Compound 48/80 and substance P induced release of histamine and serotonin from rat peritoneal mast cells

TATIANA IRMAN-FLORJANC and F. ERJAVEC
Department of Pharmacology, Medical Faculty, University of Ljubljana, Vrazov trg 2, 61000 Ljubljana, Yugoslavia

Abstract

The effect of substance P and compound 48/80 on histamine and serotonin release from not isolated and isolated mast cells have been compared in experiments in vitro. The response of not isolated and isolated mast cells were virtually identical. The release of both amines, in response to 48/80 and substance P, was dose-dependent. The percentage of histamine released by 48/80 was significantly higher than the percentage of serotonin, the difference being higher at lower concentrations of compound 48/80 after 15 min of incubation. Substance P also showed a tendency to higher efficiency for histamine than for serotonin release. In contrast to 48/80, the dose-response curves for histamine and serotonin release were parallel. These results support the view that the ratio between histamine and serotonin release depends on the liberator used. They also showed that this ratio can depend on the concentration of the agent inducing secretion. The results indicate that substance P as well as 48/80 act rather selectively as histamine liberators and that there is some difference in releasing properties of 48/80 and substance P.

Introduction

The presence of serotonin (5-HT) and histamine (Hi) stores have been demonstrated in mast cells of the rats [1, 2]. The data about the ratio between Hi and 5-HT content in rat mast cells vary, and it was found that the content of Hi as well as 5-HT in peritoneal mast cells of the rat depends on the age and the body weight of the animals [3, 4]. In response to the appropriate stimulus, mast cell can be activated to secrete both amines, Hi as well as 5-HT. It was reported that under the influence of certain agents greater amounts of one amine than the other can be released [5, 6].

In order to further evaluate Hi release in relation to 5-HT release, we studied the responses of rat peritoneal mast cells to substance P (SP) in comparison to compound 48/80. In our experiments the spontaneous loss of Hi and 5-HT, and the release of both amines from mast cells by 48/80 and SP were studied with cells in mixed peritoneal cell suspension and with isolated mast cells.

Materials and methods

Reagents

The extracting and incubation fluid used had the following composition: NaCl, 145 mM; KCl, 2.7 mM; CaCl2, 0.9 mM; 10% (v/v) Serensen phosphate buffer (Na2HPO4 and KH2PO4), 67 mM, (pH 7.2); glucose 0.1% and 0.05% bovine serum albumin (BSA). The buffered salt for resuspension and for washings was composed of NaCl, 145 mM, Tris-HCl buffer, 10 mM (pH 7.3) and 0.05% BSA.

Gum arabic stock solution prepared in deionized water had 1.090 specific gravity, pH was adjusted to 7.4 with saturated NaOH and NaCl was added to give a final concentration 0.2%. The lower layer was made by diluting 0.8 ml of the stock solution with 0.2 ml of 0.9% NaCl, and the upper layer by diluting 0.7 ml of the stock solution with 0.35 ml 0.9% NaCl.

Methods

Mast cells were obtained from male albino rats (280–320 g). After sacrifice, extracting fluid (10 ml/rat) was injected intraperitoneally. After 90 sec gentle massage of the abdomen, the fluid was withdrawn and collected in ice-cold polycarbonate tubes. In most experiments cells from 2–5 rats were pooled. The fluid was then centrifuged at 40 g for 3 min. The pellet was resuspended in fresh extracting fluid.

In other experiments, mast cells were isolated from the intraperitoneal fluid of rats by the gum arabic density gradient centrifugation procedure [7]. The collected fluid from the abdominal cavity was centrifuged at 40 g for 3 min. The cell pellet was dispersed in another buffered salt solution and layered over two layers of gum arabic solution. The samples were centrifuged at 650 for 10 min at 4°C. Mast cells, precipitated in the bottom of the tube, were washed 3 times with the buffered salt solution to be used for incubation. After the third washing and centrifugation at 100 g (2 min) mast cells were resuspended in the same volume of incubation medium as was the volume of peritoneal fluid obtained from the rats. Aliquots of 2 ml were then used for incubations.

Incubations were carried out for 15 min at 37°C without liberator, with 48/80 (0.05–0.5 μg/ml bath medium) or with SP (2–40 μg/ml bath medium). Some additional incubations were carried out for 30 min. At the end of incubation the secretion was stopped by placing the tubes in an ice-cold bath.

The supernatant and mast cells were diluted with 0.4 N perchloric acid and centrifuged. The supernatants were assayed for Hi [8] and 5-HT [9–11] on an Aminco-Bowman spectrofluorometer. For Hi determinations extraction procedure was omitted. Hi and 5-HT release were calculated as a percentage of the total content, corrected for spontaneous release in each case. Separate determinations or mean values of at least three determinations are shown in the figures. The release of Hi and 5-HT was compared by paired t-test for separate concentrations of 48/80 or SP.

Results

The mast cells, isolated on gum arabic, retained most of original Hi and 5-HT content. Recovery was 80–90%. Total Hi recovered during separation procedure was in most cases significantly higher than 5-HT.

Further loss of Hi and 5-HT occurred during incubation procedure. The amount of the spontaneous Hi or 5-HT release did not differ regardless whether not isolated or isolated mast cells were used. From both kinds of mast cells spontaneous Hi release was usually higher than spontaneous 5-HT release. In most experiment spontaneous Hi release was less than 5% and 5-HT release less than 4%.

Both agents used (48/80 and SP), were effective in inducing Hi and 5-HT release from isolated and not isolated rat mast cells. The release of Hi and 5-HT by 48/80 and SP from mast cells was dose-dependent. We observed considerable differences in the reactivity of mast cells from different pools of the cells obtained from different animals.

Compound 48/80 produced significant release of both
amines, yet it was more effective on Hi than 5-HT liberation (Fig. 1). The percentage of 5-HT released by 48/80 was less than that of Hi. The difference was significant by paired t-test ($p < 0.001$) at all concentrations of 48/80. The comparison of the differences between Hi and 5-HT release, at different concentrations of 48/80, showed that the ratio between amines released was not constant. At lower concentrations of 48/80 the release of Hi was higher than of 5-HT but the differences in the release of both amines were smaller at higher concentrations of the liberator (Fig. 2).

**Figure 1**
Histamine (Hi) and serotonin (5-HT) release (%) from not isolated rat mast cells by different concentrations of 48/80. The cells were incubated for 15 min at 37°C. The results represent average values from 3–5 experiments. All values are corrected for spontaneous release. The difference between Hi and 5-HT release was significant by paired t-test ($p < 0.001$) at all concentrations of 48/80.

**Figure 2**
Dose-response curves for the release of histamine (Hi) and serotonin (5-HT) from not isolated rat mast cells in response to 48/80. The cells were incubated for 15 min at 37°C. Each point represents the mean of 2–4 determinations from each individual experiment. The values are corrected for spontaneous release.

**Figure 3**
Histamine (Hi) and serotonin (5-HT) release (%) from not isolated rat mast cells by different concentrations of substance P (SP). The cells were incubated for 15 min at 37°C. The results represent average values from 4 experiments. All values are corrected for spontaneous release. The difference between Hi and 5-HT release was significant by paired t-test ($p < 0.001$) at all concentrations of 48/80.

**Figure 4**
Dose-response curves for the release of histamine (Hi) and serotonin (5-HT) from not isolated rat mast cells in response to substance P (SP). The cells were incubated for 15 min at 37°C. Each point represents the mean of 2–3 determinations from each individual experiment. The values are corrected for spontaneous release. The difference between Hi and 5-HT release was significant by paired t-test ($p < 0.001$) at all concentrations of SP.