1 Acknowledgment. J.M.G.-G. was supported by a grant from the Comision Asesora de Investigacion Cientifica y Tecnica, Madrid, Spain.
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0014-4754/84/101149-03$1.50 + 0.20/0

Stimulation of histamine synthesis in rat mast cells by compound 48/80 in vitro

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Summary. The histamine content of rat peritoneal fluid cells is doubled within 20 min by 0.5 µg/ml of compound 48/80. Histamine catabolism inhibitors do not reproduce this effect; cells pre-incubated with α-fluoromethylhistidine are unresponsive to compound 48/80 which therefore activates pre-formed histidine decarboxylase rather than 'inducing' it. Non-mast cells showed no change after treatment with compound 48/80.
Key words. Rat mast cells; histamine synthesis; compound 48/80.

Previous work[1] has shown that rats given relatively high doses of adrenaline present a rapid rise of up to 50% in skin and lung histamine, attributed to the stimulation of histamine synthesis in tissue mast cells. Adrenaline has also been shown to enhance mast cell ability to act on substituted arginine or tyrosine esters[2] and to activate rat plasma kallikrein[3]. Since mast cells exposed to compound 48/80 presented similiar activities, the present work was designed to investigate whether, like adrenaline, compound 48/80 stimulates mast cells to synthesize histamine rapidly in vitro.

Materials and methods. Peritoneal fluid cells were harvested from Wistar rats and incubated in Krebs Ringer phosphate buffer at 37 °C under air as previously described[2]. Histamine was assayed fluorimetrically[4] omitting the purification procedure[5]. Mast cells were separated by centrifugation into 38% bovine serum albumin[6,2]. Compound 48/80, aminoguanidine, histamine diphosphate and o-phtaldialdehyde were obtained from Sigma Chem. Co., St. Louis, USA. Compound SK+F 91488 (S- 4-(dimethylaminobutyl) isothiourea) and α-fluoromethylhistidine were gifts from Dr M.E. Parsons; Smith, Kline and French, Welwyn Garden, Herts, U.K. and Dr J. Kollonitsch; Merck, Sharp and Dohme, West Point, Pa, USA, respectively.

Results. Table 1 shows that incubation of rat peritoneal fluid cells with compound 48/80 leads to an increase in both free and total histamine of the incubates; it is clear, therefore, that the percentual increase in the free form of the amine cannot, as is often stated, be solely due to the release of pre-formed, cell-bound histamine. Although presenting fairly pronounced variability from one animal to another, these findings were quite reproducible; they appeared somewhat as a surprise, in view of the lack of reference to similar observations in the numerous reports available on the effects of compound 48/80 on rat peritoneal fluid cells[7,8]. The data presented in figure 1 offer an explanation for this lack; they show that in contrast to histamine release, the build-up of histamine in compound 48/80-incubates is a rather sluggish process which only acquires momentum after 5–10 min, i.e. long after the period required for maximal histamine release has elapsed. Since most previous studies using compound 48/80 as a histamine releaser from rat peritoneal fluid cells employed incubation periods of less than 10 min, it is not difficult to understand why histamine enrichment of such incubates was overlooked.

It appeared to be important to investigate whether histamine accumulation due to compound 48/80 could be due to an arrest of the eventual destruction of the amine during prolonged incubation. Table 2 shows this to be improbable, firstly

Table 1. Increases in histamine in rat peritoneal fluid cell suspensions exposed to compound 48/80 in vitro

Treatment	Histamine (µg/flask) In medium (free H)	percent of total	In cells	Total
Buffer	0.12 ± 0.01	8.4 ± 1.1%	1.32 ± 0.29	1.44 ± 0.29
	|		|	|
Compound 48/80	$p < 0.01$		NS	$p < 0.01$
0.5 µg/ml,	|		|	|
10 min	2.26 ± 0.17	58.4 ± 5.7%	1.60 ± 0.21	3.86 ± 0.20

Following incubation cells were separated from incubation media by centrifugation, washed and recentrifuged; washings were added to the incubation medium. Results are averages of 5 experiments; $p <$ was determined by Student's t-test on paired samples.

Table 2. Lack of effect of aminoguanidine (AG) and compound SK + F 91488 on basal histamine and inhibition by α-fluoro methyl histidine (FMH) of increased histamine observed following compound 48/80 action on rat peritoneal fluid cells

Cell treatment[1]	Histamine (µg/flask)	Significance (vs untreated control)
Buffer	3.92 ± 0.59	
Buffer, 60 min	3.58 ± 0.32	
αFMH, 60 min	4.22 ± 1.44	$p > 0.20$
AG + SKF 91488	4.39 ± 0.72	$p > 0.20$
Buffer, 40 min; 48/80	6.10 ± 0.94	$p < 0.025$
αFMH, 20 min; 48/80	5.26 ± 1.00	$p > 0.10$
αFMH, 40 min; 48/80	3.32 ± 0.83	$p > 0.20$

[1] Except were otherwise stated, incubations were performed for 20 min at the following concentrations (µg/ml): AG, 50; compound SK F 91488, 100; αFMH, 50; compound 48/80, 0.5. Results are averages of 5 experiments.

Table 3. Mast cell origin of the increased levels of histamine observed in rat peritoneal fluid cell suspensions incubated with compound 48/80

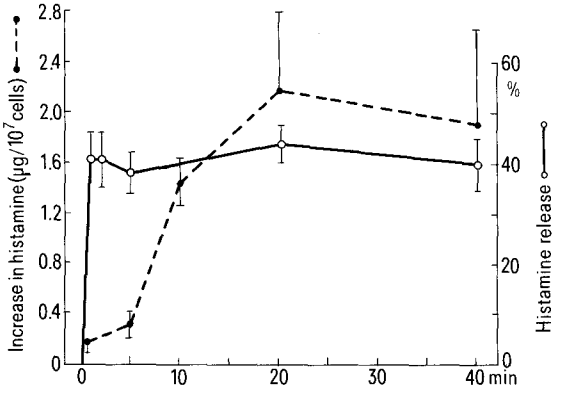
Fraction	Cell content ($\times 10^5$/ml) Mast	Other	Histamine (μg/flask) Controls	48/80 treated	
Mast cell enriched	1.58	0.67	0.55 ± 0.15	1.82 ± 0.58	$p < 0.01$
Non-mast cell	0.22	4055	0.14 ± 0.04	0.35 ± 0.11	$p > 0.10$

Peritoneal cells, fractionated using bovine serum albumin[6], were exposed for 5 min to 0.5 μg/ml of compound 48/80. Results are averages of 3 experiments. Cell counts were performed as previously described[2].

because total histamine did not change in cells incubated in buffer and secondly, because no increase in histamine similar to that evoked by compound 48/80 could be produced in incubates containing aminoguanidine, a potent inhibitor of oxidative deamination[8], plus compound SK+F 91488, a specific inhibitior of N-methyl histamine transferase[9]. In contrast, α-fluoromethylhistidine, a suicide-type, highly selective inhibitor of mammalian histidine decarboxylase[10] added to cell suspensions 40 min prior to compound 48/80, entirely blocked increases in total histamine. Alkylating, electrophilic, suicide-type inhibitors of amino acid decarboxylases present a characteristic lag period for their action[10]. This property was also evident in the present studies and helps to characterize the histamine-increasing effect of compound 48/80 as a result of the stimulation of histamine synthesis.

It has been shown that mast cells contain the histidine decarboxylase activity of rat peritoneal fluid cells[11]. A suitable fractionation[6] yields a fraction highly enriched in mast cells and another one containing few or no such cells, but practically all other cells present in the starting fluid[2]. Table 3 shows that compound 48/80 evokes a statistically significant increase in histamine in incubates containing mast cells but not in incubates containing the other cells of rat peritoneal fluid. Thus, enhanced histamine synthesis following exposure to compound 48/80 must be considered an expression of rat mast cell activity.

Discussion. Although it has been previously reported that following exposure to compound 48/80, rat peritoneal fluid cells acquire an increased ability to decarboxylate tracer amounts of histidine[12], this finding has rarely, if ever, been further mentioned in the literature. This omission may be due to the fact that the results of studies conducted at the tracer level do not reflect the extent to which observed changes in cell enzymes affect cell composition. The present findings, by showing that compound 48/80, at the concentration usually employed to effect substantial histamine release, stimulates rat peritoneal fluid mast cells to synthesize more than twice their resting content of histamine, may help to bring this phenomenon into proper focus. They contradict the rather firmly estblished con-



Histamine increase and histamine release evoked by 0.5 μg/ml compound 48/80 in rat peritoneal fluid cells in vitro. Results are averages of 5 experiments each performed with the cells of a different rat. Histamine increase represents the content in compound 48/80-treated, minus that in buffer-incubated cell suspensions. Histamine release represents the percentage of total histamine present in the incubation fluid.

cept[13, 14] that increased formation of histamine in animal tissues in the result of the 'induction' i.e., synthesis of histidine decarboxylase. The fact that in order to prevent histamine synthesis, α-FMH has to act on mast cells prior to compound 48/80, makes it clear that this inhibitor must be blocking pre-existing histidine decarboxylase, which it does in the gradual manner characteristic of suicide-type inhibitors[10].

It remains to be determined whether histamine synthesis occurring in compound 48/80-treated mast cells is consequent to, or coincident with cell degranulation and amine release, 2 well-known effects of the compound on such cells[7]. The first alternative would express a reparative change; it is improbable, because little of the histamine synthesized under the influence of compound 48/80 was retained by the cells (table 1). Related, earlier studies[1] using adrenaline as a stimulant for rapid histamine synthesis in the skin of intact rats, have shown that the newly-formed amine remains for less than 10 min in this organ, its loss coinciding with an evanescent rise of histamine in the circulation[15]. The possibility that rapidly synthesized and rapidly exported tissue mast cell histamine contributes towards immediate hypersensitivity responses to iatrogenic or immunogenic stimuli is worth considering. Observations showing that sensitized human leuckocytes present an increased histamine-forming capacity following exposure to antigen[16] and that normal human leucocytes do so in the presence of adrenaline[17], offer leads worth following in further research on this subject.

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