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DIFFERENTIAL RELEASE OF SEROTONIN WITHOUT COMPARABLE HISTAMINE UNDER DIVERSE CONDITIONS IN THE RAT MAST CELL*

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Abstract—Pretreatment of mast cells with various psychotropic agents was shown to permit preferential release of serotonin without substantial release of histamine or massive degranulation. Differential release involved both endogenous, granule-stored serotonin, and exogenous radiolabeled serotonin that had been taken up by the cell. This phenomenon occurred in mast cells stimulated to secrete with suboptimal concentrations of the classic mast cell secretagogue compound 48/80, was associated with drugs of several different structures and known mechanisms of action, and could be inhibited by certain prostaglandins. Furthermore, differential release of serotonin occurred in mast cells of retired breeders without the use of drugs or other exogenous agents. Light microscopic studies of mast cells undergoing differential release showed minimal degranulation, indicating that most of the serotonin release did not occur via classic exocytosis. The ability of mast cell to selectively release serotonin, by a mechanism unlike that occurring in allergic anaphylactic secretion, constitutes one of the first instances of differential release from secretory cells, suggests a new mechanism of release of secretory products, and expands the potential role of mast cells in the pathophysiology of the body.

Stimulus–secretion coupling leading to exocytosis dependent on calcium and metabolic energy is considered the established mechanism of secretion for most exocrine, endocrine and neural cells [1]. Mast cells, which have been used increasingly as a model secretory system [1, 2], contain hundreds of secretory granules that store a great variety of biologically active substances [3, 4]. Because of mast cell involvement in allergic diseases [5, 6] and its suspected role in other pathophysiological states [7], there has been great interest in the pharmacological modulation of its secretory activity [8, 9].

Two of the most common mediators stored in mast cell granules of several animal species are the vasoactive amines histamine and serotonin. Since histamine and serotonin may have divergent functions in delayed-type hypersensitivity, we proposed that these amines may undergo differential release from mast cells [10, 11]. We reported subsequently that the tricyclic antidepressant amitriptyline hydrochloride could inhibit histamine release from mast cells stimulated by compound 48/80 or IgE antibody and specific antigen, while permitting release of serotonin [12]. Under these conditions, exocytosis of secretory granules appears to be largely prevented, but serotonin is released by an unknown process still requiring calcium and metabolic energy [13]. Separate *in vivo* experiments have also revealed that mast cell stimulation with an antigen-specific factor from thymic derived lymphocytes (T cells) results in serotonin-dependent changes in delayed-type hypersensitivity with minimal degranulation of tissue mast cells and pharmacologic evidence of perferential release of serotonin compared to histamine [14, 15]. In the current study we have investigated the mechanism of differential release from mast cells activated *in vitro* with the non-specific secretagogue compound 48/80 in the presence of a variety of psychotropic drugs that permit differential release; we have also investigated the possibility that there may exist physiologic substances or situations that are capable of inducing or permitting a similar type of differential release.

METHODS AND RESULTS

Materials. The agents used in our experiments were purchased or obtained as follows: lithium chloride, pargyline hydrochloride and nialamide from Sigma, St. Louis, MO; lidocaine hydrochloride from Cistra, Worcester, MA; aminoguanidine hydrochloride from Aldrich, Milwaukee, WI; chlorpromazine hydrochloride from Ciba-Geigy, Summit, NJ; disodium cromoglycate (Cromolyn) from Fisons, Medford, MA; hydroxyzine hydrochloride from Pfizer, Groton, CT; amitripytline hydrochloride from Merck, Sharp & Dohme, Rahway, NJ, and from Siegfried-Ganes, Carlstadt, NJ; cyproheptadine hydrochloride monohydrate from Merck, Shape & Dohme; fluoxetine from Lilly, Indianapolis, IN; promethazine hydrochloride from Wyeth, Philadelphia, PA; PGE_1 from Sigma; PGE_2 and $PGF_{2\alpha}$ from PL Biochemicals, Milwaukee, WI.

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Various psychotropic drugs and differential release of serotonin from mast cells. Rat peritoneal mast cells were collected from 500 g Sprague–Dawley male rats (Charles River) in 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes)-buffered Locke's solution, pH 7, and were purified (90% purity) over Metrizamide [16]. They were then first preincubated with [^{3}H]serotonin (New England Nuclear) for 60 min at 37° to load them with this releasable amine [17]; after washing and resuspension in the same Locke's solution, all drugs were added at 4°, the cells were allowed to reach 37° (1 min), and they were further incubated for 5 min at 37° in a shaking water bath. Figure 1 shows that mast cells stimulated with

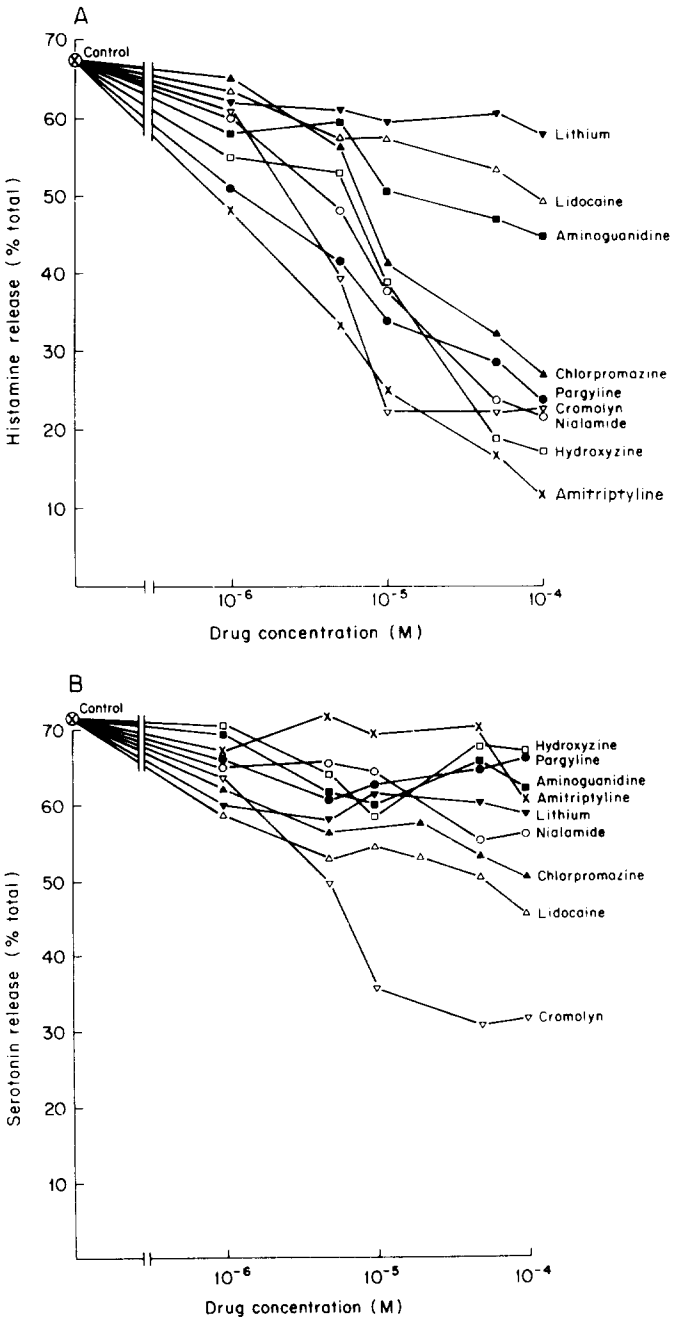

Fig. 1. Effects of antidepressant agents on 48/80-induced histamine (A) and serotonin (B) release from purified rat peritoneal mast cells. To measure mast cell secretion, approximately 10^5 purified mast cells/ml were incubated in each case, first with the antidepressant or other agent at the appropriate concentrations as indicated for 5 min and then with 48/80 (0.5 μg/ml) for 5 more min at 37°. To measure serotonin release, cells were preincubated with [^{3}H]serotonin as described under Methods and Results and were then incubated with the various agents as described. The serotonin released was measured by liquid scintillation counting in duplicate portions [17]. The histamine released was assayed fluorometrically in duplicate portions [18]. Similar dose–response curves were obtained in two other experiments.

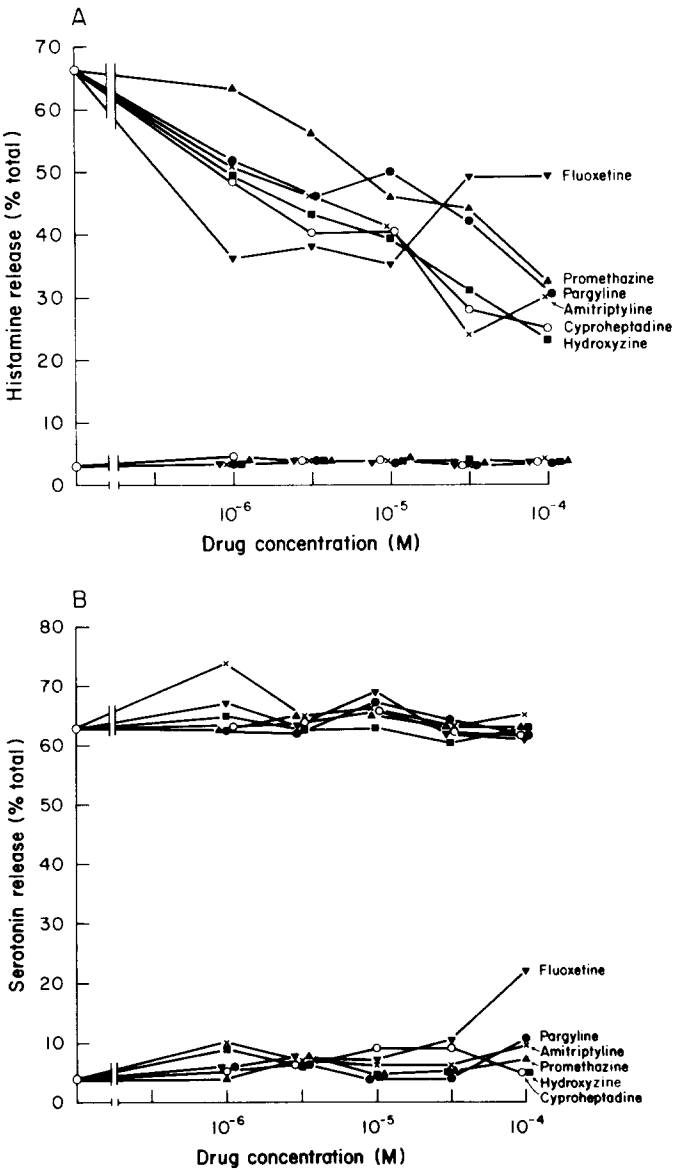

Fig. 2. Effects of antidepressant and other agents on 48/80-induced (upper) and basal (lower) release of histamine (A) and serotonin (B) from purified rat peritoneal mast cells. The conditions of the experiments were those described in the legend of Fig. 1. Results represent the mean of three separate experiments; S.D. bars have been deleted to simplify the already crowded figure.

the classic mast cell secretagogue compound 48/40 (0.5 μg/ml) released both histamine and [^{3}H]serotonin (control points), assayed fluorometrically [18] and with liquid scintillation counting respectively. Preincubation with pargyline hydrochloride, nialamide, hydroxyzine hydrochloride, or amitriptyline hydrochloride for 5 min at 37° caused inhibition of histamine release (Fig. 1A), but only a slight reduction in serotonin release (Fig. 1B). Of these drugs, the first two are monoamine oxidase inhibitors, hydroxyzine hydrochloride has antihistaminic and antipruritic properties as well as antidepressant properties, and amitriptyline hydrochloride is a tricyclic antidepressant. Aminoguanidine hydrochloride, a diamine oxidase inhibitor, had no significant effect on the secretion of either serotonin or histamine, nor did lithium chloride, the carbonate salt of which is used in manic-depressive and other syndromes (Fig. 1). The local anaesthetic agent lidocaine caused a modest reduction in the secretion of both serotonin and histamine, while the membrane-active antipsychotic agent chlorpromazine caused a partial reduction of histamine and to a smaller extent of serotonin secretion (Fig. 1). Finally, the anti-allergic compound disodium cromoglycate (cromolyn) caused marked inhibition of both serotonin and histamine secretion. These results made it fairly obvious that the ability to permit differential release is not shared indiscriminately by psychotropic agents, nor is it a property of drugs of common structure or function.

We repeated these studies with some of the drugs that permitted differential release and also examined their effect on basal secretion (Fig. 2 lower curves).

Table 1. Effect of amitriptyline on mast cell secretion*

	Secretion (% total)	
	Serotonin	Histamine
Control	2.9 ± 2.6	3.8 ± 2.5
Amitriptyline	11.2 ± 9.0†	7.9 ± 7.6
48/80	65.7 ± 12.3	59.1 ± 15.3
48/80 + Amitriptyline	63.2 ± 12.0	22.5 ± 12.0

* Mast cells (90% purity, 10^5 cells/ml) were first incubated in Hepes-buffered Locke's solution with 5 μCi/ml [^{3}H]serotonin (29.8 Ci/mmole, New England Nuclear) for 1 hr at 37° and were washed twice. They were then incubated (10^5 cells/tube) with 5×10^{-5} M amitriptyline for 5 min followed by an additional 5-min incubation with compound 48/80 (0.5 μg/ml). The histamine released was assayed fluorometrically while the serotonin released was measured by liquid scintillation counting, both in duplicate portions. Results represent the mean ± S.D. of fourteen separate experiments.

† $P < 0.05$ vs corresponding values for histamine.

In these experiments we also used the serotonin uptake inhibitor fluoxetine and the serotonin receptor antagonist cyproheptadine hydrochloride. None of the agents used had any measurable effect on basal histamine release and they only caused about 10% serotonin release at the highest concentration used (10^{-4} M). Fluoxetine induced about 20% serotonin release (Fig. 2B), but it also showed a paradoxical decrease in its ability to inhibit evoked histamine release (Fig. 2A). Cyproheptadine resembled amitriptyline in its ability to permit differential release of serotonin (Fig. 2). It therefore appeared that the ability to permit differential release was not directly related to the mechanism of serotonin uptake, although receptors binding serotonin could be involved.

Optimal conditions for demonstration of drug-induced differential release of serotonin. In some experiments, amitriptyline in high concentrations (5×10^{-5} M) caused some differential release of serotonin directly (Table 1), while still higher concentrations (10^{-4} M) induced release of both histamine and serotonin that was not cytotoxic. Lodoxamide, a water-soluble cromolyn-like drug, was also found to cause (5×10^{-5} M) some differential release of serotonin on its own (results not shown). The concentration of the mast cell activating agent was quite important in permitting the optimal expression of differential release. Amounts of 48/80 in excess of 0.5 μg/ml apparently induced secretion that was too massive for amitriptyline to preferentially inhibit histamine release (Table 2). Furthermore, if mast cells were kept in calcium- and magnesium-free solution before stimulation—at which point calcium ions were then introduced—the extent of secretion induced was apparently much stronger; as a result, less differential release occurred even with an amount of 48/40 (0.1 μg/ml) that was known to permit differential release when divalent cations were present throughout the incubation period (Table 2).

Effect of age or weight of rats on differential release of serotonin. The ability to permit differential release of serotonin appeared to be related to the age (or weight) of the animal and possibly to the extent of maturation and/or aging of the mast cells used. In an experiment designed to investigate this aspect, we used 225-g rats, which were received at six different dates, as well as retired breeders; at the time we used them, the rats ranged in weight from 225 to 650 g, the latter of which included the retired breeders. These results showed that differential release was best permitted in rats over 500 g (Fig. 3A). In addition, in mast cells from retired breeders basal serotonin release was much higher than in the rest of the rats (24% serotonin vs 1% histamine); this may be one instance where differential release was induced *without* treatment or pretreatment with any pharmacologic agent. Amitriptyline-induced serotonin release was also considerably higher (39 vs 1.6%). When these mast cells were stimulated with 48/40 alone (without any pretreatment with drugs such as amitriptyline), there was only slight differential release of serotonin (Fig. 3B).

Table 2. Effect of strength of agonist and calcium manipulation on differential release from purified mast cells*

		Release (% total)			
		Locke's solution only		Locke's solution plus amitriptyline	
Conditions	48/80 (μg/ml)	Serotonin	Histamine	Serotonin	Histamine
Locke's		5.9	4.0	7.3	6.0
Locke's	1.0	81.3	79.8	82.4	77.3
Locke's	0.5	53.4	51.6	65.1	22.6
Locke's	0.1	45.5	36.0	44.9	11.5
Ca^{2+}-free Locke's	0.1	64.8	60.9	69.3	40.2

* The conditions of this experiment are those described in Table 1. The concentration of compound 48/80 was varied as indicated and the sample labeled "Ca^{2+}-free" was kept in Ca^{2+}- and Mg^{2+}-free Locke's solution until the addition of the agonist (0.1 μg/ml 48/80) at which point the appropriate amount of $CaCl_2$ was also introduced to make the final concentration of Ca^{2+} that present in the Locke's solution used otherwise (1 mM). The amitriptyline was used at 5×10^{-5} M. The results represent total release (no basal release has been subtracted).

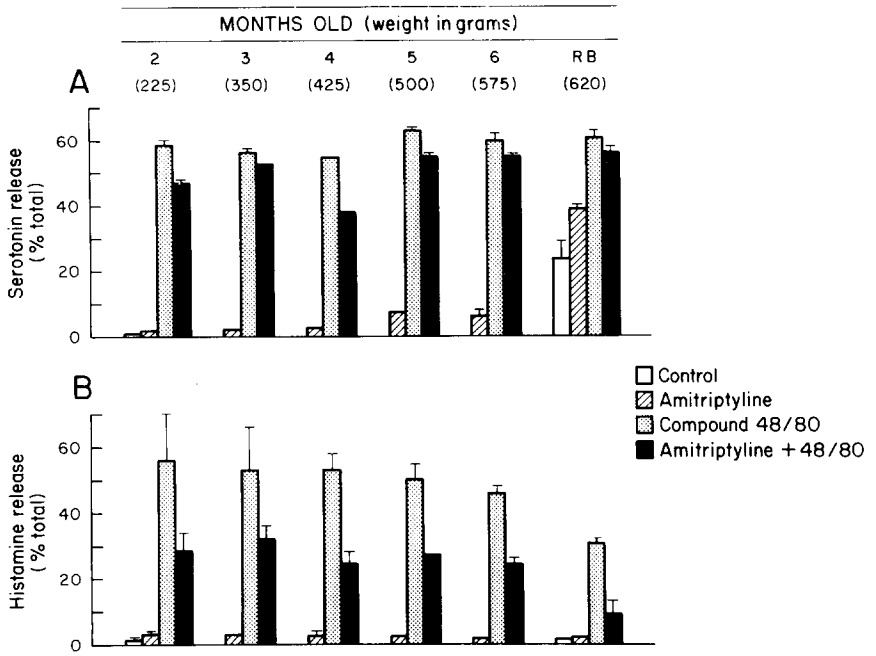

Fig. 3. Effect of age on mast cell release of serotonin (A) and histamine (B) from purified rat peritoneal mast cells. The conditions of these experiments are those described in Table 1. Male Sprague–Dawley rats were received (Charles River) at approximately 225 g (2 months old) and were then grown in the animal care facilities under routine care. The age of the animals is indicated at the top of the vertical bars with RB denoting retired breeders (received from Charles River at about 350 g); the number in parentheses underneath each age indicates the approximal weight of the rats in grams. The bar graphs represent the mean ± S.D. of two separate experiments.

Modulation of differential release of serotonin from mast cells. It was also of interest to investigate whether *differential release* of serotonin could be either induced or inhibited when it occurred by some physiologic substances. In these experiments, mast cells were preincubated with various prostaglandins at 10^{-5} M for 5 min, and were then exposed to amitriptyline (10^{-5} M) for 1 min before challenge with 48/40 (0.5 μg/ml) for an additional 5 min (Table 3). Prostaglandin E_1 (PGE_1) did not have any appreciable effect on the release of either histamine or serotonin, except for about 10% basal release of both. Prostaglandin E_2 (PGE_2) also increased the basal release (about 10%) of both serotonin and histamine, while $PGF_{2\alpha}$ had no effect on spontaneous release of either histamine or serotonin on its own. However, both PGE_2 and $PGF_{2\alpha}$ inhibited the 48/80-induced secretion *and* the differential release of serotonin (Table 3). It is of interest that leukotriene D_4 (LTD_4) at 10^{-7} M caused some differential release of serotonin (26.9 vs 2.9% histamine) on its own (results not shown), indicating that such a process may be operable under physiological conditions. Increasing the time of preincubation did not appear to have any effect on the ability of amitriptyline to cause differential inhibition of histamine release. When a similar, second dose of amitriptyline was added 25 min after the first dose and 5 min prior to stimulation with 48/80, the ability of amitriptyline to preferentially permit serotonin release was reduced by about 30% (Fig. 4). This finding may be interpreted as a limited form of tachyphylaxis, especially

Table 3. Effect of prostaglandins on regular and differential release of mast cell secretory products*

Drug (conc)	Secretion (% total)					
	Control (basal)		Regular (48/80)		Differential (amitriptyline + 48/80)	
	5-HT	Histamine	5-HT	Histamine	5-HT	Histamine
None	1.9 ± 0.5†	2.9 ± 0.3†	73.9 ± 0.2	63.4 ± 3.1	49.5 ± 2.2	13.5 ± 0.2
Amitriptyline (10^{-5} M)	6.8 ± 0.6	2.8 ± 0.3				
PGE_1 (10^{-5} M)	10.5 ± 0.3	13.1 ± 0.2	50.1 ± 1.8	51.3 ± 6.9	49.4 ± 2.8	10.0 ± 2.2
PGE_2 (10^{-5} M)	10.5 ± 1.3	12.0 ± 0.4	5.8 ± 0.3	6.8 ± 1.1	6.6 ± 0.1	6.2 ± 0.5
$PGF_{2\alpha}$ (10^{-5} M)	6.0 ± 0	7.4 ± 1.9	5.6 ± 0.6	7.0 ± 0.5	26.6 ± 2.0	16.3 ± 2.0

* Purified rat peritoneal mast cells were preincubated with the agent tested (at 10^{-5} M) for 5 min at 37° (control basal secretion), followed by 0.5 μg/ml 48/80 for another 5 min (regular release), or by 5×10^{-5} M amitriptyline for 1 min and then by 0.5 μg/ml 48/80 for 5 min (differential release). The conditions of these experiments were those described in Fig. 1. The results represent the mean ± S.D. of two separate experiments.

† These values which constitute basal release have not been subtracted from the rest of the values listed in this table.

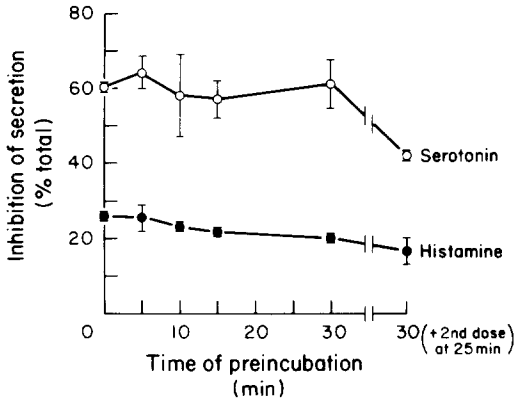

Fig. 4. Effects of length of preincubation and repeated application of amitriptyline on its ability to permit differential release of serotonin but not histamine from purified rat peritoneal mast cells. The conditions of these experiments were those described in Table 1, except for the fact that amitriptyline (5×10^{-5} M) was added to mast cells for a total length of preincubation as noted. The last point on the figure indicates that, following 25-min preincubation with 5×10^{-5} M amitriptyline, a second similar dose of amitriptyline was added (total concentration now 10^{-4} M) and preincubation was allowed to proceed for an additional 5 min at 37° (total preincubation was again 30 min). The results represent the mean ± S.D. of two separate experiments.

since continuous preincubation with 10^{-4} M amitriptyline for 30 min did not reduce the amount of serotonin released differentially (results not shown).

Differential release of endogenous serotonin from mast cells. Release of radiolabeled serotonin, incorporated into mast cell granules from the extracellular medium, has been used increasingly instead of histamine for quantitation of mast cell secretion [19–21]. Although this method has found wide acceptance because it has so far been shown to parallel the release of histamine, our results indicate that serotonin can be released in the absence of substantial histamine release and in the absence of demonstrable exocytosis of secretory granules. However, the possibility still remained that differential release may be due to some unknown ability of exogenous radiolabeled serotonin to bind to some location from which it could be displaced without exocytosis of secretory granules. Consequently, we measured endogenous (performed and granule-stored) serotonin, both in supernatant fractions and in cell pellets of mast cells undergoing differential release of serotonin, with the use of a high performance liquid chromatograph (HPLC) calibrated previously for serotonin [22]. In one of four similar experiments, where there was about 11% release of histamine and 72% release of [^{3}H]serotonin, there was approximately 67% release of endogenous serotonin (Fig. 5). These results indicate that stimulation of mast cells with 48/80 in the presence of amitriptyline resulted in a fairly similar release of both exogenous and endogenous serotonin.

Light microscopic observations on mast cells undergoing differential release. We had shown earlier that differential release depended on calcium and metabolic energy [13], but secretion by classical compound exocytosis did not appear likely since there was no concomitant histamine release. Exocytosis of secretory granules was investigated by selective staining with the cationic dye ruthenium red which, by virtue of its relative inability to cross the plasma membrane and its high affinity for the negatively charged proteoglycans of the secretory granule core, binds to and stains only those granules exposed to the extracellular fluid [23]. This technique showed that depression of histamine release by amitriptyline was accompanied by a substantial reduction in apparent granule exocytosis [13]. The morphology of mast cells treated with amitriptyline, with and without subsequent stimulation with compound 48/80, was also studied after staining thick sections of purified mast cell pellets with Richardson's stain. Thin (0.5 to 1.0 μm) sections of cell pellets containing purified mast cells treated with amitriptyline (5×10^{-5} M) for 5 min at 37° showed minimal degranulation, but with some swelling of individual granules and variability of granule staining. These cells were almost indistinguishable from samples treated with amitriptylene alone for 5 min prior to challenge with 48/80. In contrast, mast cells stimulated with 48/80 (0.5 μg/ml) for 5 min at 37°, without prior exposure to amitriptyline, showed extensive degranulation by compound exocytosis (Fig. 6).

DISCUSSION

Mast cells have often been considered a curiosity because of the plethora of biologically active molecules which, although stored in their secretory granules, do not appear to be released under physiologic conditions. Our present results indicate that mast cells may indeed be able to release certain of these molecules under conditions where overt degranulation would be minimal and, therefore, difficult to detect. In particular, differential release of serotonin was observed under the following conditions: (a) pretreatment of mast cells by certain psychotropic and other agents before induction of secretion; (b) stimulation by certain high concentrations of the psychotropic agent alone; (c) spontaneous differential release in retired breeders in the absence of exogenous agents; and (d) possibly, stimulation by leukotriene D_4. Some recent preliminary communications also reported that cyproheptadine [24] and hydroxyzine [25] inhibit histamine release and degranulation. However, these studies did not include measurements of serotonin release. The ability of some tricyclic antidepressant agents to inhibit basophil secretion of histamine has also been reported [26].

It appears possible to propose that secretory granule alterations induced by a variety of pharmacologic or physiologic conditions set the stage for differential release of mediators which occurs when an otherwise "usual" secretagogue triggers the mast cells. The fact that this differential release still requires calcium and energy [13] may imply the existence of transport *vesicles* which release their selective contents by exocytosis, or the binding of selective mediators by specific transport proteins; both processes would be expected to require calcium and

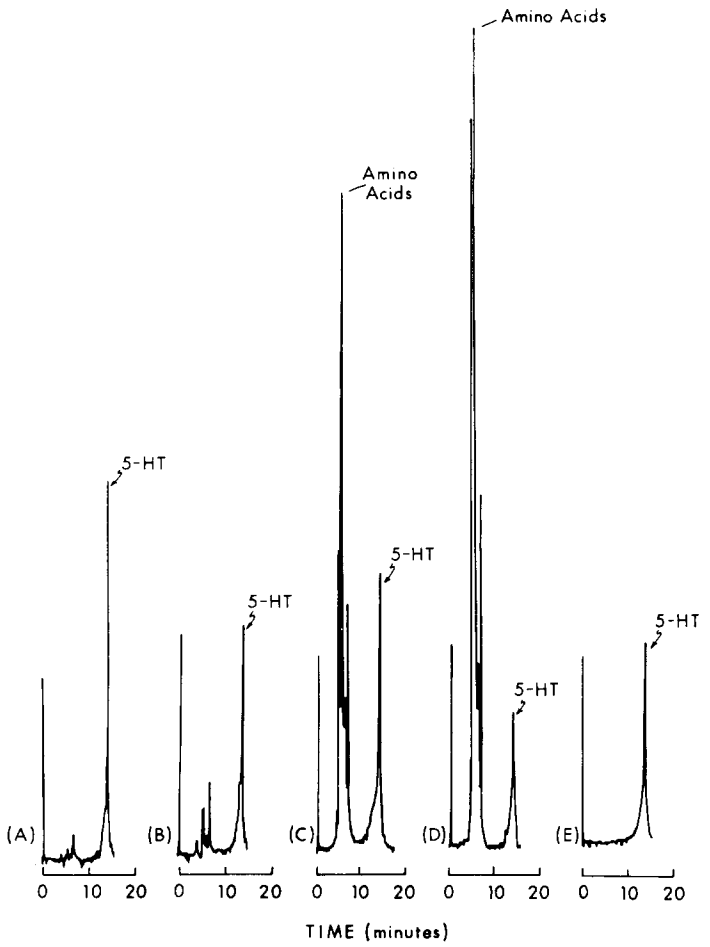

Fig. 5. Effect of amitriptyline on secretion of endogenous mast cell serotonin quantitated using high performance liquid chromatography (HPLC). Chromatographs were obtained sequentially of the following: (A) 5 nmoles serotonin (5-HT, oxalate salt, Sigma) assayed directly as control; (B) 2.5 nmoles 5-HT as above to calibrate the column; (C) supernatant fluid of approximately 3×10^5 purified mast cells preincubated with amitriptyline (10^{-4} M) for 5 min and then stimulated with compound 48/80 (0.5 μg/ml) for 5 more min at 37° followed by boiling for 5 min in 100 mM HCl to precipitate the protein fraction; (D) cell pellet of mast cells used and treated in (C) above; (E) 2.5 nmoles 5-HT as in (B) above. All samples were eluted from a Partisil-10 micron SCX cation exchange column (4 × 250 mm, Whatman) using a linear gradient of 0.01 M sodium acetate to 0.05 M sodium acetate in 1 M sodium nitrate, pH 4.6, at 55°, at a flow rate of 0.8 ml/min and at an initial pressure of 520 psi. Post-column detection of 5-HT was carried out fluorometrically using an *o*-phthaldialdehyde reagent [22].

energy to proceed. Indeed, careful electron microscopic evaluation of cytoplasmic vesicles in mast cells undergoing differential release has captured images of possible *vesicular* exocytosis and endocytosis [27]. Calcium may also be needed for amitriptyline, or any agent permitting differential release, to bind to the plasma or other membrane site. For instance, calcium is needed for the binding of disodium cromoglycate to a plasma membrane site on the mast cell [28].

Mast cell differential release of serotonin may have occurred under yet more conditions, but evidently went unnoticed [29–32]; furthermore, serotonin release was shown recently to be suppressed considerably less than that of beta-hexosaminidase when dexamethasone was used to inhibit secretion from mast cells [33]. There is also evidence that enzyme activity believed to be present in mast cell secretory granules may be "released" differentially: (a) kininase-like activity was measured in levels disproportionately higher than other molecules released during activation of basophils, and it was suggested that it may be released differentially [34]; (b) cathepsin-like activity could be measured *without* any histamine release or degranulation when mast cells were incubated with epinephrine [35]; moreover, (c) protease-like activity was released by intestinal mucosal mast cells without any evidence of degranulation when sensitized rats were challenged *in vivo* with an extract from *Nippostrongylus brasiliensis* worms [36]. Clearly, in these latter studies it is even harder to reconcile the ability of mast cells to permit measurements of enzyme activity *without* obvious degranulation since the secretory products involved are large *intragranular enzyme* proteins.

The possible existence of physiologic conditions

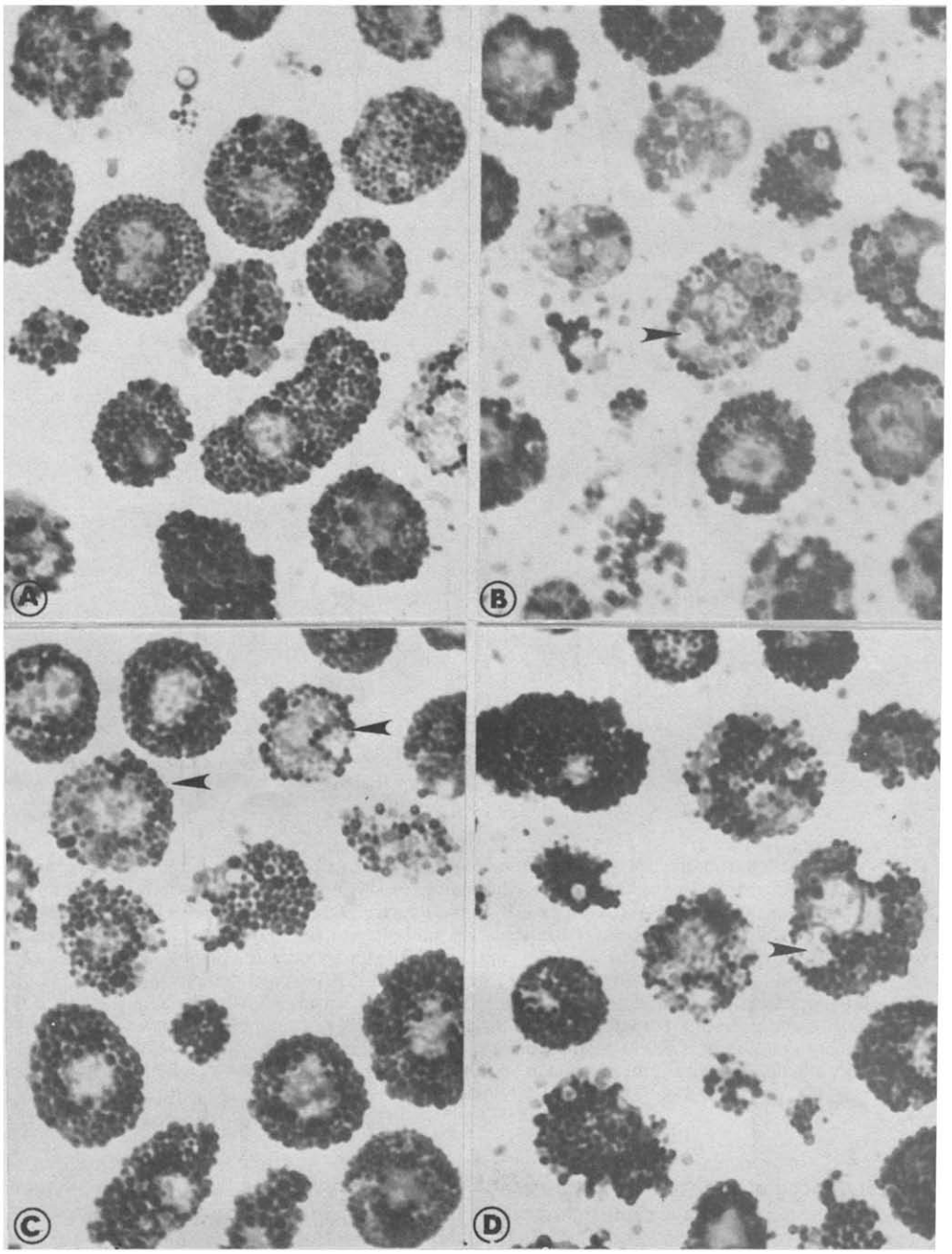

Fig. 6. Light micrographs of purified mast cells taken from one micron section of cell pellets embedded in epon and stained with Richardson's stain. Magnification, 4000. (A) Control, untreated mast cells showing granules of uniform size (approximately 1 micron in diameter) and staining characteristics. (B) Mast cells treated with 0.5 μg/ml compound 48/80 for 5 min at 37° prior to cell fixation. The granules have increased in size, there is more variation in staining, the granules are frequently released from the cells, and the cell cytoplasm contains large vacuoles (arrow). (C) Mast cells treated with 5×10^{-5} M amitriptyline for 5 min at 37° prior to fixation. Approximately half the cells show some swelling of granules and variability of granule staining (arrows). (D) Mast cells treated with 5×10^{-5} M amitriptyline for 5 min followed by 0.5 μg/ml 48/80 for 5 more min at 37° prior to cell fixation. Two-thirds of the cells show granule alterations in size and staining. Many of the cells show large cytoplasmic vacuoles (arrow).

which would either permit differential release of serotonin or could inhibit such release would be of particular importance because it could indicate the ability of the body to control selective release of secretory products. It was therefore of interest that PGE_2 and $PGF_{2\alpha}$ strongly inhibited both regular and differential release from mast cells, and that leukotriene D_4 appeared to permit differential basal release of serotonin. Such findings become relevant in view of the fact that prostaglandins and leukotrienes can be released from mast cells and other inflammatory cells [37–39], and that LTD_4 has been reported to inhibit mast cell histamine release [40]. It was also of interest that: (a) differential release of serotonin could be induced more readily in mast cells from older rats, and that (b) mast cells from retired breeder rats were able to release serotonin differentially *in vitro* without any drug pretreatment. Although there is no obvious explanation for this phenomenon, there is evidence that mast cells from retired breeder rats, in contrast to those from younger rats, *do not* support tumor angiogenesis: an implication for partially or totally defective release of secretory products [41]. The sizes of mast cells and of their secretory granules generally increase with the age of the rat [42] and their morphology indicates irregular to bizare shapes with increasing age [43]. Such changes may imply functional alterations leading to decreased or altered release of secretory products. Indeed, there is evidence that the largest mast cells in aged rats undergo only limited secretion by exocytosis in response to compound 48/80 [44] and that basophils from older patients show decreased ability to degranulate [45]. Furthermore, about 10% of any mast cell population appears to be refractory to secretion; such cells have been shown to be devoid of surface filapodia and were termed "prune-like" [46]. It could well be that, although these different populations of mast cells do not undergo secretory granule exocytosis, they are still capable of differential release of certain mediators.

The fact that mast cells undergoing differential release show only minimal degranulation may indicate either that their ability to fully respond to secretagogues has been altered or that different pathways of secretion may be employed. In this respect, secretory granule shape alterations and the appearance of vesicles, some of which contain electron-dense material, may imply the preferential utilization of a vasicular shuttle mechanism [27]. A similar mechanism has been described in the basophil [47] and may operate from either all or from specific secretory granules [48–50]. In our present experiments, one could argue that serotonin is preferentially "extracted" from granule core proteoglycans, to which it is attached electrostically, by high affinity serotonin binding proteins in the cytoplasm or in vesicular structures. We have actually reported the occurrence of specific serotonin-binding proteins in rat mast cells and basophil leukemia cells [51]. Their possible participation in differential release could help explain our finding that endogenous serotonin is secreted to the same extent as exogenous, radiolabeled serotonin.

It is noteworthy that a number of the agents shown here to permit differential release of serotonin bind to and affect the action of calmodulin. Calmodulin has been established as: (a) the calcium-specific regulator of cell function [52, 53], and has been proposed as, (b) the coupling factor of the two second messengers, calcium and cyclic AMP [54], (c) the intracytoplasmic receptor in endocrine cells [55], and (d) the calcium receptor specifically activating exocytosis [56]. With respect to mast cell secretion, which is commonly induced by cationic peptides and other molecules, it is of particular importance that cationic polypeptides have been shown to interact with calmodulin [57]. Moreover, it is of further interest that drugs which inhibit mast cell [56, 58] and basophil [59] secretion bind selectively to calmodulin. Such drugs inhibit even cation ionophore-induced secretion [56, 59], thus implying a step beyond activation of stimulus–secretion coupling [60].

Induction of mast cell secretion is linked to increased phosphate incorporation into three mast cell proteins [61, 62]; this incorporation is calcium dependent and can also be achieved in mast cell homogenates with the addition of calcium and calmodulin [63]. *Inhibition* of mast cell secretion by disodium cromoglycate, on the other hand, has been associated with the calcium-*independent* incorporation of radioactive phosphate into a fourth mast cell protein [64], an observation confirmed for other antiallergic compounds as well [65, 66]. It is of interest that differential release of serotonin is *not* accompanied with the phosphorylation patterns described above (results not shown). Alterations in calcium–calmodulin coupling and in phosphorylation of specific proteins could result in alternate routes of secretion permitting differential release of secretory products.

Differential release of mast cell mediators, possibly involving distinct biochemical and morphological routes, offers a functional solution to the numerous reports of secretory product co-storage which have challenged Dale's "one neuron-one neurotransmitter" principle [67, 68]. Furthermore, mast cell involvement in pathophysiological states in which release of mediators may proceed differentially [7, 14, 69] clearly expands the potential role of mast cells in health and in disease.

Addendum—While this paper was under review, our finding that amitriptyline selectively inhibits histamine but not serotonin release in response to 48/80 [12, 13] was confirmed and extended to release also induced by neurotensin, somatostatin and substance P [70].

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