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CHARACTERIZATION OF HISTAMINE SECRETION INDUCED BY ANTHRACYCLINES IN RAT PERITONEAL MAST CELLS

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Abstract—The histamine-releasing activity of three anthracyclines, adriamycin, daunomycin and epirubicin, has been tested on rat peritoneal mast cells.

The three drugs induced a marked and dose-dependent histamine secretion, in a noncytotoxic manner. The release was not sustained by extracellular calcium but was largely dependent on intracellular stores of this cation. This function was blocked by extremes of temperature (0 and 45°), was very rapid and virtually complete within 10 sec. Treatment of mast cells with theophylline or disodium cromoglycate significantly reduced the secretory response to anthracyclines.

On the basis of these results it is clear that the stimulant effect of anthracyclines is a true exocytotic response and thus is very similar to that of the classic mast cell secretagogue, compound 48/80.

It has recently been suggested that anthracycline-associated cardiac toxicity may be mediated by vasoactive substances and particularly by histamine [1–4]. These studies have shown that, in various animal models, the acute and chronic toxic effects of these antineoplastic drugs are related to histamine and cathecholamine release and no toxic cardiac effects are noted in the presence of antihistamines and antiadrenergics.

We have recently demonstrated that adriamycin induces an important histamine release from rat peritoneal cells *in vitro*, in a noncytotoxic manner [5]. Histamine may be released without cellular disruption by a number of diverse agents [6]; some of these, such as dextran, concanavalin A and ionophore A23187 act in a similar manner to the immunologically induced mechanism in so far as they probably transiently increase the permeability of cellular membrane to calcium ions; hence this release is calcium-dependent such as the anaphylactic reaction. Other inducers, such as 48/80 and polylisine, on the contrary, promote the release of histamine also in the absence of extracellular calcium, probably mobilizing intracellular stores of this ion; in fact cells deprived of this reserve by treatment with a chelating agent become unresponsive.

The present study was undertaken with the aim of defining the characteristics of the release of histamine promoted by anthracyclines on rat peritoneal mast cells *in vitro*.

MATERIALS AND METHODS

Mixed peritoneal cells were recovered from 200 to 400 g male Sprague–Dawley rats (Charles River, Italy) by lavage of the peritoneal cavities with saline solution at 37°. The physiological solution had the following composition: 1.54×10^{-1} M NaCl, 2.7×10^{-3} M KCl, 9×10^{-4} M $CaCl_2$, 5.6×10^{-3} M D-glucose, human serum albumin 1 g/l and 10% by volume of a Sörensen buffer containing 3×10^{-2} M $Na_2HPO_4 \times 7H_2O$ and 3.5×10^{-2} M $NaH_2PO_4 \times H_2O$. The pH of the solution was adjusted to 7.2 with 1 N NaOH before use. To determine the dependence of the release on extracellular calcium, this ion was omitted from the incubation medium. To examine the requirement for intracellular calcium, EDTA 2 mM was added to the calcium-free medium.

The cells were sedimented by centrifugation at 200–250 *g* for 10 min, the supernatant fraction was removed and cells were resuspended in buffered medium. A pooled suspension from more rats was employed for a day's experiment (final cell suspension 2 ml of solution per rat). The cell suspension contained approximately 10% mast cells and was used without further purification, because only the mast cells in such a suspension contained histamine [7].

Four hundred μl aliquots of cells were allowed to equilibrate for 5 min in a metabolic shaker with gentle mechanical agitation at the stated temperature, and then a solution (10 μl) of the releas agent was added. To determine the kinetics of the release process, the reaction was terminated after fixed periods of time by the addition of 3.6 ml of ice-cold buffer. This procedure rapidly cooled the sample and diluted the secretagogue to essentially ineffective levels. The effect of various inhibitors was also examined; cells were pretreated with theophylline (1 mg/ml) for 30 min, before stimulation. Disodium cromoglycate (0.5 mg/ml) was added to the cells simultaneously with the releasing agents.

Samples were incubated in quadruplicate for stated experimental times. Cells were separated from supernatants by centrifugation at about 200 *g* for 3 min. The cell pellets were resuspended in 400 μl of the saline solution and allowed to stand in a boiling water bath for 10 min to release residual histamine; the supernatants of controls were processed

similarly. All the samples were assayed for histamine by the fluorimetric method of Shore *et al.* [8], omitting the extraction step. The amount of histamine released was calculated as a percentage of the total histamine present in the control suspensions. All values were corrected for the spontaneous release (approximately 5%) occurring in the absence of the inducers.

Cell viability was estimated at the end of the incubation period by the trypan blue dye exclusion test.

Average and S.E. of the means were calculated; statistical evaluation of results was carried out using Student's *t*-test for independent samples. Values of $P < 0.05$ were considered significant.

Adriamycin, daunomycin, and epirubicin were obtained from Farmitalia Carlo Erba (Milano). Mechlorethamine, methotrexate, 5-fluorouracil, *cis*-platinum, compound 48/80, cromolyn sodium, theophylline, histamine dihydrochloride and *o*-phthaldialdehyde were purchased from Sigma Chemical Co. (St. Louis, MO). Lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) was kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD).

RESULTS

The three tested anthracyclines induced a marked and dose-dependent histamine release. Dose–response curves are presented in Fig. 1. The effective molar concentrations of the anthracyclines producing 50% of the maximal effect, calculated by the method of least squares (x = log dose of drug; y = percentage of control) with the aid of a computer, were 7.8×10^{-5} M for epirubicin, 7.7×10^{-5} M for adriamycin and 1.6×10^{-4} M for daunomycin. Cytotoxicity was estimated at the end of the incubation period by the trypan blue dye exclusion test. The highest dose tested (200 μg/ml) was toxic for less than 25% of whole cell population, while at lower doses, cell viability was similar to that of controls. All other antineoplastic drugs were tested at two doses: the higher induced a 25% toxicity in the whole cell population, while, for the lower dose, cellular viability was comparable to that of controls. At these doses none of other antineoplastic drugs induced a histamine release comparable to that caused by anthracyclines (Fig. 2).

Incubation of peritoneal mast cells for 30 min in absence of calcium did not significantly reduce secretion induced by the three anthracyclines or compound 48/80. Preincubation for 2 hr with 2 mM EDTA produced a powerful inhibition of the responses to these secretagogues (Fig. 3).

Histamine release induced by the anthracyclines was elevated at 37°, but markedly depressed at 0° and 45°. The spontaneous secretion was unaffected over this temperature range (Fig. 4).

The kinetics of histamine release are presented in Fig. 5. The three anthracyclines induced an extremely rapid release, which was virtually complete in 10 sec.

Figure 6 shows the effect of theophylline and cromolyn on the histamine release produced by the three anthracyclines. The two substances significantly reduced histamine release produced by anthracyclines.

DISCUSSION

In the present work the three anthracyclines tested, adriamycin, daunomycin and epirubicin, were found to be liberators of histamine from rat peritoneal cells.

This effect is similar to that of other classic non-cytolytic mast cell secretagogues, as judged by trypan blue dye exclusion test and by the dependence of the phenomenon on temperature and calcium presence [9].

The secretory response to the anthracyclines persisted in the absence of extracellular calcium but was almost abolished by pretreatments known to deplete intracellular calcium, such as a 2 hr preincubation with 2 mM EDTA, suggesting therefore that it is

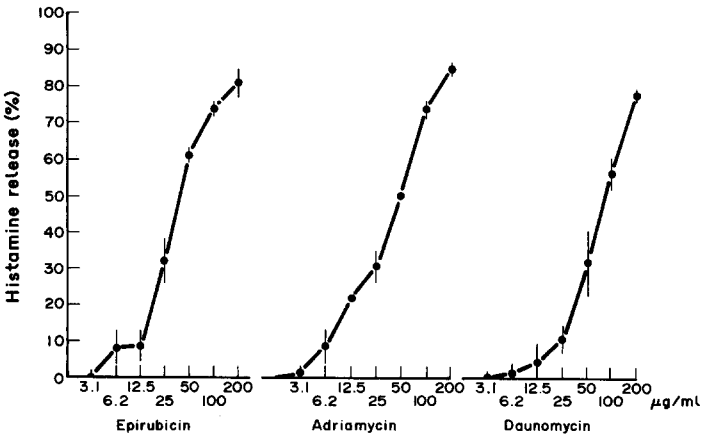

Fig. 1. Dose response curves for release of histamine by epirubicin, adriamycin and daunomycin (incubation period: 10 min). The points are the means of four separate experiments and the vertical bars show S.E.M.

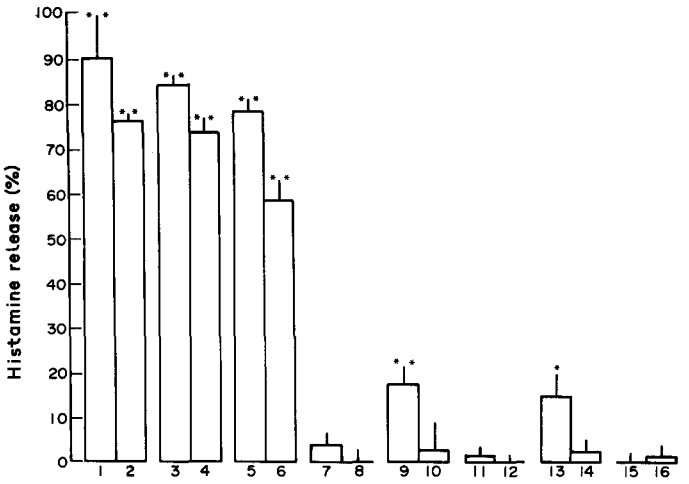

Fig. 2. Histamine release from rat peritoneal mast cells induced by the three anthracyclines and by other antineoplastic agents (incubation period: 10 min). The higher dose tested for each drug was slightly cytotoxic (≤25% dead cells), while, for the lower dose, cellular vitality was comparable to that of controls (trypan blue dye exclusion test): 1, epirubicin 200 μg/ml; 2, epirubicin 100 μg/ml; 3, adriamycin 200 μg/ml; 4, adriamycin 100 μg/ml; 5, daunomycin 200 μg/ml; 6, daunomycin 100 μg/ml; 7, mechlorethamine 100 μg/ml; 8, mechlorethamine 50 μg/ml; 9, methotrexate 1000 μg/ml; 10, methotrexate 500 μg/ml; 11, 5-fluorouracil 1000 μg/ml; 12, 5-fluorouracil 500 μg/ml; 13, *cis*-platinum 500 μg/ml; 14, *cis*-platinum 250 μg/ml; 15, lomustine 100 μg/ml; 16, lomustine 50 μg/ml. Columns represent the means from four experiments and vertical bars show S.E.M. *P < 0.05 vs control; **P < 0.01 vs controls.

probably due to cellular stores of this ion. In this respect the stimulant effects of anthracyclines resembles that of 48/80 and other basic polypeptides [10, 11], but contrasts with that of antigens, where secretion is sustained largely by extracellular calcium [6, 12].

In addition, the release induced by anthracyclines was extremely rapid and virtually complete in 10 sec; comparable results have been obtained with compound 48/80 and such kinetic behaviour appears to be characteristic of basic inducers [13].

This histamine releasing action is peculiar of this class of antineoplastic agents, as the other antitumor substances tested at equitoxic doses have not shown to induce any comparable histamine release; it is therefore clear that the stimulant effect of anthracyclines is a true esocytotic response, which appears to resemble closely the action of other classic mast cell secretagogues such as compound 48/80. However, anthracyclines are effective in releasing large amounts of histamine only at very high concentrations and hence are about 100 times less active, on a weight basis, than compound 48/80. The range of agents capable of eliciting histamine release is impressive, however, the majority of potent histamine releasers that are active both *in vivo* and *in vitro* are basic compounds. The anthracyclines are not included in this chemical class, nevertheless the histamine release initiated by anthracyclines and 48/80 have many features in common.

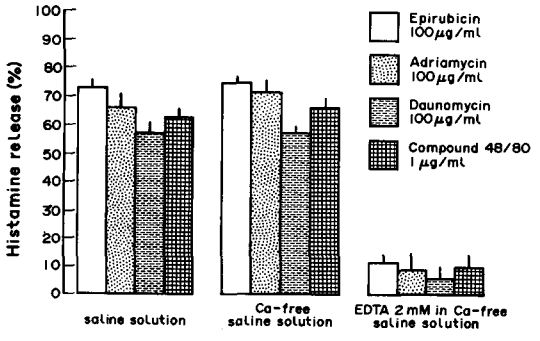

Fig. 3. Effect of various treatments that lower cell calcium levels on mast cell secretion. Mast cells were incubated for a period of 2 hr in the indicated solution. After addition of the releasing agent, secretion was allowed to proceed for 10 min. Columns represent the means from four experiments and vertical bars show S.E.M.

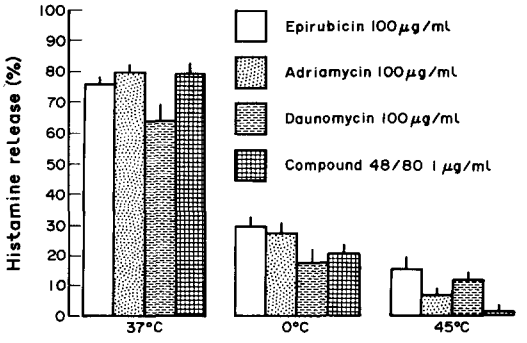

Fig. 4. Effect of temperature on histamine secretion induced by a 10 min incubation with anthracyclines and compound 48/80. Columns represent the means from four experiments and vertical bars show S.E.M.

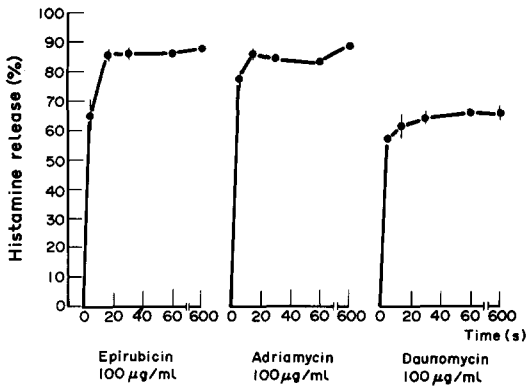

Fig. 5. Time courses of the effects of the three anthracyclines on histamine secretion from rat peritoneal mast cells. The points are the means from four experiments and vertical bars are S.E.M.

This characteristic releasing action can confirm the observations of other workers: Herman and Young [1] have demonstrated significant increases in plasma histamine concentrations in dogs coinciding with an important hypotensive response after an infusion of various anthracyclines; Bristow *et al.* [2] showed that the acute cardiovascular effects of anthracyclines in dogs are entirely related to the release of histamine and cathecholamines and to increased prostaglandin synthesis; also in rabbits [3] chronic cardiac effects may be related to histamine and cathecholamine release as pretreatment with antihistamines and antiadrenergics prevents the majority of cardiac damages. Also subacute anthracycline cardiac toxicity may be related to the release of vasoactive substances, indeed, in rabbits [4], pretreatment with cromolyn sodium, a mast cell stabilizer, produced significant protection against adriamycin mediated subacute cardiotoxicity, and completely prevented histamine release induced by the drug in isolated heart preparations.

That anthracycline-induced cardiotoxicity may be mediated by cardiac histamine release is further supported by histopathologic findings which are quantitatively identical to those produced by an histamine treatment [4, 14].

This study has demonstrated that anthracyclines are able to induce a pronounced release of histamine from rat peritoneal mast cells, through a mechanism of action resembling that of the classic basic secretagogues such as compound 48/80, and supports the hypothesis that histamine release could play a crucial role in the pathogenesis of anthracycline cardiomyopathy.

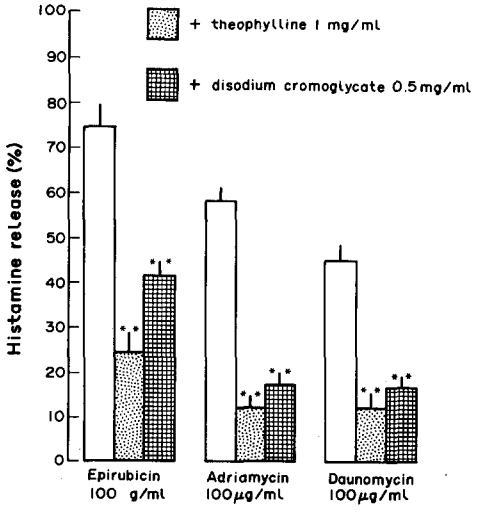

Fig. 6. Effect of theophylline and disodium cromoglycate on histamine release induced by a 10 min incubation with the three anthracyclines (100 μg/ml). Columns are the means of four experiments and vertical bars show S.E.M. $^{**}P < 0.01$ vs the anthracycline alone.

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