Heterogeneous effects of protamine on human mast cells and basophils

V. Patella, A. Ciccarelli, B. Lamparter-Schumert, A. de Paulis, M. Adt and G. Marone

Summary
To investigate the mechanisms of anaphylactoid reactions to protamine, we have examined the in vitro effects of increasing concentrations of protamine (10⁻⁶–3 x 10⁻⁴ mol litre⁻¹) on the release of preformed (histamine and tryptase) and de novo synthesized (peptide leukotriene C₄ (LTC₄) or prostaglandin D₂ (PGD₂)) mediators from human basophils and mast cells isolated from lung parenchyma, heart, skin and synovial tissues. Protamine 10⁻⁵–3 x 10⁻⁴ mol litre⁻¹ induced release of histamine, but not de novo synthesis of LTC₄ from basophils. At concentrations from 10⁻⁵ to 3 x 10⁻⁴ mol litre⁻¹ it induced histamine release from human heart (mean 6.5 (SEM 1.5) %), skin (17.7 (4.1) %) and to a lesser extent from synovial mast cells, but not from lung mast cells. Protamine also caused the release of tryptase from heart mast cells (12.8 (3.2) μg/10⁷ cells), but did not induce de novo synthesis of LTC₄ and PGD₂ from lung and skin mast cells. In these experiments cross-linking of IgE by anti-IgE caused release of LTC₄ or PGD₂ from human basophils or mast cells. These results demonstrate that protamine acted as an incomplete secretagogue, causing the release of pre-formed mediators from human basophils and mast cells. (Br. J. Anaesth. 1997; 78: 724–730).

Key words
Allergy. Histamine. Pharmacology, protamine.

Protamine sulphate is a basic protein extracted from fish sperm heads.¹ The use of i.v. protamine to reverse heparin anticoagulation has increased in the past decade with the advent of cardiopulmonary bypass technology, cardiac catheterization, haemodialysis and leukapheresis.²⁻⁴ However, protamine may be a major cause of morbidity and mortality in these procedures.²⁻⁴⁻¹⁰ During the past decade, reports of adverse reactions associated with i.v. protamine (rash, urticaria, bronchospasm, hypotension and increased pulmonary artery pressure) have also increased.²⁻⁶

The mechanism(s) by which protamine produces adverse reactions is still not completely understood. It has been suggested that they are caused by complement activation,⁵¹¹¹² inhibition of serum carboxypeptidase,¹³ thromboxane production¹⁴¹⁵ and anti-protamine IgE antibody via type I immediate hypersensitivity reactions.²⁶ It has also been suggested that protamine induces or potentiates histamine release from human basophils and mast cells.¹⁰⁻¹⁸

Techniques are now available to isolate large numbers of mast cells from human lung parenchyma,¹⁹ heart,²⁰ skin²¹ and synovial tissues.²² These techniques have led to the demonstration that human basophils and mast cells isolated from different anatomical sites vary markedly in their morphological, biochemical and functional responses.¹⁹⁻²⁵ These cells differ with respect to the releasing activity of various stimuli and release of different preformed (histamine and tryptase) and de novo synthesized mediators (peptide leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂)).²⁶

Preliminary evidence suggests that human basophils and mast cells may also vary in their histamine releasing capacity when challenged with general anaesthetics. For example, thiopentone activates skin mast cells²⁴ but not human basophils. It should be emphasized that previous studies have focused on the in vitro histamine releasing capacity of protamine¹⁶⁻¹⁸ but have not evaluated its possible effects on de novo synthesis of chemical mediators.

To investigate the mechanisms of anaphylactoid reactions to protamine, we have tested in vitro to see if protamine induces the release of preformed and de novo synthesized mediators from human basophils and mast cells isolated from human heart, lung parenchyma, skin and synovial tissues.

Materials and methods

REAGENTS

The following were purchased: 60% HClO₄ (Baker Chemical Co., Deventer, The Netherlands); piperazine-N,N’-bis (2-ethanesulphonic acid), hyaluronidase, collagenase type II, chymopapain, elastase type I, synthetic PGD₂, (Sigma Chemical Co., St Louis, MO, USA); Hanks balanced salt solution (HBSS) and fetal calf serum (FCS) (Gibco, Vincenzo Patella, MD, Anna Ciccarelli, MD, Amato de Paulis, MD, Grazia Marone, MD, Division of Clinical Immunology and Allergy, University of Naples Federico II, School of Medicine, Via S. Pansini 5, 80131 Napoli, Italy. Barbara Lamparter-Schumert, MD, Monica Adt, MD, Department of Anaesthesiology, German Heart Institute Berlin, Berlin, Germany. Accepted for publication: February 27, 1997. Correspondence to G. M.
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Grand Island, NY, USA; deoxyribonuclease I and pronase (Calbiochem, La Jolla, CA, USA); RPMI 1640 with Hepes buffer 25 mmol litre⁻¹, Eagle’s minimum essential medium (Flow Laboratories, Irvine, Scotland); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); protamine chloride hydrate (Roche SpA, Milan, Italy); (H)-LTC4 39.3 Ci mmol⁻¹ and (H)-PGD2 210 Ci mmol⁻¹ (New England Nuclear, Boston, MA, USA). Rabbit anti-human-Fce antibody was kindly donated by Drs Teruko and Kimishige Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA). The rabbit anti-PGD2 antibody was a generous gift from Dr L. M. Lichtenstein (The Johns Hopkins University, Baltimore, MD, USA). The rabbit anti-LTC4 was kindly provided by Dr E. J. Kusner (Zenea, Wilmington, DE, USA). The tryptase radioimmunoassay kit (Pharmacia Tryptase RIACT 50, Pharmacia Diagnostics AB, Uppsala, Sweden) was kindly donated by Kabi Pharmacia SpA (Milan, Italy).

BUFFERS

The Pipes buffer used in these experiments was a mixture of Pipes 25 mmol litre⁻¹, NaCl 110 mmol litre⁻¹, KCl 5 mmol litre⁻¹, pH 7.37, referred to as “P”. P2CG contains, in addition to P, CaCl2 2 mmol litre⁻¹ and glucose 1 g litre⁻¹ and gelatine 1 g litre⁻¹ in addition to P, pH 7.37.

HISTAMINE RELEASE FROM HUMAN BASOPHILS

Informed consent was obtained from all subjects and approximately 50 ml of blood were obtained and diluted into a final concentration of EDTA 0.008 mol litre⁻¹ and 1.1% Dextran 70. The procedures for isolation of peripheral blood basophils and in vitro mediator release have been described in detail elsewhere. The cell-free supernatant was assayed for histamine with an automated fluorometric technique. Net percentage release was calculated by subtracting histamine released spontaneously from unstimulated samples (mean 3.6 (SEM 0.9) %) from total histamine released from cell samples lysed with 2% perchloric acid. The difference between replicate histamine measurements was less than 10%.

ISOLATION AND PARTIAL PURIFICATION OF HUMAN HEART MAST CELLS (HHMC)

The heart tissue used in this study was obtained from patients (36–65 yr) undergoing heart transplantation at the Deutsches Herzzentrum, Berlin, mostly for cardiomyopathy. The explanted heart was immersed immediately in cold (4°C) cardioplegic solution, shipped to Naples by air (4°C) and processed within 5–18 h of removal. Fat tissue, large vessels and pericardium were removed from the heart (100–400 g). The tissue, was minced finely into 2–5-mm fragments, suspended in P buffer (10 ml/g of wet tissue) and washed three times by centrifugation (the first time 150 × g, 4°C, 8 min and twice at 150 × g, 22°C, 8 min). The heart fragments were filtered through a 150-μm pore Nytex cloth (Tetko, Inc., Elmsford, NY, USA) and incubated for 15 min at 37°C under constant stirring in P buffer containing 10 mg collagenase/g of wet tissue. Three additional cycles of enzymatic digestion were performed, with a new preparation of collagenase each time. After the last enzymatic digestion, the cell suspension was centrifuged (150 × g, 22°C, 8 min) and filtered first through a 150-μm pore Nytex cloth and then through a 60-μm pore Nytex cloth to remove large particles and large cells (mostly myocytes). Lastly, cells were washed twice in PGMD (P 25 mmol litre⁻¹, NaCl 110 mmol litre⁻¹, KCl 5 mmol litre⁻¹, Mg 1 mmol litre⁻¹, gelatin 1 g litre⁻¹, DNase 20 mg litre⁻¹; pH 7.37) by centrifugation at 150 × g, 22°C for 8 min. Cell pellets were resuspended in 250 ml of P buffer containing 2% BSA and centrifuged (25 × g, 22°C, 2 min) to remove sedimented myocytes. Myocytes (>100 μm long) were pelleted and discarded; supernatants containing endothelial cells, fibroblasts and mast cells were then collected and centrifuged (150 × g, 22°C, 8 min). At this stage of purification, Alcian blue-positive cells amounted to <1% of the total cells. HHMC were partially purified by flotation through a discontinuous Percoll gradient prepared by mixing 9 parts Percoll and 1 part 10 × P solution. This mixture was then diluted with isotonic P to give Percoll concentrations of 40, 50, 60, 70, 80 and 90%. The cell suspension was overlaid on the Percoll gradient in 50-ml polypropylene tubes and the mixture centrifuged at 350 × g, 22°C, for 20 min. The cells found at the interface between the 60–70% and 70–80% fractions were removed and washed twice with P. Mast cells in these populations ranged from 0.1 to 16%, with an average of 14.3 (2.4) %. The enzymatic dispersion of tissue yields ranged between 2 and 6 × 10⁴ mast cells per gram of heart tissue.

PURIFICATION OF HUMAN LUNG MAST CELLS (HLMC)

Human lung tissue (20–80 g) was obtained from patients undergoing thoracotomy and lung resection, mostly for lung cancer. General anaesthesia was induced using the following drugs: droperidol with fentanyl and atropine (premedication); droperidol with fentanyl, thiopentone, suxamethonium and pancuronium (anaesthesia). Lung parenchymal mast cells were isolated as described previously. This technique yielded between 3 and 8 × 10⁵ mast cells/g of lung tissue, and purities ranged between 1% and 8%.

PURIFICATION OF HUMAN SKIN MAST CELLS (HSMC)

Skin (15–65 g) was obtained from either mastectomies for breast cancer or elective cosmetic surgery procedures. General anaesthesia was induced using the following drugs: thiopentone, pancuronium, atropine, fentanyl, ethrane or fluorane. Tissue was placed immediately in Eagle’s MEM at 4°C and
used within 1 h. HSMC were isolated as described previously. This technique yielded between 1 and $7 \times 10^5$ mast cells/g of wet tissue, and purities ranged between 1% and 4%.

**PURIFICATION OF HUMAN SYNOVIAL MAST CELLS (HSMC)**

Synovial tissue (22–35 g) used in this study was obtained from patients (27–66 yr) with osteoarthritis or rheumatoid arthritis, undergoing synovectomy at the Division of Orthopaedic Surgery. Extradural anaesthesia was induced with mepivacaine i.v. and atropine, fentanyl and benperidol were given systemically. Resected joint tissue was placed immediately in Eagle’s MEM at 4°C and processed within 2 h of removal using a technique described recently. Fat, cartilage and fibrous tissue were removed, and the tissue was minced finely with scissors into 2–5-mm fragments, suspended in P buffer (10 ml/g of wet tissue) and washed twice by centrifugation at 150 g for 8 min at 4°C and then at 22°C. The minced synovium was incubated for 45 min at 37°C in a shaking water bath with chymopapain 1 mg ml$^{-1}$ and pronase 0.5 mg ml$^{-1}$ in 1 ml of Tyrode’s buffer/g synovial tissue. Fragments of remaining tissue were digested for another 45 min at 37°C with collagenase 1 mg ml$^{-1}$. The resulting cell suspensions were pooled, filtered through 200-μm pore Nytex cloth (Tetko, Elmsford, NY, USA), centrifuged at 200 $\times$ g, 22°C for 8 min, and washed twice with P buffer. Cells were washed three times in P and resuspended in P2CG. This technique yielded between 1 and $9 \times 10^5$ mast cells/g of wet tissue, and purities ranged between 1% and 8%.

**MEDIATOR RELEASE FROM HUMAN MAST CELLS**

Mast cells (approximately $3 \times 10^4$ cells per tube) were resuspended in P2CG, and 0.3 ml of the cell suspension was placed in $12 \times 75$ mm polyethylene tubes and warmed to 37°C; 0.2 ml of each prewarmed releasing stimulus was added, and incubation was continued at 37°C for 30 min. At the end of this step, the reaction was stopped by centrifugation at $1000 \times g$ 22°C for 2 min, and the cell-free supernatants were stored at $-70°C$ for subsequent assay of histamine, tryptase, LTC$_4$ and PGD$_2$ content. The cell-free supernatants were assayed for histamine with an automated fluorometric technique. To calculate histamine release as a percentage of total cellular histamine, “spontaneous” release from mast cells (2–12% of the total cellular histamine) was subtracted from both numerator and denominator. The total histamine content in mast cells was obtained by cell lysis with 8% HClO$_4$. All values are based on the means of duplicate or triplicate determinations. Replicates differed from each other by less than 10% in histamine content.

**RIA OF TRYPTASE, LTC$_4$ AND PGD$_2$**

Total tryptase content was assessed by lysis induced by incubating cells with 0.1% of Triton X-100. Tryptase was analysed by a solid-phase radioimmunoassay (Pharmacia Tryptase RIACT 50). PGD$_2$ was analysed on 100-μl fractions obtained from the supernatant fluids. The samples were stored at $-70°C$. LTC$_4$ and PGD$_2$ were analysed by RIA within 18 h of the experiment to minimize degradation of the compounds.

**STATISTICAL ANALYSIS**

Results are mean (SEM). Statistical analysis was performed by two-way non-parametric analysis of variance (Friedman’s chi-square test). The extended Tukey test was used for multiple comparisons. The level of statistical significance was $P<0.05$.

**Results**

In the first series of experiments we evaluated the effects of increasing concentrations ($10^{-6}–3 \times 10^{-4}$ mol litre$^{-1}$) of protamine on histamine release from human basophils. Protamine caused concentration-dependent histamine release from basophils from 11 normal donors (table 1). In one experiment, protamine released less then 5% of histamine content but in all other experiments release varied from 5% to 26%. The release caused by protamine reached a plateau between $10^{-5}$ and $3 \times 10^{-5}$, with less release above these concentrations. Anti-IgE, used as a

<table>
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<tr>
<th>Experiment No.</th>
<th>Histamine release (%)</th>
<th>Protamine (mol litre$^{-1}$)</th>
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<tr>
<td></td>
<td></td>
<td>$10^{-6}$</td>
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<tr>
<td>10</td>
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<td>3</td>
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<tr>
<td>Mean (SEM)</td>
<td>1.2 (0.5)</td>
<td>2.1 (0.6)</td>
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</table>
Protamine induces histamine release

Positive control in these experiments, induced 28.9 (5.4) % of histamine release. There was no correlation between the maximum release caused by protamine and anti-IgE ($r_s = 0.01; \text{ns}$). Cell viability in the presence of protamine was always $\geq 95\%$ as detected by trypan blue exclusion.\textsuperscript{28}

In the second series of experiments we compared the effect of protamine on histamine release from HHMC and HLMC. Protamine $10^{-5}$–$3 \times 10^{-4}$ mmol litre$^{-1}$ induced histamine release from HHMC in a concentration-dependent manner (fig. 1) with maximum release between 3% and 17% (8.8 (1.4) %) ($P < 0.01$ compared with spontaneous release). In none of nine HLMC preparations did protamine induce the release of more than 5% of histamine content (fig. 1).

In view of the functional heterogeneity of human mast cells isolated from different anatomical sites,\textsuperscript{20} we evaluated the effects of increasing concentrations ($10^{-5}$–$3 \times 10^{-4}$ mmol litre$^{-1}$) of protamine on histamine release from mast cells from skin and synovial tissues. These concentrations of protamine induced histamine release from HSMC (table 2) and to a lesser extent from HSyMC (table 3). The maximum release varied between 7% and 25% (HSMC) and 12% and 13% (HSyMC).

Tryptase is a neutral protease that is a selective marker for mast cells.\textsuperscript{31} The secretory granules of all mature human mast cells contain large amounts (approximately 24.2 (4.3) $\mu g/10^6$ HHMC of tryptase).\textsuperscript{20,22} We investigated if activation of HHMC with protamine caused release of tryptase in addition to histamine. Tryptase was released in parallel with histamine from HHMC whereas protamine did not induce release of histamine and tryptase (<5%) from HLMC in three experiments (fig. 2).

### Table 2  Effect of increasing concentrations of protamine on histamine release from HSMC

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Protamine (mol litre$^{-1}$)</th>
<th>Histamine release (%)</th>
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<tr>
<td></td>
<td>10$^{-6}$</td>
<td>$3 \times 10^{-6}$</td>
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<tr>
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<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>1.0 (1.0)</td>
<td>2.8 (1.4)</td>
</tr>
</tbody>
</table>

### Table 3  Effect of increasing concentrations of protamine on histamine release from HSyMC

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Protamine (mol litre$^{-1}$)</th>
<th>Histamine release (%)</th>
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<tbody>
<tr>
<td></td>
<td>10$^{-6}$</td>
<td>$3 \times 10^{-6}$</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3</td>
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<tr>
<td>Mean (SEM)</td>
<td>0(0)</td>
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Different experimental models have shown that some of the adverse effects of protamine are caused by generation of eicosanoids.\textsuperscript{32,33} We therefore compared production of the newly formed lipid mediators from human basophils and mast cells activated by protamine $10^{-5}\text{–}3\times10^{-4}\text{ mmol litre}^{-1}$ and anti-IgE $10^{-2}\text{–}3\times10^{-1}\text{ µg ml}^{-1}$. In this group of experiments protamine induced concentration-dependent release of histamine from basophils, but did not stimulate de novo synthesis of LTC\textsubscript{4} by these cells (fig. 3). Anti-IgE stimulated release of large amounts of LTC\textsubscript{4}. Protamine also did not induce de novo synthesis of PGD\textsubscript{2} or LTC\textsubscript{4} from HHMC and HSMC, whereas anti-IgE stimulated the release of these eicosanoids (data not shown).

**Discussion**

Our results indicate that protamine is an incomplete secretagogue inducing only release of preformed mediators (histamine and tryptase) from human basophils and mast cells without stimulating de novo synthesis of eicosanoids. Protamine activated not only peripheral blood basophils, but also mast cells isolated from human heart, skin and synovial tissues, but not from lung parenchyma.

Intravascular administration of high concentrations of protamine is used increasingly to reverse heparin anticoagulation during cardiac catheterization,\textsuperscript{4} cardiothoracic and vascular surgical procedures\textsuperscript{34,35} and after dialysis\textsuperscript{36} and leukapheresis.\textsuperscript{37} I.v. protamine can cause acute reactions such as rash, urticaria, bronchospasm, hypotension, cardiovascular collapse, pulmonary hypertension and even death.\textsuperscript{2,4,7–9} These adverse reactions are thought to be caused by multiple mechanisms, including anaphylactic\textsuperscript{2–6} and anaphylactoid reactions.\textsuperscript{11–14,16–18} Intravascular administration of protamine causes rapid and massive exposure of basophils to this agent. It is therefore likely that the release of histamine from peripheral blood basophils might at least partly explain some of the adverse effects of protamine in some patients.

Intravascularly administered compounds rapidly expose circulating basophils and tissue mast cells to very high concentrations that tend to decline according to their pharmacokinetics. Thus we investigated the effects of a wide range of concentrations of protamine which must include concentrations reached during intravascular administration of the compound. The percentage of histamine release from basophils caused by protamine varied greatly from donor to donor, presumably because of the influence on basophil releasibility.\textsuperscript{19,23,38} This may partly explain the wide variability of protamine-induced adverse effects in patients given this drug intravascularly.\textsuperscript{6} At first sight, the bell shape of the dose–response curve of protamine on release of histamine from basophils might be surprising. However, other stimuli (anti-IgE, antigens, etc.) can produce a similar dose–response curve.\textsuperscript{20}

Although it has been demonstrated in different experimental models that the adverse reactions to protamine are to some extent caused by synthesis of eicosanoids and other lipid mediators,\textsuperscript{32,33} protamine did not induce release of LTC\textsubscript{4} and PGD\textsubscript{2} from human basophils and mast cells in vitro. Possibly in vivo protamine induces the release of lipid mediators from other cells.

Radiocontrast media, which can cause anaphylactoid reactions, also act as incomplete secretagogues on human basophils and mast cells\textsuperscript{26} and pretreatment with histamine H\textsubscript{1} and H\textsubscript{2} receptor antagonists reduces the incidence and severity of adverse reactions to these agents.\textsuperscript{39} In the light of our results, it might be appropriate to investigate if these antagonists also reduce the incidence of anaphylactoid reactions to protamine.

Protamine caused a concentration-dependent release of tryptase from HHMC, HSMC and HSyMC. This neutral protease, present in the cytoplasmic granules of all human mast cells,\textsuperscript{20,22,31} can activate complement leading to the formation of anaphylatoxins (C3a and C5a).\textsuperscript{40} Complement activation and C3a formation can occur during in vivo administration of protamine\textsuperscript{11–14} and tryptase release from mast cells might therefore contribute, as an amplification factor, to the pathogenesis of the generalized reactions to protamine, through activation of the complement system. Tryptase has a longer half-life than histamine in plasma.\textsuperscript{31} Therefore, it may be useful to measure plasma concentrations of tryptase in order to identify adverse reactions caused by protamine in humans.

Our results showed that protamine can induce release of vasoactive and proinflammatory mediators from HHMC in vitro. Coronary artery spasm, hypotension and shock can occur during intravascular administration of protamine.\textsuperscript{8,9} Mast cells have been demonstrated clearly within human heart tissue\textsuperscript{20,28} around coronary vessels and in the shoulder region of atherosclerotic human coronary vessels.\textsuperscript{41} During intravascular administration of
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Protamine, cardiac mast cells are likely to be exposed to high concentrations and our findings suggest that in vivo they can thus be activated to release histamine, tryptase and possibly other mediators. Histamine exerts significant cardiovascular effects in humans and vasoactive mediators released locally from coronary and cardiac mast cells might therefore contribute to the pathogenesis of severe adverse reactions to protamine.

Unlike HHMC and HSMC, HLMC were not activated by protamine in vitro. However, transient increases in pulmonary artery pressure are frequent when protamine is administered i.v. It is therefore possible that the pulmonary reactions to protamine involve alternative mechanisms such as direct activation of basophils, tryptase-mediated formation of anaphylatoxins that in turn activate C5a receptors on HSMC and HHMC, or other unknown mechanisms. Our results were obtained with preparations of mast cells isolated from different tissues of patients receiving several drugs before and during surgical procedures. Consequently, we cannot exclude the possibility that some of the heterogeneous effects of protamine on the release of mediators from mast cells isolated from different tissues might be influenced by the in vivo treatment of the cell donors.

In conclusion, these findings suggest that protamine selectively activated different types of human mast cells and basophils, acting as an incomplete secretagogue. These results are of interest for several reasons. They contribute to a better understanding of the pathogenesis of immediate generalized reactions caused by rapid injection of protamine and they also suggest that anti-H1 and anti-H2 blockers might be useful in attenuating anaphylactoid reactions to this substance. Finally, they indicate that measurements of plasma tryptase might be useful to identify adverse reactions.

Acknowledgements

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References


32. Morel DR, Mo Costabella PM, Pettit J-F. Adverse cardiopulmonary effects and increased plasma thromboxane concentrations following the neutralization of heparin with protamine in awake sheep are infusion rate-dependent. *Anesthesiology* 1990; 73: 415–424.


