

Ramipril and metoprolol intake aggravate human and murine anaphylaxis: Evidence for direct mast cell priming

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Background: Cofactors contribute to the elicitation of anaphylaxis. β -Blockers and angiotensin-converting enzyme (ACE) inhibitors are widely used cardiovascular drugs. We specially designed a mouse model to further analyze the cofactor potential of these drugs.

Objective: We sought to test the hypothesis that β -blockers and ACE inhibitors alter the risk for severe anaphylaxis and to pinpoint the associated mechanism.

Methods: The risk factor potency of cardiovascular drugs on the severity of anaphylaxis in patients from German-speaking countries was analyzed. *In vivo* interaction of the cardiovascular drugs metoprolol (β -blocker) and ramipril (ACE inhibitor) with the anaphylactic response was determined. Mast cell (MC) mediators (histamine, serotonin, leukotriene C₄, prostaglandin D₂, and mouse mast cell protease 1) were quantified in serum. Bone marrow–derived cultured MCs served to identify whether the therapeutics targeted MCs directly.

Results: Our anaphylaxis database indicated a higher risk of severe anaphylaxis after monotherapy with β -blockers or ACE inhibitors, which was more pronounced when both drugs were combined. This was confirmed in our mouse model. While single therapeutics had either no significant (ramipril) or a modestly aggravating (metoprolol) effect, their combined administration exacerbated anaphylactic symptoms potently and simultaneously enhanced MC mediators, hinting at MCs as direct targets. In fact, Fc ϵ RI-mediated MC histamine release was synergistically increased by metoprolol/ramipril or metoprolol/bradykinin (the latter increased after ACE inhibitor intake), whereas the substances had no significant effect on their own. MC priming was particularly pronounced when Fc ϵ RI aggregation was in the suboptimal range, reflecting common clinical settings.

Conclusion: β -Blockers and ACE inhibitors synergistically aggravate anaphylaxis at least partly by decreasing the threshold of MC activation. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Anaphylaxis, β -blocker, angiotensin-converting enzyme inhibitor, cofactor, mast cells, cardiovascular medication

Abbreviations used

ACE:	Angiotensin-converting enzyme
BMcMCs:	Bone marrow–cultured mast cell
cAMP:	Cyclic AMP
LT:	Leukotriene
MC:	Mast cell
mMCP-1:	Mouse mast cell protease 1
PGD ₂ :	Prostaglandin D ₂
PK:	Protein kinase
PSA:	Passive systemic anaphylaxis

Anaphylaxis is a severe IgE-mediated and mast cell (MC)–dependent hypersensitivity reaction with a potentially fatal outcome. Together with cutaneous and gastrointestinal symptoms, the respiratory and cardiovascular systems are frequently involved.^{1–3} The most prevalent elicitors of anaphylaxis are food, drugs, and venom,^{4–6} but reactions do not always occur on contact with the offending allergen, even in the same patient.

In fact, about 30% of reactions are elicited under specific circumstances only, and these observations have led to the concept of so-called augmenting factors or cofactors, which need to combine with the triggering allergen for anaphylaxis to ensue.^{7–10} Drugs are an important group of relevant cofactors.⁵ They can interact at several levels and through different mechanisms with the anaphylactic response. The best understood examples are cases in which the drug itself acts as the allergen (eg, β -lactams), so that MC degranulation is triggered like a classical type I allergic reaction.¹¹ Another group of therapeutics (eg, opioids) activate MCs directly in an IgE-independent manner.^{12,13} The third and largest group comprises a heterogeneous collection (eg, nonsteroidal anti-inflammatory drugs or most contrast media), which generally do not degranulate MCs directly^{14,15} but which can nevertheless affect anaphylactic responses; their mechanisms of action are poorly defined thus far. β -Blockers and angiotensin-converting enzyme (ACE) inhibitors are commonly used in the management of cardiovascular diseases^{16,17} and supposedly belong to the latter category. Their use has increased over the last decades,^{18,19} but the intersection of these drugs with anaphylaxis is not well understood. The question of whether single cardiovascular drugs serve as potentiating factors in anaphylaxis is likewise still a matter of debate.^{20–23} Cardiovascular medication is commonly prescribed as a combination of drugs from different categories because single substances are often insufficient to control blood pressure appropriately.^{24,25} The possibility that such cardiovascular drugs might aggravate anaphylactic responses when used alone or in combination has not been studied in detail thus far. Here we first assess the potential role of β -blockers and ACE inhibitors in the context of anaphylaxis based on data extracted from our extensive registry for

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German-speaking countries,⁸ showing that both therapeutics display modest yet discernible anaphylaxis-promoting effects on their own and more pronounced effects if combined. After establishing a mouse model to investigate the role of potential cofactors on anaphylactic responses, we confirm the above effects from epidemiologic data in the mouse and furthermore show that the β -blocker metoprolol and the ACE inhibitor ramipril have modest anaphylaxis-promoting activities as single substances but display clearly potentiating effects when administered in combination. In search of the underlying mechanism, we identify MCs as direct targets of the drugs, which are primed for enhanced responses to Fc ϵ RI aggregation. Effects preferentially occur when the degree of Fc ϵ RI cross-linking is in the suboptimal range, presumably reflecting the scenario most commonly encountered in clinical settings.

METHODS

Anaphylaxis registry, data extraction, and analysis

The anaphylaxis registry contains data from patients with severe allergic reactions entered by allergists using a standardized online questionnaire, as described previously.³ We analyzed data sets from 4783 patients collected in 2006–2014 and defined 4 groups according to their anaphylaxis severity grades. Severity grading was based on the clinical reaction patterns assessed before first aid treatment was given. The groups were stratified for the variables of use of antihypertensive medication either as monotherapy or in combination or patients without antihypertensive medication.

Mice

Female BALB/c wild-type mice (10–12 weeks old) were obtained from Charles River (Sulzfeld, Germany). Animals were kept under specific pathogen-free conditions in a temperature-controlled environment with free access to standard chow and water. All experiments were approved by the local State Office of Health and Social Affairs.

Passive cutaneous anaphylaxis

Mice received ramipril (2.5 mg/kg; kindly provided by Sanofi-Aventis, Frankfurt, Germany) through drinking water and metoprolol (30 mg/kg dissolved in water; Sigma-Aldrich, Hamburg, Germany) by means of oral gavage, either alone or in combination, for 14 days. Sham-treated mice received water instead. The average amount of daily fluid intake was monitored for 2 weeks before the experiment and during the experiment every day to ensure the mice received the correct dose of ramipril. On day 14, mouse anti-trinitrophenyl-IgE (anti-TNP-IgE; BD Bioscience Pharmingen, San Jose, Calif) was injected subcutaneously (3 ng) into the right ear and PBS alone into the left ear. Twenty-four hours later, mice were challenged with 7 μ g of TNP-BSA (Biocat, Heidelberg, Germany) together with 1% Evans Blue administered intravenously. After 30 minutes, mice were killed, and vascular permeability was visualized based on blue staining of the ear. Skin samples were taken, and Evans blue was extracted with dimethylformamide. The amount of dye was quantified based on OD₆₀₀ measurement.

Passive systemic anaphylaxis

Mice were passively sensitized with anti-TNP-IgE administered intravenously (7 μ g), followed by an intravenous injection of TNP-BSA 24 hours later at the dosages shown in the figure legends. Changes in rectal temperature were assessed for 70 minutes with a digital thermometer (Physitemp Instruments, Clifton, NJ).

After preoptimization of the passive systemic anaphylaxis (PSA) model, mice received oral ramipril and metoprolol alone or in combination for 14 days. Sham-treated mice received water instead. Mice were then sensitized with mouse anti-TNP-IgE, followed 24 hours later by challenge with the

optimized dose of TNP-BSA (7 μ g). Anaphylaxis was determined as above, and plasma samples were collected.

Quantification of MC mediators in serum

Levels of histamine (LDN, Nordhorn, Germany), serotonin (IBL, Hamburg, Germany), leukotriene (LT) C₄, prostaglandin D₂ (PGD₂; Cayman Chemical, Ann Arbor, Mich) and mouse mast cell protease 1 (mMCP-1; eBioscience, Frankfurt, Germany) were measured by using ELISA, according to the manufacturers' instructions.

MCs

Bone marrow specimens were obtained from female BALB/c mice and cultured in IMDM medium (PAA, Cölbe, Germany) in the presence of mouse recombinant IL-3 (ImmunoTools, Friesoythe, Germany) for 4 to 6 weeks at 37°C and 5% CO₂ in a humidified atmosphere to obtain bone marrow–cultured mast cells (BMCMCs), which were identified as MCs by the expression of c-Kit and Fc ϵ RI. BMCMCs were sensitized with anti-TNP-IgE (1 μ g/mL) overnight, washed with PAG-CM buffer (PIPES albumin glucose buffer, pH 7.5, containing 3 mmol/L CaCl₂ and 1.5 mmol/L MgCl₂), and preincubated with different concentrations of ramiprilat (active form of ramipril, kindly provided by Sanofi-Aventis), metoprolol, or bradykinin (Sigma-Aldrich) alone or in several combinations for 20 minutes at 37°C to investigate the effect of cardiovascular medication on histamine release. Drug concentrations were based on clinical plasma concentrations achievable during antihypertensive medication.^{26–28} Cells were activated by the addition of 2 concentrations of anti-IgE (0.4 or 4 μ g/mL; BD Pharmingen, San Jose, Calif) or kept in PAG-CM only (spontaneous release) for 25 minutes at 37°C. Calcium ionophore A23187 (2 μ mol/L; Calbiochem, San Diego, Calif) was used as a positive control to assess the degree of MC release. Total histamine content of the cells was determined after cell lysis with 1% perchloric acid (complete). Histamine release was assessed in cell supernatants by using an autoanalyzer (Borgwald Technik, Hamburg, Germany).^{29,30} Net histamine release was calculated as follows:

$$\text{Net histamine release (\%)} = \frac{[(\text{Stimulated release} - \text{Spontaneous release}) / \text{Complete}] \times 100.$$

Statistical analysis

Logistic regression to compute crude and adjusted odds ratios was performed with STATA software, version 12 (StataCorp, College Station, Tex). All other statistical analyses were performed with PRISM 5.0 software (GraphPad Software, La Jolla, Calif) by using nonparametric 1-way ANOVA, followed by the Bonferroni test. Data are shown as means $\pm$ SEMs. A *P* value of less than .05 was considered statistically significant.

RESULTS

Increased risk for severe anaphylaxis in patients taking ACE inhibitors and β -blockers

It is still controversial whether monotherapies with either β -blockers or ACE inhibitors aggravate anaphylaxis. Therefore we analyzed the effect of β -blocker and ACE inhibitor intake on the severity of anaphylaxis using clinical data from the anaphylaxis registry. A summary of anaphylactic cases collected in 2006–2014 is presented in Table I. We performed a risk factor analysis, which included 4 groups of anaphylactic patients, classified into severity grades according to Ring and Messmer.³¹ Our results show a slight increase in odds ratios for patients who used the combined drugs (Fig 1, A) if severity grades I/II and III/IV were used as dependent variables. This effect was more pronounced when patients with the most severe reactions were analyzed in comparison with patients with less severe

TABLE I. Elicitors and cofactors of anaphylaxis

	Severity grades				Total	
	I	II	III	IV		
Total cases (%)	189 (4)	2756 (58)	1722 (36)	116 (2)	4783	100%
Children (female/male)	47 (24/23)	596 (203/393)	470 (153/317)	10 (2/8)	1123	23%
Adults (female/male)	142 (83/59)	2160 (1280/880)	1252 (702/550)	106 (43/63)	3660	77%
Elicitors (% of total no. in line 1)						
Insect	92 (49)	1410 (51)	807 (47)	58 (50)	2367	49%
Food	40 (21)	638 (23)	535 (31)	9 (8)	1222	26%
Drugs	36 (19)	442 (16)	228 (13)	38 (33)	744	16%
Other	1 (1)	98 (4)	41 (2)	4 (3)	144	3%
Unknown	20 (11)	168 (6)	111 (6)	7 (6)	306	6%
Cofactor involvement						
Yes (% of total no. in line 1)	79 (42%)	1113 (40%)	733 (43%)	72 (62%)	197	42%
Cofactors (% of no. with cofactor involvement)						
Exercise	46 (58)	519 (47)	329 (45)	21 (29)		
Drugs (ACE-i, BB, At2, ASA, PPI)	32 (41)	488 (44)	342 (47)	46 (64)		
Alcohol	3 (4)	73 (7)	63 (9)	3 (4)		
Infection	6 (8)	80 (7)	58 (8)	1 (1)		
Other (stress, menses, others)	13 (16)	202 (18)	128 (17)	17 (24)		
First-line treatment by professionals						
Yes (% of total no. in line 1)	84 (44)	1451 (53)	926 (54)	77 (66)	2538	53%
Adrenaline (% of no. with first-line treatment)	1 (1)	238 (16)	236 (25)	51 (66)	526	21%

Anaphylaxis severity was assessed according to the method of Ring and Messmer.³¹ Data sets from January 2006 to March 2014 were depicted. In total, 4960 cases were reported to the anaphylaxis registry from Germany, Switzerland, and Austria. For 177 cases, the use of ACE inhibitors or β -blockers was unknown, and thus 4783 cases could be analyzed. ACE-i, ACE inhibitor; ASA, acetylsalicylic acid; At2, angiotensin 2 inhibitor; BB, β -blocker; PPI, proton-pump inhibitor.

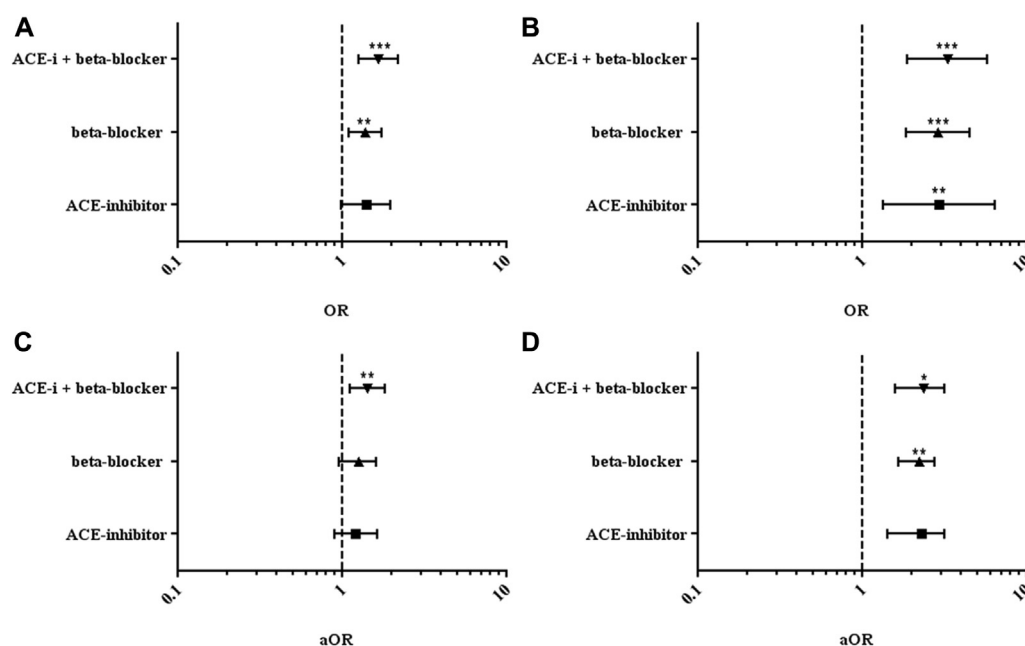


FIG 1. Risk factor calculation of anaphylactic symptoms. **A**, Risk factor calculation for ACE inhibitor (ACE-i) and β -blocker intake with severity grade I/II ($n = 2945$) versus III/IV ($n = 1883$) as dependent variables (crude analysis). **B**, Risk factor calculation for ACE inhibitor and β -blocker intake with severity grade I to III ($n = 4667$) versus severity grade IV ($n = 116$) as dependent variables (crude analysis). **C**, Risk factor calculation as indicated in Fig 1, A, adjusted for sex and age. **D**, Risk factor calculation as indicated in Fig 1, B, adjusted for sex and age. Crude odds ratios (OR) and adjusted odds ratios (aOR) are shown (log 10-scale). ORs and aORs of greater than 1 indicate increased risk of severe anaphylactic symptoms. * $P < .05$, ** $P < .01$, and *** $P < .001$.

reactions (Fig 1, B). When considering other variables with a well-known effect on anaphylaxis, such as sex and age, the results still indicate moderate effect sizes (Fig 1, C and D).

Together, the results suggest that the combined use of these drugs modestly but significantly increases the risk for severe anaphylaxis.

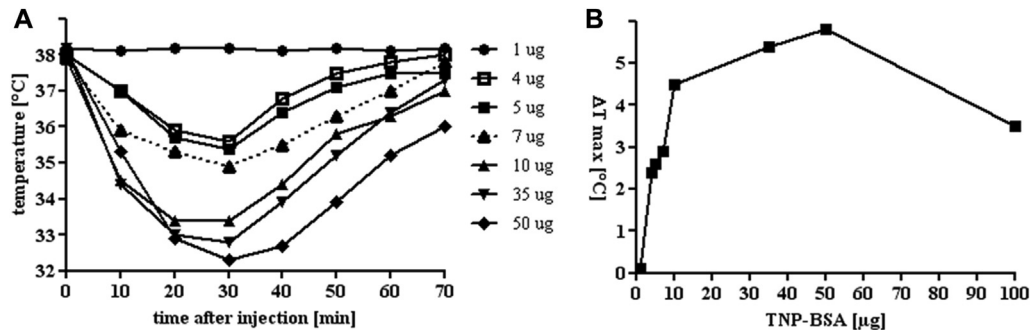


FIG 2. Dose response of TNP-BSA. **A**, Mice were administered anti-TNP-IgE mAb to sensitize MCs and, after 24 hours, injected with different doses of TNP-BSA. Rectal temperature was assessed and is shown as a function of time. **B**, Maximum temperature decrease was calculated for each dose.

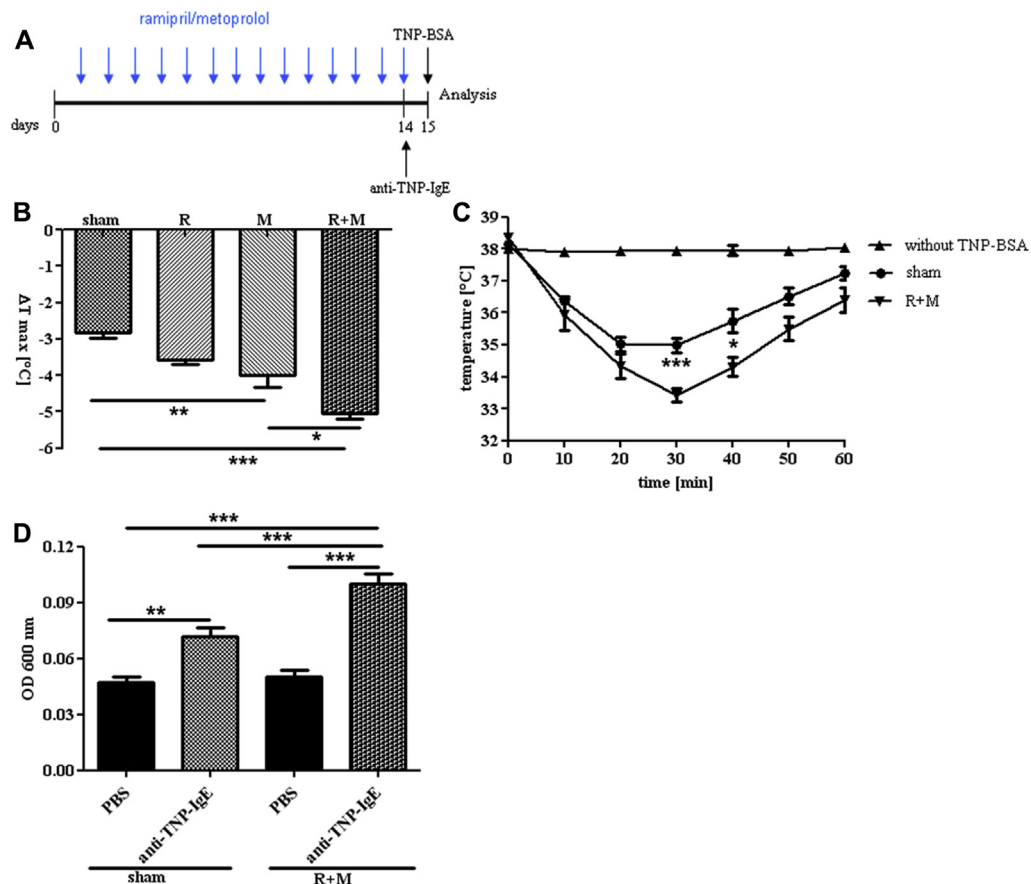


FIG 3. Pretreatment with ramipril (*R*) plus metoprolol (*M*) aggravates PSA and passive cutaneous anaphylaxis (PCA). **A**, BALB/c mice received oral ramipril and metoprolol alone/in combination or water as a negative control for 14 days. PSA was induced as described in the Methods section. **B**, Maximum temperature decrease was calculated. **C**, Body core temperature as a function of time. **D**, PCA was induced as described in the Methods section. Data are represented as means $\pm$ SEMs ($n = 4-6$). * $P < .05$, ** $P < .01$, and *** $P < .001$.

Model of suboptimal anaphylaxis for the study of cofactor potency

It was crucial to identify an allergen dose that, by itself, elicited anaphylaxis in a suboptimal manner to determine the proanaphylactic effect of cofactors in a mouse model. To achieve this, we used a model of PSA in which the dose of the transferred TNP-specific IgE was kept constant and the allergen varied over a broad range of doses. We observed that even small dose differences

resulted in substantial changes in the anaphylactic response, as measured by the decrease in body core temperature. For example, there was a profound difference between 5 and 7 μ g of TNP-BSA (Fig 2, A). At high doses, a further increase did not result in an increment of anaphylaxis but rather led to a reduction in the maximum temperature decrease when compared with the optimum at 50 μ g. The resultant bell-shaped curve is typically observed in patients with allergic reactions (Fig 2, B), but to our

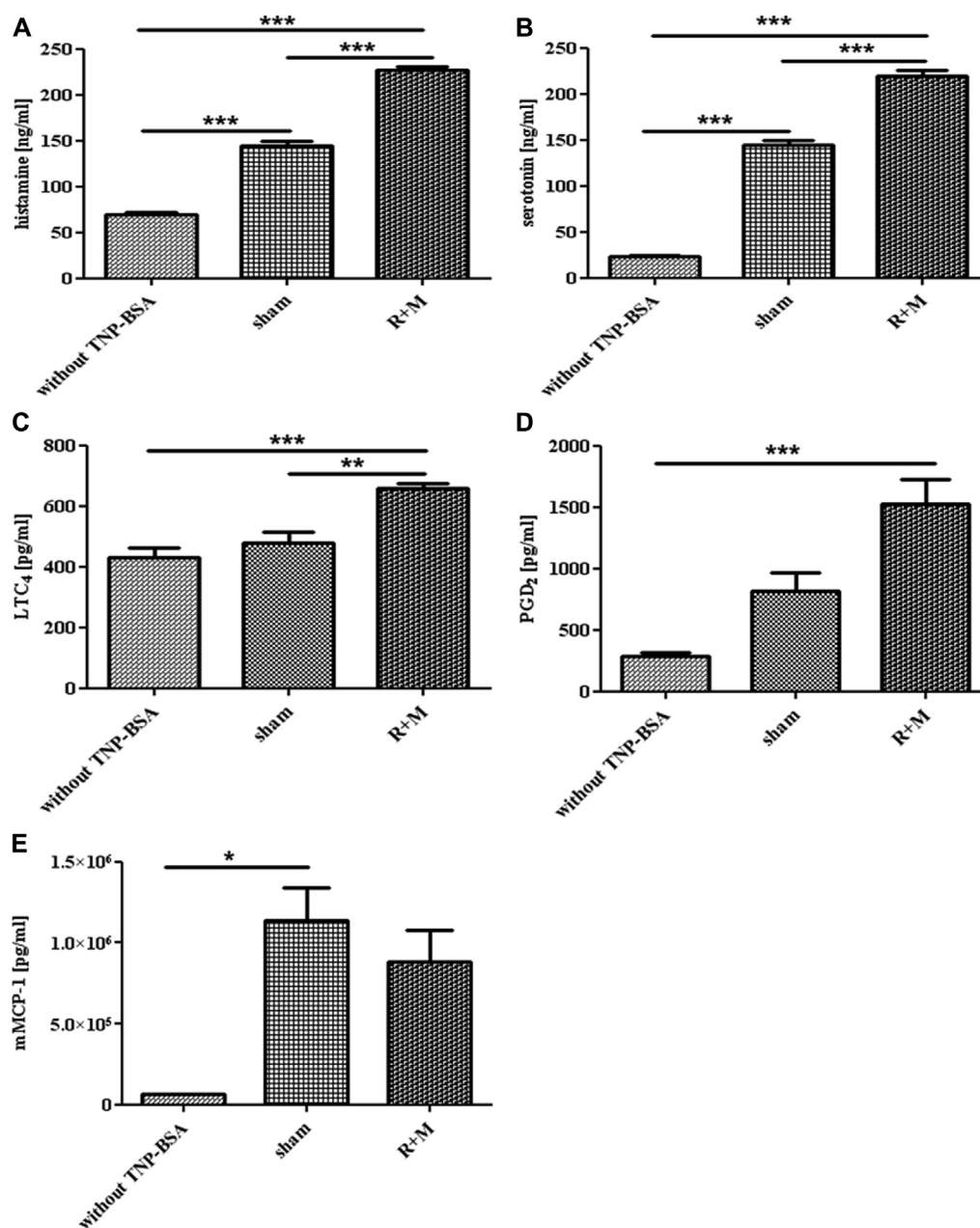


FIG 4. Pretreatment with ramipril and metoprolol (*R+M*) increases levels of MC mediators during anaphylaxis. **A-E**, Plasma levels of various MC mediators were measured by means of ELISA. Data are shown as means $\pm$ SEMs ($n = 4-6$). * $P < .05$, ** $P < .01$, and *** $P < .001$.

knowledge, this has not been formally proved in a model of anaphylaxis. TNP-BSA (7 μ g) induced a temperature decrease of 3°C (Fig 2, A), which was considered adequate for the study of cofactor activity in the forthcoming experiments.

Ramipril and metoprolol cotreatment aggravates anaphylaxis

To assess whether cardiovascular drugs can augment anaphylactic symptoms in a controlled *in vivo* setting, we treated mice with metoprolol, ramipril, or both drugs combined for 14 days (to mimic long-term treatment in patients with cardiovascular diseases) before induction of PSA (Fig 3, A). As shown in Fig 3, B,

ramipril-treated mice exhibited a slight enhancement in hypothermia compared with the sham-treated group (-0.75°C). Although there was a significant decrease in the temperature of mice treated with metoprolol alone (-1.18°C), it was the combination of both drugs that caused the clearest aggravation of hypothermia, resulting in a decrease in core body temperature by an additional 2.22°C (Fig 3, B and C). Together, the data indicate that a single cardiovascular drug can already exacerbate anaphylaxis but that the combined treatment results in a more pronounced augmentation. To determine whether systemic symptoms are reflected by local MC responses, we used the model of passive cutaneous anaphylaxis. Also in this model prior treatment with ramipril and metoprolol increased anaphylactic serum extravasation (Fig 3, D). On

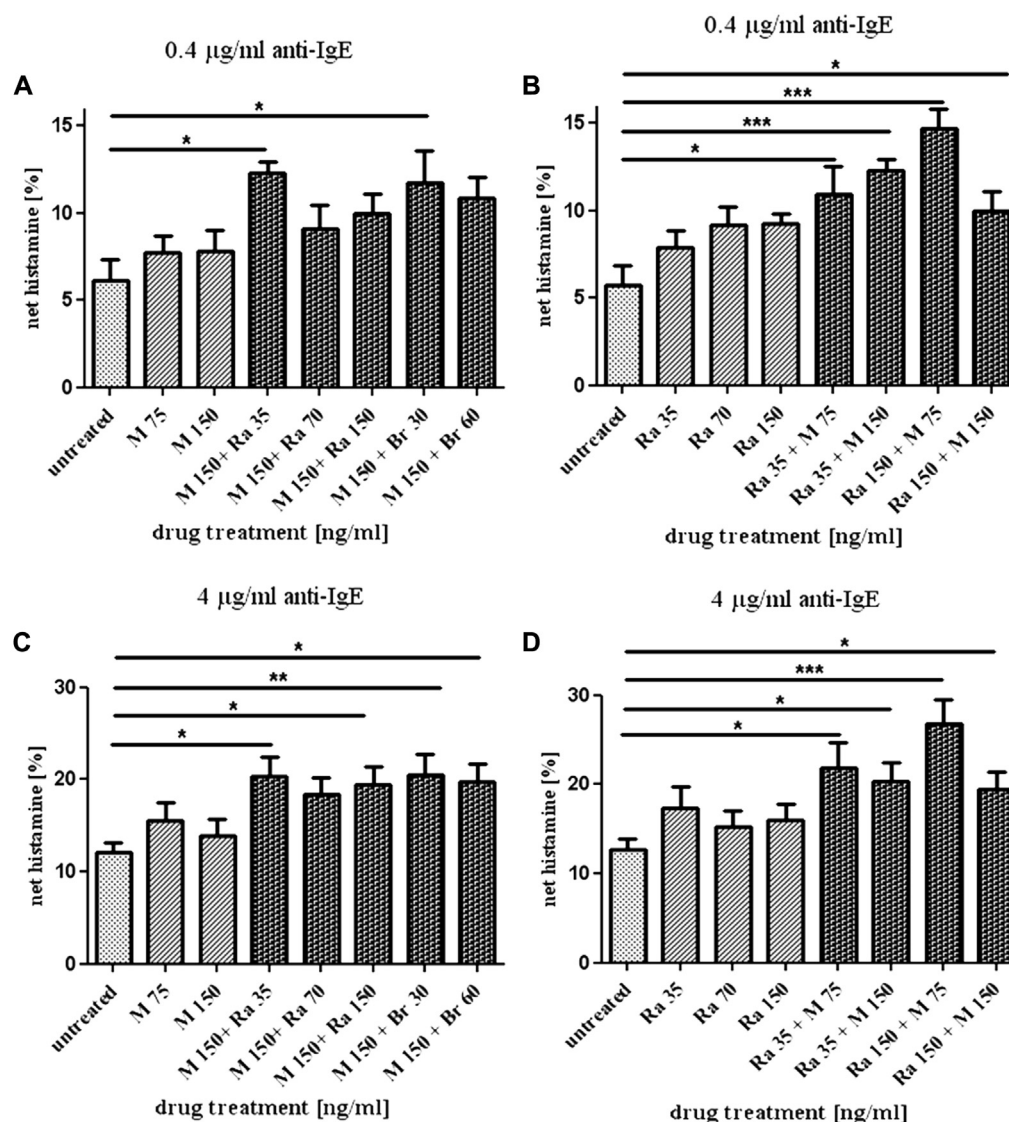


FIG 5. Ramiprilat (*Ra*) and metoprolol (*M*) decrease the threshold for MC activation in a synergistic fashion. BMcMCs were sensitized with IgE for 24 hours, pretreated with antihypertensive drugs for 20 minutes, and activated with 0.4 µg/mL (A and B) or 4 µg/mL (C and D) anti-IgE for a further 25 minutes. Released histamine was quantified from the supernatants and is shown as a percentage of total cellular histamine content. Data are shown as means $\pm$ SEMs ($n = 10-14$). * $P < .05$, ** $P < .01$, and *** $P < .001$ versus control (anti-IgE only).

the basis of these observations, we conclude that the combination of ramipril and metoprolol aggravates anaphylactic responses in systemic but also local IgE-dependent models.

Ramipril and metoprolol enhance MC mediator release during systemic anaphylaxis

MC activation results in the release of various soluble mediators, which can be measured in serum during an anaphylactic episode. These include preformed granule-associated factors, such as histamine, serotonin, and MC-specific proteases, as well as the lipid-derived mediators LTC₄ and PGD₂, which are rapidly generated from arachidonic acid. Here we first verified that these mediators are induced in our PSA model. As shown in Fig 4, PSA was indeed associated with increased levels of histamine and serotonin, as well as increased production of LTC₄ and PGD₂, although LTC₄ levels were only slightly increased. An

even more pronounced increase was found for mMCP-1 in anaphylactic versus sham-treated mice (Fig 4, E). Thus even though anaphylaxis was elicited at a suboptimal level, it was nevertheless mirrored by the accumulation of typical MC mediators in serum. Treatment of mice with ramipril and metoprolol led to clear increments in the levels of histamine, serotonin, LTC₄, and PGD₂. Surprisingly, the mMCP-1 level was slightly (yet not significantly) decreased in drug-treated mice. Together, the aggravated anaphylactic response on ramipril and metoprolol intake (Fig 3) is reflected by the accumulation of mediators released by MCs during an anaphylactic episode (Fig 4).

Ramipril and metoprolol exert proanaphylactic effects by priming MCs

Having found that levels of MC mediators are increased in anaphylactic mice receiving antihypertensive medication, we

addressed the underlying mechanism. Two potential explanations might have accounted for the increased concentrations of MC mediators in drug-treated anaphylactic mice: (1) a direct interaction of the drugs with MCs to directly influence their activation or (2) indirect effects on MC responses through action on neighboring cells and cell-cell communication networks. We addressed the first possibility and treated MCs with metoprolol, ramiprilat, or bradykinin (a vasodilator levels of which are increased after ACE inhibitor intake), either alone or in different combinations and then stimulated by augmenting concentrations of anti-IgE. An identical panel of treatments in the absence of anti-IgE served to determine whether the substances alone could trigger MC degranulation. The degree of MC activation was assessed based on histamine release.

Calcium ionophore A23187 served as a positive control of MC activation (net histamine release, 31.4% to 42.3%; data not shown). Neither the drugs nor bradykinin triggered histamine release when used in the absence of anti-IgE (data not depicted).

Single treatments with the drugs slightly enhanced the MC degranulation triggered by FcεRI without reaching statistical significance. A constant concentration of metoprolol (150 ng/mL) combined with ramiprilat elicited a clearly stronger MC activation than the control without drug pretreatment (Fig 5, A and C). Bradykinin alone slightly increased IgE-mediated histamine release without reaching statistical significance (data not shown). Again, the combination of metoprolol and bradykinin potentiated anti-IgE-mediated activation (Fig 5, A and C). The drug combinations enhanced MC degranulation by up to 92% versus the control on activation with 0.4 μg/mL anti-IgE (Fig 5, A), whereas the increase was less pronounced if the antibody was upscaled to elicit stronger responses on its own (maximum increase by 69%; Fig 5, C). Similar results were obtained in the panels in which both metoprolol and ramiprilat concentrations varied (Fig 5, B and D). Again, the release was significantly enhanced with various combinations of ramiprilat/metoprolol, and this was likewise more pronounced when FcεRI cross-linking was lower (by 156% at 0.4 μg/mL vs 111% at 4 μg/mL anti-IgE). This suggests that the therapeutics preferentially prime MCs when activation by allergen is in the suboptimal range, which is consistent with clinical observations. Overall, we conclude that metoprolol/ramiprilat or metoprolol/bradykinin combination acts directly on MCs to augment their releasability through FcεRI, potentially explaining the increased anaphylactic response on treatment with these drugs (Figs 3 and 4).

DISCUSSION

β-Blockers or ACE inhibitors have been associated with anaphylactic responses, but the data remain somewhat controversial regarding monotherapy with either group of therapeutics. In clinical settings patients commonly receive drug combinations for full control of hypertension.^{24,25} Drug combinations might well act synergistically to increase the small proanaphylactic action of each substance, but little is known about such drug-drug interactions in the context of anaphylaxis. Here the effect of the drugs on symptom severity in anaphylactic patients was analyzed, and patients receiving combined medication with β-blockers and ACE inhibitors were found to be at higher risk for severe anaphylactic symptoms. This effect was diminished when the data were adjusted for sex and age. As we previously reported, age itself is a risk factor for the development of severe anaphylactic symptoms,³ which can explain the lower effect of the drugs on the

age-adjusted data. Nevertheless, our results suggest that these drugs can increase the severity of anaphylactic symptoms, especially when different therapeutics are combined, even though more studies need to be done to accurately calculate the relative risk imposed by the substances.

A mouse model was established that allowed for the identification of cofactors of anaphylaxis to further elucidate whether cardiovascular drugs influence anaphylactic responses. We noticed that even small differences in the dose of the triggering allergen had a substantial effect on the severity of the ensuing anaphylactic response, making it crucial to use a dose producing suboptimal anaphylaxis in the absence of the potentiating factor. Using such preoptimized conditions, we found that ramipril and metoprolol, when administered individually, augmented the severity of anaphylaxis but that the degree of this potentiation was rather weak (not significant in the case of ramipril), fitting the observations in anaphylactic patients. A remarkable effect was achieved with both drugs combined, indicating that it is the concomitant use of different substances that might ultimately create the conditions to set off a clinically relevant anaphylactic reaction.

The present study verifies in a mouse model what has been known from clinical observations for quite a while, namely that several augmenting factors can unite to ultimately decrease the threshold for anaphylaxis to take effect.¹⁰ The individual cofactors can act either at the level of effector cells or at the level of the “shock tissue” reacting to the mediators released from MCs and basophils.

We tested the hypothesis that the medication alters effector cell responsiveness. Because the PSA model used is MC dependent,³² the levels of MC mediators commonly released after FcεRI cross-linking³³ were assessed. Histamine, serotonin, and PGD₂ levels increased after PSA elicitation, even under the suboptimal conditions used here, whereas LTC₄ levels were only slightly increased, suggesting that the unstable molecule LTC₄ might have been metabolized to its degradation products (LTD₄ and LTE₄) at the time of analysis.^{33,34} After treatment with ramipril and metoprolol, levels of MC mediators (including LTC₄) increased versus those seen in untreated anaphylactic mice, indicating that the 2 drugs combined augmented the activatory potential of MCs in a generalized fashion. The only exception was mMCP-1, levels of which increased during anaphylaxis but were slightly reduced (rather than increased) by ramipril and metoprolol. mMCP-1 is specifically produced by MCs in the gut, whereas high amounts of PGD₂ and especially LTC₄ are derived from lung MCs.^{35,36} The results raise the interesting possibility that MCs in the different tissues might react heterogeneously to the medication, hinting at MC variability among organs regarding sensitivity to these drugs. Future studies will be required to address this subject.

The strong association between the severity of anaphylaxis and the increase in levels of MC mediators (except for mMCP-1) implied that the former resulted from an altered potential of MCs to become activated after antihypertensive medication. We addressed this issue by studying the effect of the drugs on MC activation *in vitro*. Neither metoprolol nor ramiprilat had an effect on histamine release in the absence of FcεRI aggregation (data not shown); thus the antihypertensive treatment by itself was unable to degranulate MCs. However, histamine release was enhanced after drug treatment when MCs were triggered by FcεRI aggregation. Although the individual drugs had little effect on FcεRI-mediated degranulation, their combination had an enhancing effect. This effect was more pronounced when allergen

concentrations were low. ACE inhibitors are suspected to modulate allergic responses through the stabilization of bradykinin,³⁷⁻³⁹ and therefore we also tested the effect of bradykinin. Bradykinin slightly increased FcεRI-induced degranulation, and again, this effect was potentiated by metoprolol. Taken together, ACE inhibitors seem to augment MC responses either through direct interaction with MCs or through stabilization of bradykinin, which then secondarily increases MC releasability.

MCs are known to express functional β -adrenoceptors, the activation of which leads to G protein-mediated adenylylase stimulation and subsequent cyclic AMP (cAMP) accumulation. This is followed by activation of the cAMP/protein kinase (PK) A cascade, a negative regulator of allergen-induced MC responses,⁴⁰⁻⁴² and inhibition of PKC. Because β_1 -adrenoceptors are expressed by MCs, it is conceivable that the β_1 -antagonist metoprolol mediates its proanaphylactic effect through inhibition of the cAMP/PKA cascade. MC histamine release critically depends on calcium influx⁴³ and translocation of PKC to the cell membrane.^{44,45} Interestingly, the ACE inhibitor captopril was reported to activate MCs by increasing their permeability for calcium, which led to a direct influx of extracellular calcium into the cytosol to enhance exocytosis.^{46,47} We assume that ramiprilat can act in a similar manner. In addition, ACE inhibition can lead to an accumulation of bradykinin and kinin-assisted mediator release from MCs.^{48,49}

It is important to realize that the above interactions of the therapeutics with MCs are probably weak, require high doses, and do not commonly occur in clinical settings. In fact, the β -blocker alone, or when combined with ramipril, did not induce an MC-dependent response in mice in the absence of allergen (data not illustrated). In addition, the drugs (either alone or in several combinations) did not induce MC degranulation on their own (data not depicted). Even when combined with FcεRI triggering, the drugs had a subtle effect when used as single substances. Therefore even though individual drugs might contribute to anaphylactic responses only marginally, the combination of several suboptimal events could be sufficient to eventually trigger anaphylaxis.

Taken together, our data provide evidence that antihypertensive medication with ACE inhibitors and β -blockers augments anaphylactic reactions and that this is at least partially caused by direct MC priming. Our model might not only help to identify additional cofactors of anaphylaxis and their mechanisms, through which they operate, but also uncover drug combinations with more favorable safety profiles. This is crucial in view of the large number of patients who critically depend on daily medication with such molecules.

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Clinical implications: Patients receiving cardiovascular drugs, such as ACE inhibitors, β -blockers, or both, have an increased risk of severe anaphylactic symptoms caused by the priming of MCs toward enhanced responsiveness to Fc ϵ RI aggregation.

REFERENCES

- Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. *J Allergy Clin Immunol* 2002;110:341-8.
- Triggiani M, Patella V, Staiano RI, Granata F, Marone G. Allergy and the cardiovascular system. *Clin Exp Immunol* 2008;153(suppl 1):7-11.
- Worm M, Edenharter G, Rueff F, Scherer K, Pfohler C, Mahler V, et al. Symptom profile and risk factors of anaphylaxis in Central Europe. *Allergy* 2012;67:691-8.
- Ben-Shoshan M, Clarke AE. Anaphylaxis: past, present and future. *Allergy* 2011;66:1-14.
- Beyer K, Eckermann O, Hompes S, Grabenhenrich L, Worm M. Anaphylaxis in an emergency setting—elicitors, therapy and incidence of severe allergic reactions. *Allergy* 2012;67:1451-6.
- Helbling A, Humi T, Mueller UR, Pichler WJ. Incidence of anaphylaxis with circulatory symptoms: a study over a 3-year period comprising 940,000 inhabitants of the Swiss Canton Bern. *Clin Exp Allergy* 2004;34:285-90.
- Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008;63:226-32.
- Worm M, Babina M, Hompes S. Causes and risk factors for anaphylaxis. *J Dtsch Dermatol Ges* 2013;11:44-50.
- Hompes S, Kohli A, Nemat K, Scherer K, Lange L, Rueff F, et al. Provoking allergens and treatment of anaphylaxis in children and adolescents—data from the anaphylaxis registry of German-speaking countries. *Pediatr Allergy Immunol* 2011;22:568-74.
- Wolbing F, Fischer J, Koberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. *Allergy* 2013;68:1085-92.
- Thong BY, Yeow C. Anaphylaxis during surgical and interventional procedures. *Ann Allergy Asthma Immunol* 2004;92:619-28.
- Grosman N. Histamine release from isolated rat mast cells: effect of morphine and related drugs and their interaction with compound 48/80. *Agents Actions* 1981;11:196-203.
- Sheen CH, Schleimer RP, Kulka M. Codeine induces human mast cell chemokine and cytokine production: involvement of G-protein activation. *Allergy* 2007;62:532-8.
- Trcka J, Schmidt C, Seitz CS, Brocker EB, Gross GE, Trautmann A. Anaphylaxis to iodinated contrast material: nonallergic hypersensitivity or IgE-mediated allergy? *AJR Am J Roentgenol* 2008;190:666-70.
- Webb LM, Lieberman P. Anaphylaxis: a review of 601 cases. *Ann Allergy Asthma Immunol* 2006;97:39-43.
- Mueller UR. Cardiovascular disease and anaphylaxis. *Curr Opin Allergy Clin Immunol* 2007;7:337-41.
- Lopez-Sendon J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, et al. Expert consensus document on angiotensin converting enzyme inhibitors in cardiovascular disease. The Task Force on ACE-inhibitors of the European Society of Cardiology. *Eur Heart J* 2004;25:1454-70.
- Poulos LM. Trends in hospitalization for anaphylaxis, angioedema, and urticaria in Australia, 1193-1994 to 2004-2005. *Am Acad Allergy Asthma Immunol* 2007;120:878-84.
- Siegel D, Lopez J. Trends in antihypertensive drug use in the United States: do the JNC V recommendations affect prescribing? Fifth Joint National Commission on the Detection, Evaluation, and Treatment of High Blood Pressure. *JAMA* 1997;278:1745-8.
- Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009;124:1047-54.
- Stoevesandt J, Hain J, Kerstan A, Trautmann A. Over- and underestimated parameters in severe Hymenoptera venom-induced anaphylaxis: cardiovascular medication and absence of urticaria/angioedema. *J Allergy Clin Immunol* 2012;130:698-704.e1.
- Renaudin JM, Beau douin E, Ponvert C, Demoly P, Moneret-Vautrin DA. Severe drug-induced anaphylaxis: analysis of 333 cases recorded by the Allergy Vigilance Network from 2002 to 2010. *Allergy* 2013;68:929-37.
- Brown SG, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin Immunol* 2013;132:1141-9.e5.
- Ryan TJ, Anderson JL, Antman EM, Braniff BA, Brooks NH, Califf RM, et al. ACC/AHA guidelines for the management of patients with acute myocardial infarction: executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Acute Myocardial Infarction). *Circulation* 1996;94:2341-50.
- Liu H, Xing X, Huang L, Huang Z, Yuan H. The expression level of myocardial beta1-adrenergic receptor affects metoprolol antihypertensive effects: a novel mechanism for interindividual difference. *Med Hypotheses* 2013;81:71-2.
- Dayer P, Leemann T, Marmy A, Rosenthaler J. Interindividual variation of beta-adrenoceptor blocking drugs, plasma concentration and effect: influence of genetic status on behaviour of atenolol, bopindolol and metoprolol. *Eur J Clin Pharmacol* 1985;28:149-53.
- Grafe M, Bossaller C, Graf K, Auch-Schwelk W, Baumgarten CR, Hildebrandt A, et al. Effect of angiotensin-converting-enzyme inhibition on bradykinin metabolism by vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 1993;264:H1493-7.
- Ahimastos AA, Natoli AK, Lawler A, Blombery PA, Kingwell BA. Ramipril reduces large-artery stiffness in peripheral arterial disease and promotes elastogenic remodeling in cell culture. *Hypertension* 2005;45:1194-9.
- Siraganian RP. Automated histamine release. A method for in vitro diagnosis. *Int Arch Allergy Appl Immunol* 1975;49:108-10.
- Guhl S, Stefaniak R, Strathmann M, Babina M, Piazena H, Henz BM, et al. Bivalent effect of UV light on human skin mast cells—low-level mediator release at baseline but potent suppression upon mast cell triggering. *J Invest Dermatol* 2005;124:453-6.
- Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977;1:466-9.
- Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaR-III. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997;99:901-14.
- Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. *J Allergy Clin Immunol* 2009;124:639-48.
- Ono E, Taniguchi M, Mita H, Fukutomi Y, Higashi N, Miyazaki E, et al. Increased production of cysteinyl leukotrienes and prostaglandin D2 during human anaphylaxis. *Clin Exp Allergy* 2009;39:72-80.
- Stevens RL, Friend DS, McNeil HP, Schiller V, Ghildyal N, Austen KF. Strain-specific and tissue-specific expression of mouse mast cell secretory granule proteases. *Proc Natl Acad Sci U S A* 1994;91:128-32.
- Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode *Trichinella spiralis* in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. *J Exp Med* 2000;192:1849-56.
- Schleimer RP, Schulman ES, MacGlashan DW Jr, Peters SP, Hayes EC, Adams GK 3rd, et al. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J Clin Invest* 1983;71:1830-5.
- MacGlashan DW Jr, Schleimer RP, Peters SP, Schulman ES, Adams GK 3rd, Newball HH, et al. Generation of leukotrienes by purified human lung mast cells. *J Clin Invest* 1982;70:747-51.
- Schleimer RP, MacGlashan DW Jr, Peters SP, Pinckard RN, Adkinson NF Jr, Lichtenstein LM. Characterization of inflammatory mediator release from purified human lung mast cells. *Am Rev Respir Dis* 1986;133:614-7.
- Weston MC, Peachell PT. Regulation of human mast cell and basophil function by cAMP. *Gen Pharmacol* 1998;31:715-9.
- Xiao RP. Beta-adrenergic signaling in the heart: dual coupling of the beta2-adrenergic receptor to G(s) and G(i) proteins. *Sci STKE* 2001;104:re15.
- Peachell P. Regulation of mast cells by beta-agonists. *Clin Rev Allergy Immunol* 2006;31:131-42.
- Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000;101:558-69.
- White JR, Pluznik DH, Ishizaka K, Ishizaka T. Antigen-induced increase in protein kinase C activity in plasma membrane of mast cells. *Proc Natl Acad Sci U S A* 1985;82:8193-7.
- Kato T. Role of protein kinase A in the inhibition of human mast cell histamine release by β -adrenergic receptor agonists. *Allergol Int* 2002;53:197-203.
- Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. Ca²⁺ and protein kinase C signaling for histamine and sulfidoleukotrienes released from human cultured mast cells. *Biochem Biophys Res Commun* 1999;257:895-900.
- Razin E. Signal transduction in mast cells and basophils. New York: Springer-Verlag; 1999.
- Chakravarty N, Yu WJ. Role of intracellular calcium in histamine release from rat mast cells. *Agents Actions* 1984;14:386-91.
- Pearce FL, Ennis M, Truneh A, White JR. Role of intra- and extracellular calcium in histamine release from rat peritoneal mast cells. *Agents Actions* 1981;11:51-4.