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EFFECT OF PURIFICATION, THEOPHYLLINE AND SODIUM FLUORIDE ON HISTAMINE RELEASE PRODUCED BY ANTINEOPLASTIC DRUGS ON RAT MAST CELLS

A DISTINCTIVE MECHANISM OF ACTION FOR CARBOPLATIN

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Abstract—The antineoplastic drugs, cytarabine, ifosfamide, vinorelbine, doxorubicin, asparaginase and carboplatin, elicit histamine release from rat pleural and peritoneal mast cells. Both cell populations do not show heterogeneity in their response to stimulation by any of these drugs, with the exception of cytarabine, which activates pleural mast cells at lower concentrations. When these cells are purified with Percoll, L-asparaginase, vinorelbine and cytarabine completely lose their capability of inducing histamine release in both cell populations, while the other drugs still show the same pattern of response as with unpurified cells. These results indicate that some membrane component crucial for the action of vinorelbine, L-asparaginase and cytarabine is lost during the purification procedure. Pretreatment of the cells with theophylline completely inhibits the response to cytarabine, ifosfamide, vinorelbine and asparaginase, and inhibits the response to doxorubicin by up to 75% only. Theophylline does not change the response to carboplatin. Sodium fluoride does not change the response to any of the drugs tested, with the exception of carboplatin, in which case a complete inhibition is observed. In conclusion, carboplatin activates rat mast cells through a completely different mechanism of action with respect to the other drugs studied.

Rat mast cells, in response to many stimuli, release by exocytosis granules that contain several inflammatory mediators. These mediators, of which histamine is one of the more important, elicit the wide variety of symptoms associated with hypersensitivity reactions and inflammation [1]. There is a wide variety of drugs that release histamine from mast cells [2]. This activation is not mediated through receptors, since there is no structure–activity relationship, and the only feature common to all these drugs is their basic nature [3], such as compound 48/80, polymers of basic amino acids or neurotransmitters such as substance P. Signal transduction of these stimuli includes activation of different kinases through GTP binding proteins (G-proteins) [4] and rise in cytosolic Ca^{2+} concentrations [5]. So far, a mechanism for the mode of activation of these signaling pathways is not known, even though direct activation of G-proteins seems to be necessary for these stimuli to act [6, 7].

The occurrence of hypersensitivity reactions after the administration of cancer chemotherapeutic drugs has not received much attention, even though histamine release may be the cause of some of the adverse effects of these drugs. It is well known that L-asparaginase produces frequently hypersensitivity reactions due to the antigenic nature of this polypeptide [8]. Some authors have already reported the histamine-releasing activity of some antineoplastic drugs on rat mast cells [9–12]. In this paper, we report the histamine-releasing activity of some antineoplastic drugs not previously described as such and the effect that two major signal transduction systems, cAMP and G-proteins, exert on their action. We also report that some of these drugs require a cellular component that is lost during cell purification. Since pleural and peritoneal mast cells are pharmacologically different [13, 14], we studied each population separately.

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MATERIALS AND METHODS

Chemicals. Vinorelbine was obtained from Pierre Fabre (France), carboplatin from Almirall (Italy), ifosfamide from Funk (Germany), doxorubicin from Farmitalia (Italy), L-asparaginase from Bayer (Germany) and cytarabine from Upjohn (U.K.). Percoll® was from Pharmacia (Sweden). All other chemical were from the Sigma Chemical Co., (St Louis, MO, U.S.A.) except for orthophthalaldehyde which runs from Merck (Darmstadt, Germany).

Mast cell isolation. Mast cells were obtained by lavage of pleural and peritoneal cavities of Sprague–Dawley rats (200–400 g) as described previously [15]. Physiological saline composition was (mM): Na^+ (142.3), K^+ (5.94), Ca^{2+} (1), Mg^{2+} (1.2), Cl^- (126.1), CO_3^- (22.85), $PO_4H_2^-$ (1.2), SO_4^{2-} (1.2) giving a final osmotic pressure of 300 ± 5 mOsm/Kg H_2O. Bovine serum albumin (1 mg/mL) was added and the pH was adjusted to 7.0.

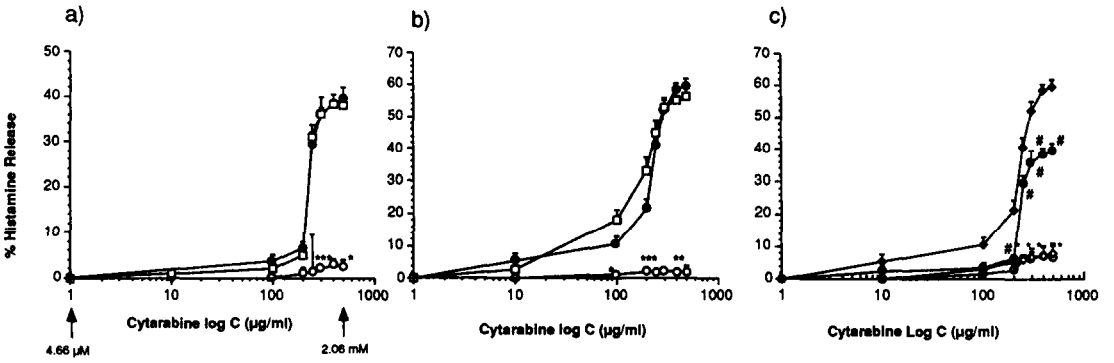

Fig. 1. Histamine release elicited by cytarabine in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. (c) Filled symbols represent unpurified cells; open symbols represent purified cells. Circles, peritoneal cells; romboids, pleural cells. Significant differences with respect to the controls are indicated by the * symbol. Significant differences between both control populations are indicated by the # symbols. Values are means ± SEM of four experiments.

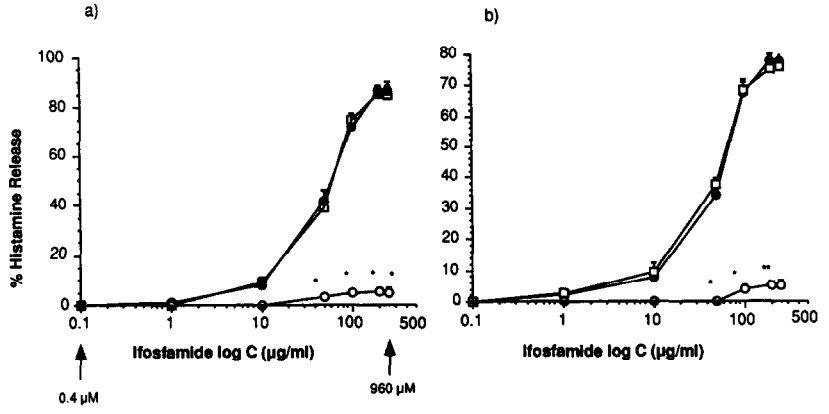

Fig. 2. Histamine release elicited by ifosfamide in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. Significantly differences with respect to the controls are indicated by the * symbol. Values are means ± SEM of four experiments.

The unpurified cellular suspension contained 4–8% mast cells, with an average of $1.5–2 \times 10^6$ mast cells/rat. Cell viability was studied by the trypan blue exclusion test [15] and was always higher than 97%. This method has been proven suitable for checking the cytotoxicity of a drug [16, 17].

Cell incubation. Twenty five microliters of a freshly prepared, concentrated solution of each drug were added to sufficient incubation medium to attain a final volume of 0.9 mL and preincubated. When the medium reached 37°, 100 µL of cell suspension, containing $1–1.5 \times 10^5$ mast cells, were added to each tube. Incubations were carried out in a bath at 37° for 10 min, and for 10 min more after addition of the stimulus.

Incubations were stopped by immersing the tubes in a cold bath. After centrifugation at 1000 g_{max} for 5 min the supernatants were collected and decanted into other tubes for histamine determination.

Appropriate controls to determine spontaneous histamine release with drug in the absence of any pretreatment were performed for every experiment.

Histamine release assay. Histamine was assayed spectrofluorometrically both in the pellet (residual histamine) and supernatants (released histamine) by Shore's method [18]. However, 0.1% orthophthaladehyde was employed in order to reduce the background while maintaining the sensitivity. For each drug, we studied the possible release of histamine caused by each excipient alone or combined as in the commercial preparation. The controls showed no histamine release elicited by the excipients. Also, the osmotic pressure generated by the combination of drugs plus excipients did not

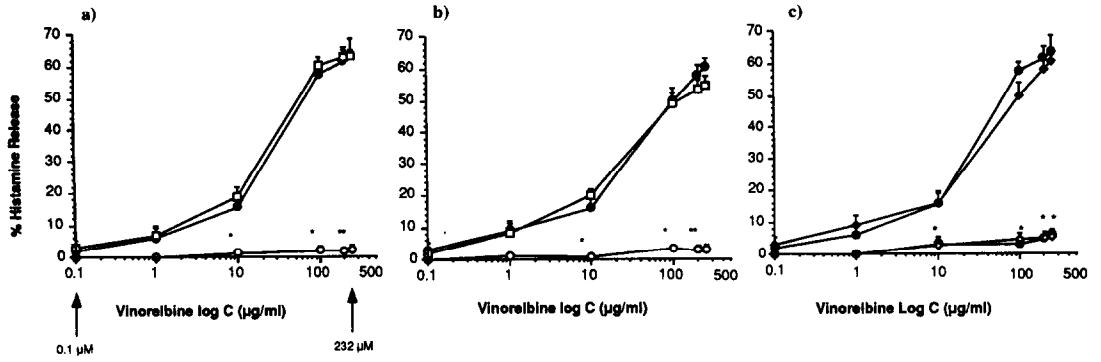

Fig. 3. Histamine release elicited by vinorelbine in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. (c) Filled symbols indicate unpurified cells; open symbols indicate purified cells. Circles, peritoneal cells; romboids, pleural cells. Significant differences with respect to the controls are indicated with the * symbol. Values are means ± SEM of four experiments.

elicit any release of histamine. Trichloroacetic acid was added (7%, final concentration) to prevent reaction because protein interferes with histamine assay. To ensure total histamine assay, pellets were sonicated for 60 sec in 0.8 mL of 0.1 N HCl. Results are expressed as percentage of histamine released with respect to the total histamine content.

Statistical analysis. Results were analysed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller was used for statistical significance. Results were expressed as means ± SEM.

RESULTS

We used the trypan blue test to check for a possible cytotoxic effect of all the drugs employed at all concentrations, and we did not find any cytotoxicity within the concentrations used. Figure 1 shows the dose–response profile obtained with treatment of rat mast cells with cytarabine. The cells from the peritoneal (Fig. 1a) and pleural (Fig. 1b) cavities released histamine with 200 and 100 μg/mL of the drug, respectively. The maximum responses were 39 ± 3% and 59.5 ± 2.5% for peritoneal and pleural cells, respectively. Pretreatment with 10 mM sodium fluoride did not change the response significantly, while pretreatment with 10 mM theophylline completely inhibited the response of both populations. Purification completely oblated the response of both populations (Fig. 1c).

Figure 2 shows the response induced by ifosfamide. There was no quantitative difference between peritoneal (Fig. 2a) or pleural (Fig. 2b) cells, and pretreatment with sodium fluoride did not change the profile of the dose–response curve. As in the case of cytarabine, theophylline completely inhibited the response of both pleural and peritoneal mast

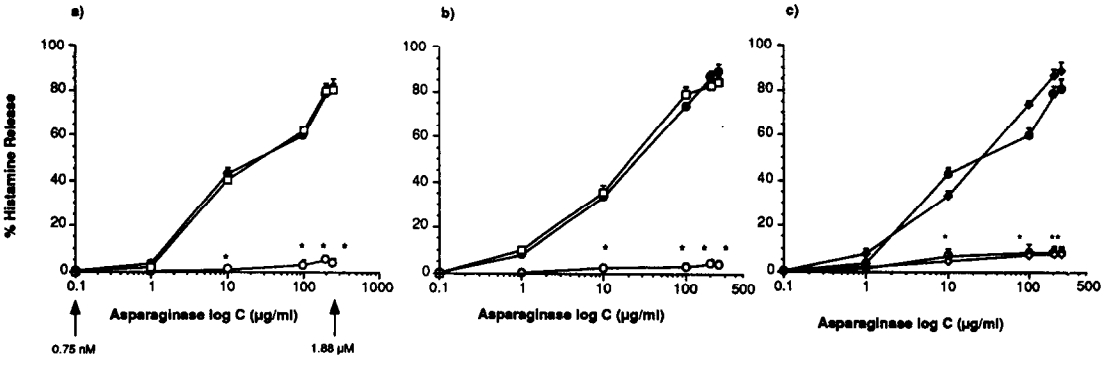

Fig. 4. Histamine release elicited by asparaginase in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. (c) Filled symbols represent unpurified cells; open symbols represent purified cells. Circles, peritoneal cells; romboids, pleural cells. Significant differences with respect to the controls are indicated with the * symbol. Values are means ± SEM of four experiments.

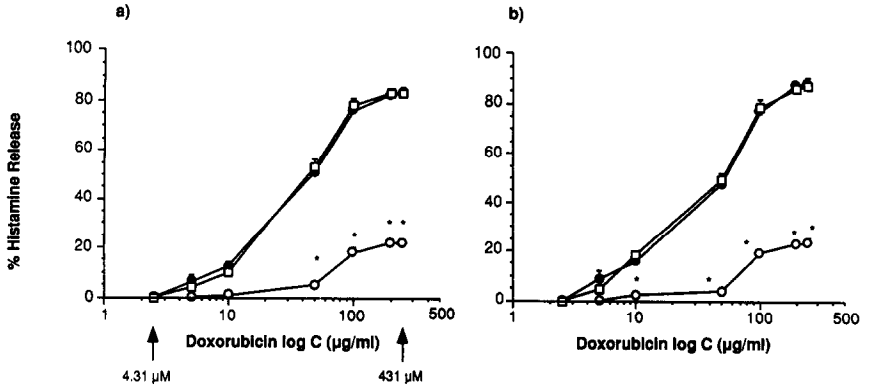

Fig. 5. Histamine release elicited by doxorubicin in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. Significant differences with respect to the control are indicated with the * symbol. Values are means ± SEM of three experiments.

cells. The response induced by ifosfamide is shifted more to the left than that of cytarabine, since the cells began to release histamine with 10 μg/mL of ifosfamide. Purification did not change the dose–response profile of the cells stimulated with ifosfamide, the maximum response being in any case close to 80% (results not shown).

A similar profile of response was obtained with vinorelbine (Fig. 3) and L-asparaginase (Fig. 4). Both drugs started to release histamine in a similar manner in pleural and peritoneal cells, lower concentrations than ifosfamide being needed. The concentration that activated histamine release was 5 μg/mL for both vinorelbine and L-asparaginase. Also, pretreatment with sodium fluoride did not change the profile of response for either drugs, while theophylline completely inhibited the response. The efficacy of the response was about 80% and 65% histamine release for L-asparaginase and vinorelbine, respectively. Nevertheless, the response to both L-asparaginase and vinorelbine was supressed almost completely after purification, the maximum release being lower than 10%. Both pleural and peritoneal mast cells showed the same pattern of response after purification.

The response elicited by doxorubicin, as shown in Fig. 5, was similar to those elicited by the other drugs for both pleural and peritoneal mast cells, and pretreatment with sodium fluoride did not affect the response. Nevertheless, theophylline did not induce a complete inhibition of the response, as was the case for cytarabine, ifosfamide, vinorelbine and L-asparaginase. There was inhibition, but at the highest concentrations of doxorubicin cells still released 20% histamine, which rendered a 75% inhibition, while the actions of the rest of the drugs were inhibited by up to 95% with respect to the controls. The maximum response was, as for ifosfamide, about 80% for both purified and unpurified pleural and peritoneal mast cells (results not shown).

A very different model of interaction was obtained when cells were stimulated with carboplatin (Fig. 6). Carboplatin elicited histamine release from purified and unpurified mast cells within the concentration values 1–300 μg/mL. The response was triggered by 100 μg/mL, and the maximum response was approximately 50%. The control response of pleural and peritoneal mast cells was similar, but in this case theophylline did not alter the response, while sodium fluoride completely inhibited the release of histamine in both pleural and peritoneal mast cells. There was no statistically significant difference between pleural and peritoneal cells, either unpurified or purified (results with purified cells not shown).

DISCUSSION

In this paper we present data obtained with six drugs representative of six different groups of agents, namely, nitrogen mustards (ifosfamide) as alkylating agents, cytotoxic antibiotics (doxorubicin), heavy metal complexes (carboplatin), enzymes (L-asparaginase), *Vinca* alkaloids (vinorelbine) and antimetabolites (cytarabine). In our systematic study of the effects of cancer chemotherapeutic agents on mast cells, we found that a large number of antineoplastic drugs elicit histamine release from rat mast cells (this paper and Refs 11 and 12). It is first of all important to point out that this paper does not try to correlate results obtained in rats with concentrations of antitumor drugs used clinically. Nevertheless, these results are important because we indicate some mechanistic effects with agents not previously described as histamine releasers *in vitro*.

The fact that these drugs elicit histamine release from mast cells can be explained as a general mechanism that resembles the action of other basic mast cells secretagogues, such as compound 48/80 [2]. If this is the case, these cancer chemotherapeutic agents may act through G-proteins [7] and the release of histamine may not be due to interaction with specific receptors [19]. This observation is further supported by the fact that the response is

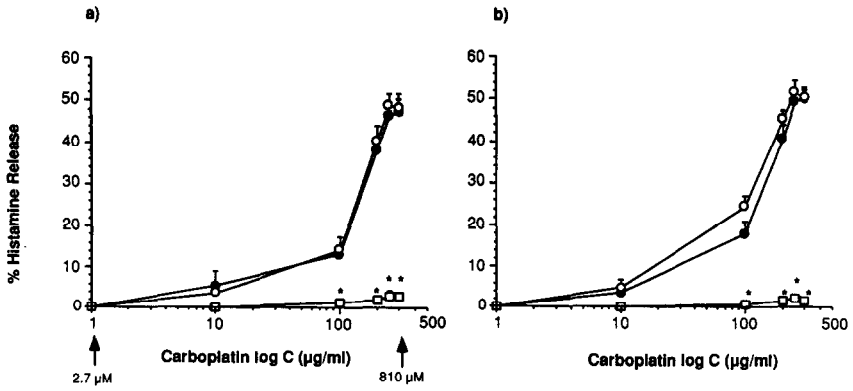

Fig. 6. Histamine release elicited by carboplatin in rat peritoneal (a) and pleural (b) mast cells. Filled circles represent control values, open circles represent the release in cells pretreated with 10 mM theophylline for 10 min and open squares represent the release of cells pretreated for 10 min with 10 mM sodium fluoride. Significant differences with respect to the control are indicated with the * symbol. Values are means ± SEM of four experiments.

very fast and independent of extracellular calcium [11, 12]. We can conclude from our results that, with the exception of cytarabine, the unspecific action of the agents we used does not show any heterogeneity in pleural or peritoneal mast cell response. Cytarabine elicits histamine release from pleural mast cells at lower concentrations than from peritoneal cells, and this higher sensitivity of the pleural population is in agreement with previous results [15].

Theophylline is a rather unspecific drug that acts on several systems, mainly adenosine receptors at lower concentrations [20] and phosphodiesterases at higher concentrations [21]. At the concentrations that we used, the main action of theophylline is the inhibition of phosphodiesterases. Since type IV phosphodiesterase seems to be the main type in rat mast cells [22], the effect is to increase intracellular cAMP levels. Nevertheless, this action is still additional to possible effects on adenosine receptors or calcium movements [23]. We can conclude that the action of the drugs we tested, with the exception of carboplatin, is inhibited by high levels of intracellular cAMP.

The release of histamine by antineoplastic drugs through non-immunological stimulation, such as by cisplatin or asparaginase, has been reported clinically [8]. Therefore, the inhibitory action of the theophylline may explain some cases where adverse effects to cancer chemotherapeutic agents were attenuated with the administration of theophylline, such as the cardiotoxicity observed with anthracyclines, although anthracycline toxicity may appear also through oxygen free-radical generation [24].

It is interesting that theophylline does not affect the response elicited by carboplatin, while sodium fluoride completely inhibits the activation of mast cells. G-proteins exist as trimers, and sodium fluoride has been reported to induce the dissociation of the α monomer from the β-γ dimer in a GTP-independent manner. This activation is attributed to the active species AlF_4, which is formed when contaminating Al is released from the glassware, and mimics the γ-phosphate of GTP at the GTP binding site on the G-protein [25]. Although the action of sodium fluoride is not specific, it is the only drug available to activate the G-proteins without resorting to the potentially artifactual membrane preparations or cell permeabilization procedures necessary for G-protein activation by GTP [26]. Therefore, we can conclude that the complete inhibition of the response obtained when mast cells were stimulated with carboplatin is mediated through some G-protein. The inhibitory activity of the pertussis-sensitive G-protein on rat mast cells has been described (27), although we cannot draw an appropriate conclusion from our results. The striking observation is rendered that carboplatin activates mast cells by a mechanism completely different from that of the other drugs tested but we are, as yet, unable to explain the mechanism.

The heterogeneity of mast cells does not permit information to be extrapolated from rat to human mast cells, even though rat mast cells are pharmacologically very similar to human skin mast cells [2]. We do not intent to validate the rat model for the obtaining of information in humans, but simply show the response of rat mast cells to these drugs. Nevertheless, the fact that histamine release induced by vinorelbine, cytarabine or L-asparaginase depends on a cellular component which is lost during purification indicates that the interaction is located on the plasma membrane, since L-asparaginase is a polypeptide and therefore does not cross the plasma membrane.

We present here the striking result that some of the drugs tested are completely dependent on some cellular factor which is lost during purification. Our results with doxorubicin are the same as those reported by Decorti *et al.* [28], who did not observe any effect of purification on histamine release and also described the inhibitory effect of theophylline. The purification of mast cells can induce the loss of membrane components, such as IgE receptors [29] or β-adrenergic receptors [15]. Therefore, we can

speculate that the loss of response to vinorelbine, cytarabine and L-asparaginase might be attributable to the lack of some plasma membrane component. Supporting this speculation is the fact that doxorubicin is not affected by the purification procedure, and there is a correlation between histamine release and the uptake of doxorubicin by rat mast cells [28]. These observations widen the knowledge of the type of interaction that occurs between cancer chemotherapeutic drugs and cells, and indicate that there is much more to be known about these interactions. Finally, our results support the prevously described non-immunological histamine release after administration of some antineoplastic drugs, i.e. L-asparaginase and cisplatin [8].

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REFERENCES

1. Chilton FH and Lichtenstein LM, Lipid mediators of the allergic reaction. *Chem Immunol* **49**: 173–205, 1990.
2. Diamant B, New insights into the mechanisms of histamine release from rat peritoneal mast cells. *Chem Immunol* **49**: 142–172, 1990.
3. Lagunoff D, Martin TW and Read G, Agents that release histamine from mast cells. *Annu Rev Pharmacol Toxicol* **23**: 331–351, 1983.
4. Kurosawa M, Protein phosphorylation and inositol phospholipid metabolism in activated mast cells. *Clin Exp Allergy* **20**: 7–12, 1990.
5. MacGlashan DJ, Single-cell analysis of Ca^{++} changes in human lung mast cells: graded vs. all-or-nothing elevations after IgE-mediated stimulation. *J Cell Biol* **109**: 123–134, 1989.
6. Aridor M, Traub LM and Sagi ER, Exocytosis in mast cells by basic secretagogues: evidence for direct activation of GTP-binding proteins. *J Cell Biol* **111**: 909–917, 1990.
7. Mousli M, Bronner C, Landry Y, Bockaert J and Rouot B, Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. *FEBS Lett* **259**: 260–262, 1990.
8. Weiss RB, Hypersensitivity reactions to cancer chemotherapy. *Semin Oncol* **9**: 5–13, 1982.
9. Decorti G, Bartoli KF, Candussio L and Baldini L, Characterization of histamine secretion induced by anthracyclines in rat peritoneal mast cells. *Biochem Pharmacol* **35**: 1939–1942, 1986.
10. Riegel EA, Kaliner M, El HAN, Ferrans VJ, Kawanami O and Herman EH, Anthracycline-induced histamine release from rat mast cells. *Agents Actions* **12**: 431–437, 1982.
11. Botana LM, Arnaez E, Alfonso A, Vieytes MR and Louzao MC, Non-immunological release of histamine from rat mast cells elicited by antineoplastic agents. Effect of drug combinations. *Cancer Chem Pharmacol*, in press.
12. Botana LM, Arnaez E, Vieytes MR, Alfonso A and Bujan MJ, Non-immunological release of histamine from rat mast cells elicited by antineoplastic drugs. *Cancer Chem Pharmacol* **23**: 23–25, 1992.
13. Pearce FL, Mast cell heterogeneity: the problem of nomenclature. *Agents Actions* **23**: 125–128, 1988.
14. Botana LM, Adrenergic agonists do not compete with the antagonist (-)3-[125I]iodocyanopindolol for binding to rat pleural or peritoneal mast cell adrenergic receptor. *Gen Pharmacol* **18**: 263–287, 1987.
15. Botana LM, Espinosa J and Eleno N, Adrenergic activity on rat pleural and peritoneal mast cells. Loss of beta-receptors during the purification procedure. *Gen Pharmacol* **18**: 141–148, 1987.
16. Johnson AR and Moran NC, Selective release of histamine from rat mast cells by compound 48-80 and antigen. *Am J Physiol* **216**: 453–459, 1969.
17. Chakravarty N, Histamine secretion from permeabilized mast cells by calcium. *Life Sci* **39**: 1549–1554, 1986.
18. Shore PA, The chemical determination of histamine. *Methods Biochem Anal* **1**: 89–97, 1971.
19. Mousli M, Bueb JL, Bronner C, Rouot B and Landry Y, G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol Sci* **11**: 358–362, 1990.
20. Sydbom A and Fredholm BB, On the mechanism by which theophylline inhibits histamine release from rat mast cells. *Acta Physiol Scand* **114**: 243–251, 1982.
21. Beavo JA and Reifsnyder DH, Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *TIPS* **128**: 150–155, 1989.
22. Bergstrand H, Lundquist B and Schurmann A, Rat mast cell high affinity cyclic nucleotide phosphodiesterases: separation and inhibitory effects of two antiallergic agents. *Mol Pharmacol* **14**: 848–855, 1978.
23. Saeki K, Ikeda S and Nishibori M, Calcium requirement for the inhibition by theophylline of histamine release from mast cells. *Life Sci* **32**: 2973–2980, 1983.
24. Klugmann FB, Decorti G, Candussio L, Grill V, Mallardi F and Baldini L, Inhibitors of adriamycin-induced histamine release *in vitro* limit adriamycin cardiotoxicity *in vivo*. *Br J Cancer* **54**: 743–748, 1986.
25. Gilman AG, G Proteins: transducers of receptor-generated signals. *Annu Rev Biochem* **56**: 615–649, 1987.
26. Strnad CF, Parente JE and Wong K, Use of fluoride ion as a probe for the guanine nucleotide-binding protein involved in the phosphoinositide-dependent neutrophil transduction pathway. *FEBS Lett* **206**: 20–4, 1986.
27. Nakamura T and Ui M, Islet-activating protein, pertussis toxin, inhibits Ca^{2+}-induced and guanine nucleotide-dependent releases of histamine and arachidonic acid from rat mast cells. *FEBS Lett* **173**: 414–418, 1984.
28. Decorti G, Klugmann FB, Candussio L, Furlani A, Scarcia V and Baldini L, Uptake of adriamycin by rat and mouse mast cells and correlation with histamine release. *Cancer Res* **49**: 1921–1926, 1989.
29. Coutts SM, Nehring R, Jariwala N, Weinryb I and Khandwala A, Loss of IgE receptors and shedding of a protease during purification of mast cells. *Int Arch Allergy Appl Immunol* **1**: 78–81, 1981.