

Immediate Hypersensitivity Elicits Renin Release from Cardiac Mast Cells

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Key Words

Angiotensin II • Immediate-type hypersensitivity •
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 β -Hexosaminidase • Norepinephrine • Cardiac anaphylaxis •
Renin inhibitor

Abstract

Background: We recently reported that murine and cavian heart mast cells are a unique extrarenal source of renin. Ischemia/reperfusion releases this renin leading to local angiotensin formation and norepinephrine release. As mast cells are a primary target of hypersensitivity, we assessed whether anaphylactic mast cell degranulation also results in renin and norepinephrine release. **Methods:** Hearts isolated from presensitized guinea pigs were challenged with antigen. **Results:** Cardiac anaphylaxis was characterized by mast cell degranulation, evidenced by β -hexosaminidase release and associated with renin and norepinephrine release. Mast cell stabilization with cromolyn or lodoxamide markedly attenuated the release of β -hexosaminidase, renin and norepinephrine. Renin inhibition with BILA2157 did not affect mast cell degranulation, but attenuated norepinephrine release. **Conclusions:** Our findings disclose that immediate-type hy-

persensitivity elicits renin release from mast cells, activating a local renin-angiotensin system, thereby promoting norepinephrine release. As renin is stored in human heart mast cells, allergic reactions could initiate renin release, leading to local angiotensin formation and hyperadrenergic dysfunction.

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Introduction

We recently reported that mast cells are a unique extrarenal source of renin [1]. We also found that in ischemia/reperfusion, renin is released from cardiac mast cells and is pivotal for activating a local renin-angiotensin system that promotes exocytotic and carrier-mediated norepinephrine release leading to reperfusion arrhythmias [2]. Mast cells are the principal target of immediate-type hypersensitivity reactions, culminating in mast cell degranulation and release of a broad spectrum of preformed mediators [3]. Accordingly, we speculated that immediate hypersensitivity may also elicit renin release from mast cells and thereby activate a local renin-angiotensin system. We tested this hypothesis in 'cardiac

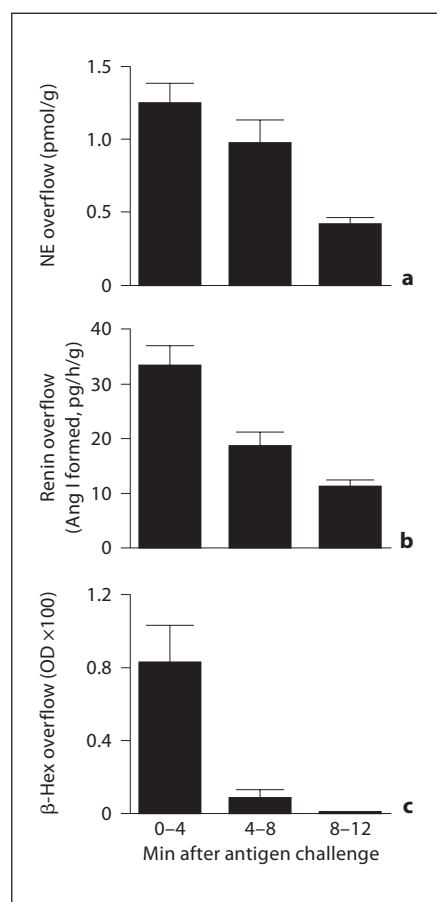


Fig. 1. Type I immediate hypersensitivity elicits the release of mast cell renin in isolated guinea pig hearts. Time course of overflows of norepinephrine (NE; **a**), active renin (i.e. angiotensin-I-forming activity; **b**) and β -hexosaminidase (β -hex, a marker of mast cell degranulation; **c**) from 8 Langendorff-perfused hearts subjected to anaphylaxis ex vivo. Hearts were excised from guinea pigs presensitized with rabbit anti-ovalbumin cytotoxic IgG and challenged with ovalbumin. Bars represent means $\pm$ SEM of coronary overflows in 3 subsequent 4-min intervals after antigen injection. Neither β -hexosaminidase nor renin were present in the coronary effluent preceding antigen, whereas a minimal amount of norepinephrine overflowed in basal conditions (0.23 ± 0.01 pmol/g/min). Ang I = Angiotensin I; OD = optical density.

anaphylaxis', an ex vivo model of immediate-type hypersensitivity which enables an analysis of mediators released from local mast cells [4–6]. We found that mast cell degranulation, elicited by antigen challenge of hearts isolated from presensitized guinea pigs, results in the release of active renin and norepinephrine, a process which is inhibited by classical mast cell-stabilizing agents and by selective renin inhibition.

Materials and Methods

Cardiac Anaphylaxis

Male Hartley guinea pigs (300–350 g; Charles River) were passively sensitized by intraperitoneal administration of 0.8 mg/ml rabbit anti-ovalbumin IgG (Rockland Immunochemicals) [5, 7]. Twenty-four hours later, the animals were anesthetized with CO₂ and euthanized by exsanguination (Institutional Animal Care and Use Committee approved). Hearts were isolated and perfused at constant pressure (40 cm H₂O) in a Langendorff apparatus (Radnoti) with Krebs-Henseleit buffer (KHB) containing NaCl 120 mM, KCl 4.7 mM, CaCl₂ 2.5 mM, MgSO₄ 1.2 mM, KH₂PO₄ 1.2 mM, NaHCO₃ 25 mM, glucose 11 mM, and EDTA 0.5 mM, equilibrated with 95% O₂ and 5% CO₂ at 37°C, pH 7.4. Control hearts were perfused with KHB for 20 min prior to antigen challenge (intra-aortic injection of ovalbumin 1 mg/0.5 ml; Sigma-Aldrich). Other hearts were continuously perfused with KHB containing cromolyn (300 μ) or lodoxamide (10 μ M), immediately upon isolation, or with BILA2157 (100 μ M), 10 min before antigen challenge. Coronary flow was measured by effluent collections every 2 min up to 12 min. All samples were assayed for β -hexosaminidase, renin and norepinephrine.

β -Hexosaminidase Assay

β -Hexosaminidase was assayed using a modification of the method of Schwartz et al. [8]. Twenty microliter samples of coronary effluent were placed in the well of a 96-well plate. Fifty microliters of substrate solution (*p*-nitrophenyl-N-acetyl- β -D-glucosaminide, 1.3 mg/ml in 0.1 M citrate buffer, pH 4.5) were added to each well and incubated for 90 min at 37°C. The reaction was stopped by adding 150 μ l of 0.2 M glycine (pH 10.7). The optical density was read at 405 nm on a spectrophotometric plate reader using SoftMax Pro 4.8. The mean optical density was calculated for each sample (samples were run in quadruplicate) and then divided by the total volume of the original sample.

Renin Assay

Coronary effluent was immediately concentrated 8-fold by centrifugal filtration (Millipore). Concentrated samples were incubated overnight with human angiotensinogen (240 nM; Calbiochem). Renin activity (angiotensin I formed) was determined by GammaCoat Plasma Renin Activity ¹²⁵I Radioimmunoassay (DiaSorin). The detection limit was approximately 0.01 pmol [2].

Norepinephrine Assay

Norepinephrine was assayed by HPLC with electrochemical detection as previously described [9]. The detection limit was approximately 0.2 pmol.

Results and Discussion

Following antigen challenge of spontaneously beating hearts isolated from presensitized guinea pigs, β -hexosaminidase, a marker of mast cell degranulation [8], was copiously released into the coronary effluent (fig. 1c). The overflow of β -hexosaminidase was maximal during the first 4 min after antigen challenge (approximately 90% of

total) and declined to the remaining 10% of total in the following 4 min. No β -hexosaminidase overflow occurred prior to antigen challenge or upon a subsequent antigen injection, verifying that a full anaphylactic reaction had taken place. Notably, antigen challenge resulted in the overflow of angiotensin-I-forming activity (fig. 1b). This activity was predominantly ascribable to renin, since it was largely (approximately 65%) blocked by the selective renin inhibitor BILA2157 (100 nM) [10] (fig. 2b). Renin overflow peaked in the first 4 min after antigen challenge (53% of total) and then declined over the next 8 min (33 and 14% of total was released in the 4–8- and 8–12-min postantigen intervals, respectively) (fig. 1b). No renin overflow occurred prior to antigen challenge or upon a subsequent antigen injection. The magnitude of renin release was comparable with that previously observed by us in isolated guinea pig hearts subjected to ischemia/reperfusion [2]. BILA2157 had no effect on coronary flow in control conditions, nor did it modify the decrease in coronary flow which is characteristic of cardiac anaphylaxis [5]. Thus, it is unlikely that renin release from local mast cells contributes to anaphylactic coronary vascular constriction. On the other hand, mast cell stabilization with lodoxamide antagonized anaphylactic coronary constriction by about 25%. This effect is consistent with an inhibition of the release of mast cell-derived vasoconstricting mediators (e.g., TxA_2 , $\text{PGF}_{2\alpha}$, LTC_4 and D_4 , and PAF) [5]. Although nitric oxide has been shown to be released from the anaphylactic heart [11], it is unlikely that its release is directly related to that of mast cell-derived renin. That renin release might indirectly affect nitric oxide production in cardiac anaphylaxis cannot be excluded on the basis of our present findings.

Following antigen challenge, there was a large increase in the overflow of norepinephrine into the coronary effluent (fig. 1a). Norepinephrine overflow peaked in the first 4 min of anaphylaxis (approximately 47% of total) and declined over the next 8 min (37 and 16% of total was released in the 4- to 8- and 8- to 12-min postantigen intervals, respectively). Norepinephrine overflow prior to antigen challenge was minimal (0.23 ± 0.01 pmol/g/min).

When hearts were pretreated with the mast cell-stabilizing agents cromolyn (300 μM) or lodoxamide (10 μM), the coronary overflow of renin following antigen challenge was 75–80% lower than that of hearts undergoing anaphylaxis without mast cell stabilization (fig. 2b). This reduction in renin release was associated with an approximately 40% decrease in β -hexosaminidase overflow in hearts perfused with each of the 2 mast cell stabilizers

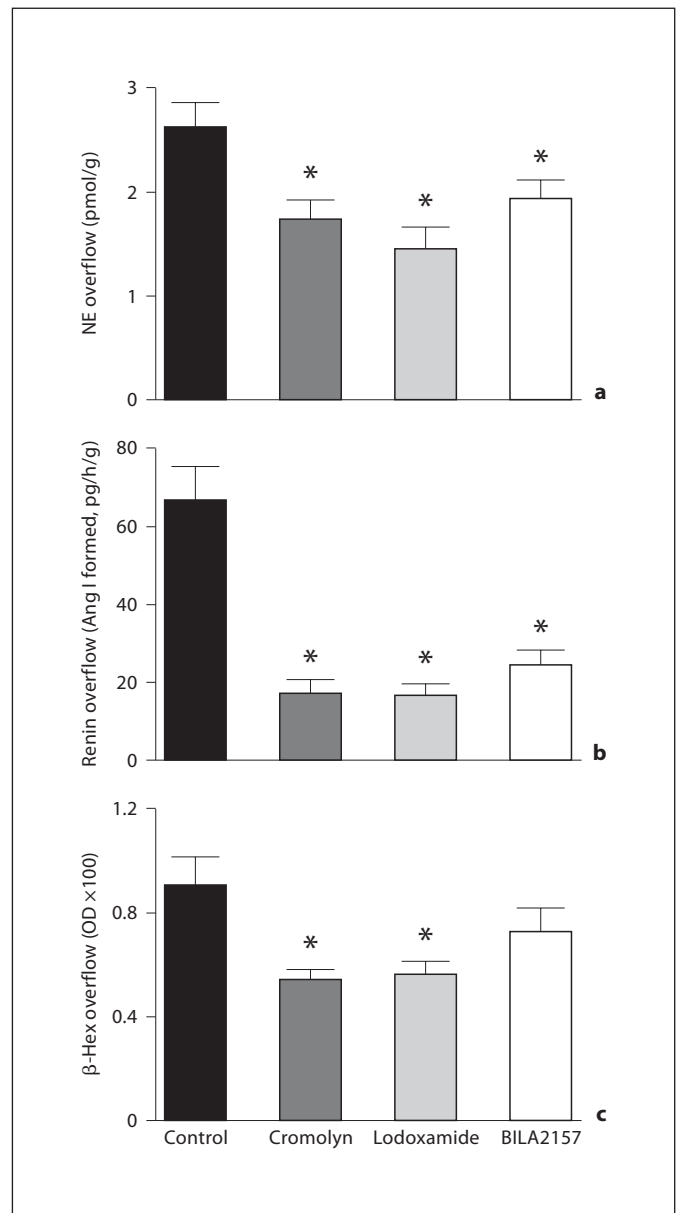


Fig. 2. Pharmacological mast cell stabilization inhibits the release of β -hexosaminidase, renin and norepinephrine from isolated guinea pig hearts undergoing anaphylaxis ex vivo, while selective renin inhibition curtails norepinephrine release. Coronary overflow of norepinephrine (NE; **a**), active renin (i.e. angiotensin-I-forming activity; **b**) and β -hexosaminidase (β -hex; **c**) from 30 Langendorff-perfused hearts exposed to anaphylaxis. Hearts were excised from guinea pigs presensitized with rabbit anti-ovalbumin cytotoxic IgG and challenged with ovalbumin in the absence (control, $n = 8$) or presence of mast cell-stabilizing agents (cromolyn, 300 μM , $n = 6$; lodoxamide, 10 μM , $n = 7$) or the selective renin inhibitor BILA2157 (100 nM, $n = 9$). Ang I = Angiotensin I; OD = optical density. Bars represent means $\pm$ SEM for overflow collected over 12 min following antigen injection. * $p < 0.05$, significantly different from control, by 1-way ANOVA with Dunnett's post-hoc test.

(fig. 2c), indicating that mast cell degranulation was inhibited. The finding that mast cell stabilization was accompanied by a marked reduction of renin release into the coronary effluent clearly establishes the mast cell as a major source of renin released in the heart by immediate-type hypersensitivity reactions. BILA2157 did not significantly affect the overflow of β -hexosaminidase (fig. 2c); indeed, one would not expect that, once released, renin would influence mast cell degranulation. This also demonstrates that BILA2157 was devoid of nonspecific effects on mast cell degranulation. It is unlikely that basophils participated in the release of renin, since the hearts were extensively perfused with KHB solution prior to antigen challenge, so as to wash out any blood cells from the coronary circulation. Furthermore, we had determined that rat basophil leukemia cells do not contain renin (unpublished observation).

The anaphylactic overflow of norepinephrine was decreased by cromolyn, lodoxamide and BILA2157, implicating mast cell-derived renin in the release of norepinephrine (fig. 2a). We had reported that the intracardiac release of mast cell-derived renin activates a local renin-angiotensin system [2], due to the presence in the interstitial fluid of angiotensinogen and angiotensin-converting enzyme [12, 13]. Once locally formed, angiotensin activates AT₁ receptors on cardiac sympathetic nerve endings promoting norepinephrine release by vesicular exocytosis and carrier-mediated nonvesicular release [14]. Interestingly, whereas the coronary overflow of renin in cardiac anaphylaxis was comparable with that previously observed in ischemia/reperfusion [2], norepinephrine overflow was 100-fold lower in cardiac anaphylaxis than in ischemia/reperfusion. One explanation is that norepinephrine release was curtailed by anaphylactic mediators such as prostanoids [5], known to reduce sympathetic transmitter release [15, 16]. More likely, in the ischemia/reperfusion model, norepinephrine release is predominantly carrier-mediated, a process initiated by Na⁺/H⁺ exchanger activation [17] and potentiated by locally formed angiotensin [2, 14]. The quantity of norepinephrine released by the carrier-mediated process is much greater than that released by exocytosis [17]. In fact, in cardiac anaphylaxis, the relatively minor release of norepinephrine is most likely exocytotic [18].

Although cardiac renin has been viewed by some as of renal origin and taken up from the circulation [19, 20], a large body of evidence supports the existence of an independent cardiac renin-angiotensin system [12, 13, 21, 22]. Our recent discovery that cardiac mast cells are a novel source of renin and that mast cells have the capacity to

synthesize renin de novo [1] is further proof of the existence of a local cardiac renin-angiotensin system.

Our present findings have potential clinical implications. Mast cells are known to accumulate in the hearts of patients who succumb to severe anaphylactic reactions [23], and immunoreactive renin is present in mast cells in the human heart (Reid, Silver and Levi, unpublished). Thus, it is conceivable that the reported association between hypersensitivity and cardiac dysfunctions [24] might be related not only to the action of traditional hypersensitivity mediators [5, 25], but also to the intracardiac release of mast cell-derived renin. The consequent activation of a local renin-angiotensin system and the powerful vasoconstricting and norepinephrine-releasing effects of angiotensin [26] could mechanistically explain the instances of coronary spasm, angina, ST-segment elevation and infarction reported in the clinical literature [27–30]. Besides ischemia/reperfusion [2] and immediate hypersensitivity (fig. 1, 2), other conditions such as stress [31] and agents known to degranulate mast cells, such as endothelin-1 [32] and thrombin [33], could conceivably elicit renin release from cardiac mast cells. Whether a local renin-angiotensin system could also be activated in these circumstances remains to be established.

In conclusion, we report the novel finding that immediate-type hypersensitivity reactions elicit local release of renin as a result of mast cell degranulation. Type I immediate hypersensitivity appears to be as powerful a stimulus for the release of renin from cardiac mast cells as ischemia/reperfusion. As renin is stored in human heart mast cells, allergic reactions could initiate renin release, leading to local angiotensin formation, and thus, to hyperadrenergic and ischemic dysfunction.

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References

- 1 Silver RB, Reid AC, Mackins CJ, Askwith T, Schaefer U, Herzlinger D, Levi R: Mast cells: a unique source of renin. *Proc Natl Acad Sci USA* 2004;101:13607–13612.
- 2 Mackins CJ, Kano S, Seyedi N, Schafer U, Reid AC, Machida T, Silver RB, Levi R: Cardiac mast cell-derived renin promotes local angiotensin formation, norepinephrine release, and arrhythmias in ischemia/reperfusion. *J Clin Invest* 2006;116:1063–1070.

- 3 Schwartz LB: Mast cells: function and contents. *Curr Opin Immunol* 1994;6:91–97.
- 4 Graver LM, Robertson DA, Levi R, Becker CG, Weksler BB, Gay WA: IgE-mediated hypersensitivity in human heart tissue: histamine release and functional changes. *J Allergy Clin Immunol* 1986;77:709–714.
- 5 Levi R: Cardiac anaphylaxis: models, mediators, mechanisms and clinical considerations; in Marone G, Lichtenstein LM, Condorelli M, Fauci AS (eds): *Human Inflammatory Disease*, Clinical Immunology. Toronto, Decker, 1988, pp 93–105.
- 6 Rubin LE, Levi R: Protective role of bradykinin in cardiac anaphylaxis. Coronary-vasodilating and antiarrhythmic activities mediated by autocrine/paracrine mechanisms. *Circ Res* 1995;76:434–440.
- 7 Feigen GA, Prager DJ: Experimental cardiac anaphylaxis. Physiologic, pharmacologic and biochemical aspects of immune reactions in the isolated heart. *Am J Cardiol* 1969;24:474–491.
- 8 Schwartz LB, Austen KF, Wasserman SI: Immunologic release of β -hexosaminidase and β -glucuronidase from purified rat serosal mast cells. *J Immunol* 1979;123:1445–1450.
- 9 Seyedi N, Mackins CJ, Machida T, Reid AC, Silver RB, Levi R: Histamine H_3 -receptor-induced attenuation of norepinephrine exocytosis: a decreased protein kinase A activity mediates a reduction in intracellular calcium. *J Pharmacol Exp Ther* 2005;312:1–9.
- 10 Simoneau B, Lavallee P, Anderson PC, Bailey M, Bantle G, Berthiaume S, Chabot C, Fazal G, Halmos T, Ogilvie WW, Poupart MA, Thavonekham B, Xin Z, Thibeault D, Bolger G, Panzenbeck M, Winkquist R, Jung GL: Discovery of non-peptidic P2-P3 butanediamide renin inhibitors with high oral efficacy. *Bioorg Med Chem* 1999;7:489–508.
- 11 Bani D, Nistri S, Mannaioni PF, Masini E: Cardiac anaphylaxis: pathophysiology and therapeutic perspectives. *Curr Allergy Asthma Rep* 2006;6:14–19.
- 12 Dostal DE, Baker KM: The cardiac renin-angiotensin system – conceptual, or a regulator of cardiac function? *Circ Res* 1999;85:643–650.
- 13 Bader M, Peters J, Baltatu O, Müller DN, Luft FC, Ganten D: Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med* 2001;79:76–102.
- 14 Reid AC, Mackins CJ, Seyedi N, Levi R, Silver RB: Coupling of angiotensin II AT_1 receptors to neuronal NHE activity and carrier-mediated norepinephrine release in myocardial ischemia. *Am J Physiol Heart Circ Physiol* 2004;286:H1448–H1454.
- 15 Boehm S, Kubista H: Fine tuning of sympathetic transmitter release via ionotropic and metabotropic presynaptic receptors. *Pharmacol Rev* 2002;54:43–99.
- 16 Levi R, Seyedi N, Schaefer U, Estephan R, Mackins CJ, Tyler E, Silver RB: Histamine H_3 -receptor signaling in cardiac sympathetic nerves: identification of a novel MAPK- PLA_2 -COX-PGE $_2$ -EP $_3$ R pathway. *Biochem Pharmacol* 2007;73:1146–1156.
- 17 Levi R, Smith NCE: Histamine H_3 -receptors: a new frontier in myocardial ischemia. *J Pharmacol Exp Ther* 2000;292:825–830.
- 18 Mest H-J, Pönicke K, Heinroth-Hoffmann I, Meissner N, Giessler C: Relevance of mediators to cardiac parameters of isolated anaphylactic guinea-pig heart. *Prostaglandins Leukot Essent Fatty Acids* 1995;53:21–29.
- 19 Danser AHJ, van Kesteren CAM, Bax WA, Tavenier M, Derkx FHM, Saxena PR, Schalekamp MADH: Prorenin, renin, angiotensinogen, and angiotensin-converting enzyme in normal and failing human hearts: evidence for renin binding. *Circulation* 1997;96:220–226.
- 20 Catanzaro DF: Physiological relevance of renin/prorenin binding and uptake. *Hypertens Res* 2005;28:97–105.
- 21 Campbell DJ: Circulating and tissue angiotensin systems. *J Clin Invest* 1987;79:1–6.
- 22 Lindpaintner K, Ganten D: The cardiac renin-angiotensin system. An appraisal of present experimental and clinical evidence. *Circ Res* 1991;68:905–921.
- 23 Perskvist N, Edston E: Differential accumulation of pulmonary and cardiac mast cell subsets and eosinophils between fatal anaphylaxis and asthma death. A postmortem comparative study. *Forensic Sci Int* 2007;169:43–49.
- 24 Criqui MH, Lee ER, Hamburger RN, Klauber MR, Coughlin SS: IgE and cardiovascular disease. Results from a population-based study. *Am J Med* 1987;82:964–968.
- 25 Marone G, Bova M, Detoraki A, Onorati AM, Rossi FW, Spadaro G: The human heart as a shock organ in anaphylaxis. *Novartis Found Symp* 2004;257:133–149.
- 26 De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T: International union of pharmacology. 23. The angiotensin II receptors. *Pharmacol Rev* 2000;52:415–472.
- 27 Kounis NG, Grapsas ND, Goudevenos JA: Unstable angina, allergic angina, and allergic myocardial infarction. *Circulation* 1999;100:156e.
- 28 Fujita Y, Chikamitsu M, Kimura M, Toriumi T, Endoh S, Sari A: An anaphylactic reaction possibly associated with an intraoperative coronary artery spasm during general anesthesia. *J Clin Anesth* 2001;13:221–226.
- 29 Goldhaber-Fiebert S, Grecu L: Postoperative ST-segment elevation: was vasospasm caused by anaphylaxis or by its treatment with epinephrine? *Ann Allergy Asthma Immunol* 2006;97:449–453.
- 30 Del Furia F, Querceto L, Testi S, Santoro GM: Acute ST-segment elevation myocardial infarction complicating amoxicillin-induced anaphylaxis: a case report. *Int J Cardiol* 2007;117:e37–e39.
- 31 Huang M, Pang XZ, Letourneau R, Boucher W, Theoharides TC: Acute stress induces cardiac mast cell activation and histamine release, effects that are increased in apolipoprotein E knockout mice. *Cardiovasc Res* 2002;55:150–160.
- 32 Maurer M, Wedemeyer J, Metz M, Piliponsky AM, Weller K, Chatterjea D, Clouthier DE, Yanagisawa MM, Tsai M, Galli SJ: Mast cells promote homeostasis by limiting endotoxin-1-induced toxicity. *Nature* 2004;432:512–516.
- 33 Dugina TN, Kiseleva EV, Glusa E, Strukova SM: Activation of mast cells induced by agonists of proteinase-activated receptors under normal conditions and during acute inflammation in rats. *Eur J Pharmacol* 2003;471:141–147.