11. N. Chakravarty and E. Zeuthen, *J. Cell Biol.* **25**, 113 (1965).
12. R. Dulbecco and M. Vogt, *J. exp. Med.* **99**, 167 (1954).
13. I. L. Thon and B. Uvnäs, *Acta physiol. scand.* **71**, 303 (1967).
14. T. B. Dunn and M. Potter, *J. natn. Cancer Inst.* **18**, 587 (1957).
15. G. H. L. Dillard, G. Brecher and E. P. Cronkite, *Proc. Soc. exp. Biol. Med.* **78**, 796 (1951).
16. P. A. Shore, A. Burkhalter and V. H. Cohn, Jr., *J. Pharmac. exp. Ther.* **127**, 182 (1959).
17. S. H. Snyder, J. Axelrod and M. Zweig, *Biochem. Pharmac.* **14**, 831 (1965).
18. H. U. Bergmeyer, in *Methods of Enzymatic Analysis*, Vol. 1, p. 481. Academic Press, New York (1974).
19. B. Diamant and D. Glick, *J. Histochem. Cytochem.* **15**, 695 (1967).
20. H. V. Ellis, III, A. R. Johnson and N. C. Moran, *J. Pharmac. exp. Ther.* **175**, 627 (1970).
21. J. P. Kaltenbach, M. H. Kaltenbach and W. B. Lyons, *Expl Cell Res.* **15**, 112 (1958).
22. B. Diamant, *Int. Archs Allergy appl. Immun.* **32**, 236 (1967).
23. E. Layne, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 3, p. 447. Academic Press, New York (1957).
24. A. Sakuma, H. Saga, R. Goshima, S. Inubushi, T. Uwabo, I. Yanagisawa and S. Torii, *Oyo Yakuri* (*Pharmacometrics*) **3**, 377 (1969).
25. R. E. Bray and P. P. VanArsdel, Jr., *Proc. Soc. exp. Biol. Med.* **106**, 255 (1961).
26. B. Uvnäs and I.-L. Thon, *Expl Cell Res.* **23**, 45 (1961).
27. P. Röhlich, P. Anderson and B. Uvnäs, *J. Cell Biol.* **51**, 465 (1971).
28. H. Yamasaki and S. Komoto, *Int. Archs Allergy appl. Immun.* **41**, 854 (1971).
29. P. Anderson, S. A. Slorach and B. Uvnäs, *Acta physiol. scand.* **88**, 359 (1973).
30. B. Högberg and B. Uvnäs, *Acta physiol. scand.* **48**, 133 (1960).
31. L. O. Boréus, *Acta physiol. scand.* **49**, 251 (1960).
32. C. Long and I. F. Penny, *Biochem. J.* **65**, 382 (1957).
33. A. Ichikawa, H. Hayashi, M. Minami and K. Tomita, *Biochem. Pharmac.* **21**, 317 (1972).
34. A. R. Johnson and N. C. Moran, *J. Pharmac. exp. Ther.* **175**, 632 (1970).
35. A. R. Johnson and N. C. Moran, *Fedn Proc.* **28**, 1716 (1969).