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Parallel secretion of endogenous 5-hydroxytryptamine and histamine from mast cells stimulated by vasoactive peptides and compound 48/80

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The peptides, neurotensin, substance P, somatostatin, and bombesin, several analogues and fragments of neurotensin and compound 48/80, all caused the secretion of both endogenous 5-hydroxytryptamine (5-HT) and histamine. There was no differential effect of any of the secretagogues tested on the secretion of 5-HT and histamine. Amitriptyline prevented the secretion of histamine in response to stimulation by neurotensin, substance P, somatostatin or compound 48/80 but was without effect on the secretion of endogenous 5-HT.

Introduction Histamine and 5-hydroxytryptamine (5-HT) are two of several biologically active compounds secreted by rat mast cells (Bloom, 1974; Foreman, 1981). Both amines are thought to be stored in secretory granules and both have pronounced, although somewhat divergent, effects on the blood vasculature, many of which are associated with the reactions of allergy and inflammation (Douglas, 1975). Recently it has been shown that pretreatment of mast cells with the tricyclic antidepressant, amitriptyline inhibits the stimulated release of histamine but not that of 5-HT (Theoharides *et al*,, 1982). From this it was suggested that the peritoneal mast cell is able to secrete differentially histamine and 5-HT. Against this background we wondered whether stimulation by different secretagogues might result in the preferential secretion of either amine. Our results, which we describe here, fail to show selective release of 5-HT or histamine. We have, however, confirmed the selective inhibition of histamine release by amitriptyline.

Methods Peritoneal mast cells were obtained from Sprague Dawley rats of either sex (300–400 g) and purified (>80% purity) as previously described (Cochrane & Douglas, 1974; Carraway *et al.*, 1982). The cells were suspended in Locke solution (composition mM: NaCl 150, KCl 5, $CaCl_2$1, HEPES (N-2-hydroxymethylpiperazin-N'-ethanesulphonic acid) 10, pH 7.2–7.4; (+)-glucose 5.6 mM; bovine serum albumin 1 mg ml^{-1}) to a concentration of approximately 2×10^5 cells ml^{-1}. For Ca-free solutions, $CaCl_2$ was omitted from the Locke solution and replaced by EGTA 0.5 mM. In some experiments, mast cells were preincubated for 2 h at 37°C in Ca-free solution containing EGTA 1 mM (Cochrane & Douglas, 1974; Carraway *et al.*, 1982). For the experiments, 450 μl aliquots of the cells in Locke were preincubated for 10 min at 37°C, the releasing agent added in a volume of 50 μl and the incubation continued for 5 min. The supernatant and cell fractions were then separated by centrifugation (300 *g* for 5 min) and each extracted with 2% perchloric acid (final concentration). Amine secretion (supernatant amine) was expressed as a percentage of total amine (cell plus supernatant) present. Histamine was determined by fluorometric assay as previously described (Cochrane *et al.*, 1975). Endogenous 5-HT was measured by high performance liquid chromatography (h.p.l.c.) with electrochemical detection as described by Sperk (1982) using an oxidation potential of 0.6 V and an injection volume of 100 μl.

Neurotensin(NT), substance P (SP), somatostatin (SRIF), Met-enkephalin, bombesin, angiotensin I angiotensin II, capsaicin and compound 48/80 were obtained from Sigma Chemical Co. (St. Louis). The NT-fragments and analogues were prepared as described previously (Granier *et al.*, 1982).

The following compounds were tested (numbers apply to Figure 1b): (1) NT^{8-13}; (2) $AcNT^{8-13}$; (3) Lys^8-NT^{8-13}; (4) Lys^9NT^{8-13}; (5) Ac_2Lys^{8-13}; (6) $Ac_2Lys^9NT^{8-13}$; (7) $AcPhe^{11}NT^{8-13}$; (8) $Ala^{12}NT^{8-13}$; (9) $Ala^{13}NT^{8-13}$; (10) $AcNT^{7-13}$; (11) NT^{6-13}; (12) Ac_2NT^{6-13}.

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Results The addition of neurotensin (NT, <Glu - Leu - Tyr - Glu - Asn - Lys - Pro - Arg - Arg - Pro - Tyr - Ile - Leu), substance P (SP), somatostatin (SRIF), or compound 48/80 to isolated mast cells induced a prompt and dose-dependent secretion of endogenous 5-HT and histamine. All agents were more effective on histamine than 5-HT liberation, but for each secretagogue the slopes of the dose-response curves for the release of the two amines were parallel ($r => 0.90$; Figure 1a). Preincubation of the mast cells in Ca-free EGTA Locke, reduced the neurotensin-induced secretion of 5-HT to control values (control = 0.5 ± 0.2; 3 μM NT 1.5 ± 0.1; 3 μM NT + Ca-free conditions = 0.4 ± 0.1; x ± s.e. mean, $n = 3$). Likewise, preincubation of mast cells in glucose-free Locke containing 2-deoxyglucose (DOG, 100 μM) and antimycin A (1 μM) or preincubation of the cells in Locke containing disodium cromoglycate (DSCG, 100 μM) inhibited the release of 5-HT in response to NT (control cells = 0.3 ± 0.1; 3 μM NT = 1.5 ± 0.3; 3 μM NT antimycin A + DOG* = 0.6 ± 0.1; 3 μM NT + DSCG* = 0.2 ± 0.1; x ± s.e.mean, $n = 3$; *$P < 0.05$ vs NT). The secretion of histamine in response to all secretagogues tested was likewise calcium- and energy-dependent.

We also compared the effects of several analogues and fragments of NT on the secretion of endogenous 5-HT and histamine. As shown in Figure 1b, the abilities of the peptides to induce secretion differed considerably, the octapeptide, NT^{6-13}, being the most active. However, the relative effectiveness of each compound on the release of the two amines was similar. We also tested angiotensin I, angiotensin II, Met-enkephalin (each at 30 μM), and capsaicin (100 μM): each failed to stimulate the secretion of either 5-HT or histamine. Bombesin (30 μM) caused a twofold increase over the basal secretion of both amines.

In addition, we examined the effect of amitriptyline (10^{-5}M) on the stimulated secretion of endogenous 5-HT and histamine. Amitriptyline largely inhibited histamine secretion in response to NT (14.0% to 5.0%, $n = 2$), Substance P (40.0% to 10.0%, $n = 2$), somatostatin ($51.3 \pm 6.8\%$ to $18.3 \pm 3.4\%$, $n = 3$) and significantly ($P < 0.05$) reduced histamine release in response to compound 48/80 ($74.7 \pm 5.9\%$ to $51.0 \pm 3.4\%$, $n = 3$; all data x ± s.e.mean). In contrast, it had no effect on the secretion of endogenous 5-HT in response to NT, substance P, somatostatin, or compound 48/80. Basal secretion of either amine was not significantly affected by amitriptyline. Similar results were obtained when the secretion of exogenously accumulated [^{3}H]-5-HT was measured (not shown).

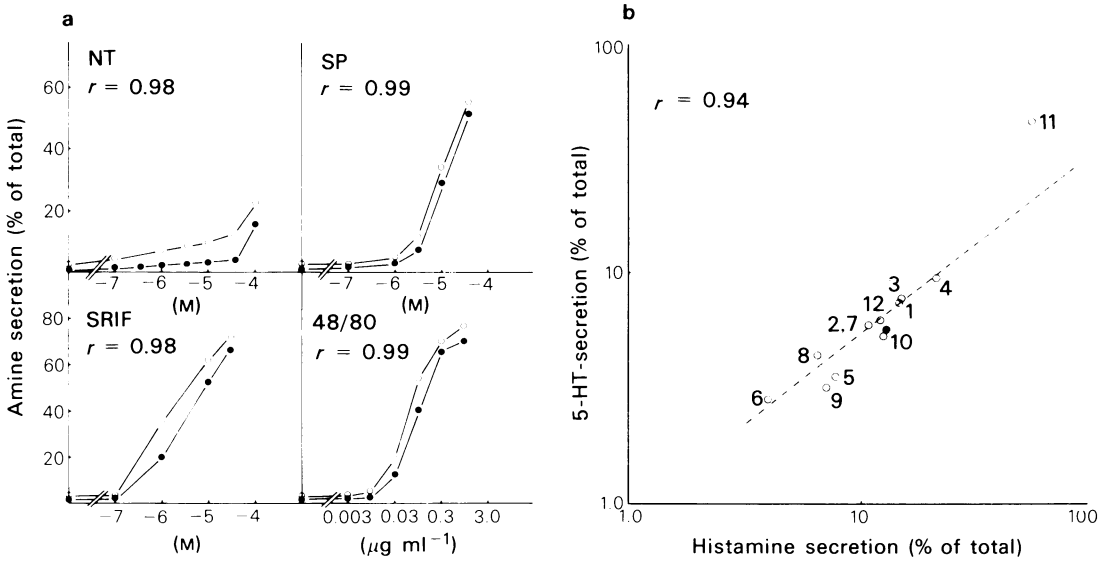

Figure 1 (a) Dose-response curves for the secretion of endogenous 5-hydroxytryptamine (5-HT, ●) and histamine (○) induced by neurotensin (NT), substance P (SP), somatostatin (SRIF), or compound 48/80. The points are the means of 3–5 experiments. For graphical reasons, s.e.mean are omitted but were always within 10%. (b) Correlation between secretion of endogenous 5-HT and histamine induced by NT (3 μM, ●) or one of twelve NT-sequence analogues or fragments (3 μM, ○). For identification of peptides see methods. All correlation coefficients were calculated using linear regression analysis.

Discussion Our results show that endogenous 5-HT and histamine are released in a parallel fashion when mast cells are stimulated by any of several non-immunological secretagogues. In each case, raising the concentration of secretagogue increased the secretion of both 5-HT and histamine with a correlation coefficient of > 0.9 (Figure 1a). Furthermore, the secretion of 5-HT elicited by any of twelve structural analogues of NT was closely correlated to the secretion of histamine ($r = 0.9$, Figure 1b). The higher percentage of histamine than 5-HT release by 48/80 and SP is in line with results published by Irman-Florianc & Erjavec (1983).

The incubation of the cells in media free of calcium, addition of metabolic inhibitors or disodium cromoglycate inhibited the stimulated secretion of both histamine and 5-HT. Moreover, those agents that failed to elicit histamine secretion, namely, angiotensin I and II, Met-enkephalin, and capsaicin, also failed to elicit the secretion of endogenous 5-HT. Taken together, our results suggest that the secretion of histamine and 5-HT are very closely coupled and may involve similar mechanisms.

We were able to confirm the observation of Theoharides *et al.*, (1982) that amitriptyline inhibits the release of histamine but not that of 5-HT in response to 48/80. In addition, we have shown that amitriptyline differentially affects the response to NT, substance P, and somatostatin in a similar manner. If differential release of 5-HT and histamine occurs physiologically, this may be brought about by as yet unkown secretagogues or there may be endogenous factors which are able to inhibit selectively the secretion of one amine and not the other.

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