VM
VIA MEDICA

ORIGINAL ARTICLE

Major contribution of vasospasm-induced coronary blood flow reduction to anaphylactic ventricular dysfunction assessed in isolated blood-perfused rat heart

Yuhichi Kuda, Yasutaka Kurata, Mofei Wang,
Mamoru Tanida, Toshishige Shibamoto

Department of Physiology II, Kanazawa Medical University, Ishikawa, Japan

Abstract

Background: *Cardiac anaphylaxis is accompanied by coronary spasm and decreased left ventricular (LV) contractility. However, it has not been determined experimentally whether LV dysfunction during anaphylaxis is induced mainly by reduced coronary blood flow (CBF) or direct negative inotropic actions of chemical mediators. To demonstrate the major role of CBF reduction in anaphylactic LV dysfunction, we determined LV contractility during anaphylaxis and forcible CBF reduction maneuver to reproduce the anaphylaxis-induced CBF reduction in isolated blood-perfused rat hearts.*

Methods: *Isolated hearts from Wistar rats in the ovalbumin-sensitized anaphylaxis, non--sensitized flow reduction, and non-sensitized time control group were subjected to coronary perfusion with blood at a constant pressure and measurements of CBF and LV pressure. Cardiac anaphylaxis was induced by intracoronary injections of ovalbumin antigen.*

Results: *In response to antigen administrations, sensitized anaphylaxis group rat hearts showed decreases in CBF and the maximum increasing rate of systolic LV pressure (dP/dt_{max}) with an increased coronary vascular resistance as evidence of coronary spasm. The non-sensitized flow reduction group rat hearts whose CBF was forcibly reduced as in anaphylaxis showed the same degree of dP/dt_{max} reduction.*

Conclusions: *The contractile failure during cardiac anaphylaxis is caused mainly by decreased CBF due to coronary spasm.* (Cardiol J 2014; 21, 1: 11–17)

Key words: anaphylactic shock, acute coronary syndrome, coronary blood flow, left ventricular contractility, cross-circulation method

Introduction

Anaphylactic shock is sometimes life-threatening and accompanied by cardiac manifestations, which are clinically characterized by acute myocardial ischemia via coronary artery spasm and left ventricular (LV) dysfunction [1–8]. Allergic activation of cardiac must cells is known to be of particular importance as a cause of the acute coronary syndrome, which is called "Kounis syndrome" [9]. Anaphylaxis-induced coronary vasoconstriction and cardiac dysfunction have also been demonstrated experimentally [10, 11]: in excised rat hearts, ovalbumin-induced anaphylaxis caused cardiac dysfunction characterized by the decreases in the systolic LV pressure (LVP) and the maximum incre-

---

asing rate of LVP (dP/dt_{max}) along with coronary vasoconstriction. These findings strongly suggest that coronary vasoconstriction-induced myocardial ischemia and reduced LV contractility are involved in the pathogenesis of anaphylactic shock. Nevertheless, it is not known to what extent the reduced coronary blood flow (CBF) or the direct negative inotropic effect of chemical mediators is implicated in the detrimental action of anaphylaxis on cardiac contractility. There is no experimental study to examine the effect of CBF reduction on cardiac contractility as a mechanistic cause of anaphylactic cardiac dysfunction.

The aim of the present study was to determine the major mechanism of the anaphylactic LV dysfunction in isolated rat hearts by a maneuver of forcible CBF control, specifically whether it is primarily due to coronary vasoconstriction-induced CBF reduction. We hypothesized that if anaphylactic cardiac dysfunction is mainly due to the decrease in CBF, an imposed CBF reduction deteriorates LV contractility to the same extent as in anaphylaxis. Contractility of isolated coronary perfused rat hearts suffering from anaphylaxis was compared with that of CBF-reduced rat hearts. The LVP, pressures of the coronary artery (CAP) and vein (CVP), and CBF were directly and simultaneously measured before and after ovalbumin challenge in antigen-sensitized Wister rat hearts, as well as in non-sensitized rat hearts with imposed CBF reduction. The dP/dt_{max} was determined during isovolumic contractions for the assessment of LV contractility without changes in preload. As a distinct feature of this study we applied the cross-circulated blood-perfusion method which permits analysis of LV mechanical works in excised whole heart preparations under more physiological conditions than crystalloid perfusion [12, 13].

Methods

Animals

Male Wistar rats (n = 21) weighing 364 ± ± 17 g were assigned to one of the 3 groups: 1) the anaphylaxis group to be sensitized with ovalbumin for cardiac anaphylaxis experiment; 2) the flow reduction group not to be sensitized but to be used for the CBF reduction experiment; 3) the non-sensitized time control group. Rats were maintained at 23°C under pathogen-free conditions on a 12:12-h dark-light cycle, and allowed food and water ad libitum. The experiments were approved by the Animal Research Committee of Kanazawa Medical University.

Antigen sensitization

Rats in the anaphylaxis group, but not in the 2 other groups, were actively sensitized by the subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund's adjuvant (0.5 mL) with 1 mg ovalbumin (grade V; Sigma Chemical Company, St. Louis, MO), as described previously [14].

Isolated cross-circulated rat heart preparation

Two weeks after the sensitization, hearts were excised and coronary perfused with blood of metabolic supporter and blood supplier rats at a constant pressure by the cross-circulation method [13]. A thin latex balloon inserted into excised heart LV was connected to a pressure transducer (Life Kit DX-312, Nihon Kohden, Tokyo, Japan) to measure LVP during isometric contractions, with a maximum systolic LVP adjusted to 120–140 mm Hg. The heart rate was maintained at 300 bpm by electrical pacing to eliminate chronotropic influences. The systemic arterial pressure of a supporter rat served as the coronary perfusion pressure of 102 ± 14 (SD) mm Hg. Arterial pH, PO_2, and PCO_2 of the supporter rat were maintained within their physiological ranges (pH = 7.3–7.4; PO_2 = 95– –100 mm Hg; PCO_2 = 35–40 mm Hg) with supplemental oxygen and sodium bicarbonate.

Measurement items

LVP, CAP, and CVP were continuously measured with pressure transducers (Life Kit DX-312, Nihon Kohden) and digitally recorded by PowerLab (AD Instruments, Castle Hill, Australia), which could determine the time derivative of LVP and dP/dt_{max} as an index of LV contractility in real time. Total CBF (mL/min/g) was continuously measured with an electromagnetic flow meter (MFV-3100, Nihon Kohden) placed in the middle of the coronary venous drainage tubing from the superior vena cava. For assessing coronary circulation, the coronary vascular resistance (CVR) was determined by the following equation:

$$CVR = (CAP – CVP) / CF \text{ [mm Hg} \times \min \times \text{g/mL].}$$

Experimental protocol

Baseline measurements of LVP, CAP, CVP and CBF (with determinations of dP/dt_{max} and CVR) were first performed before antigen injection. Following the baseline measurements, the antigen (ovalbumin 1.2 mg) dissolved in 300 μL saline was administered into the coronary artery, which yielded the forcible constriction of the left coronary
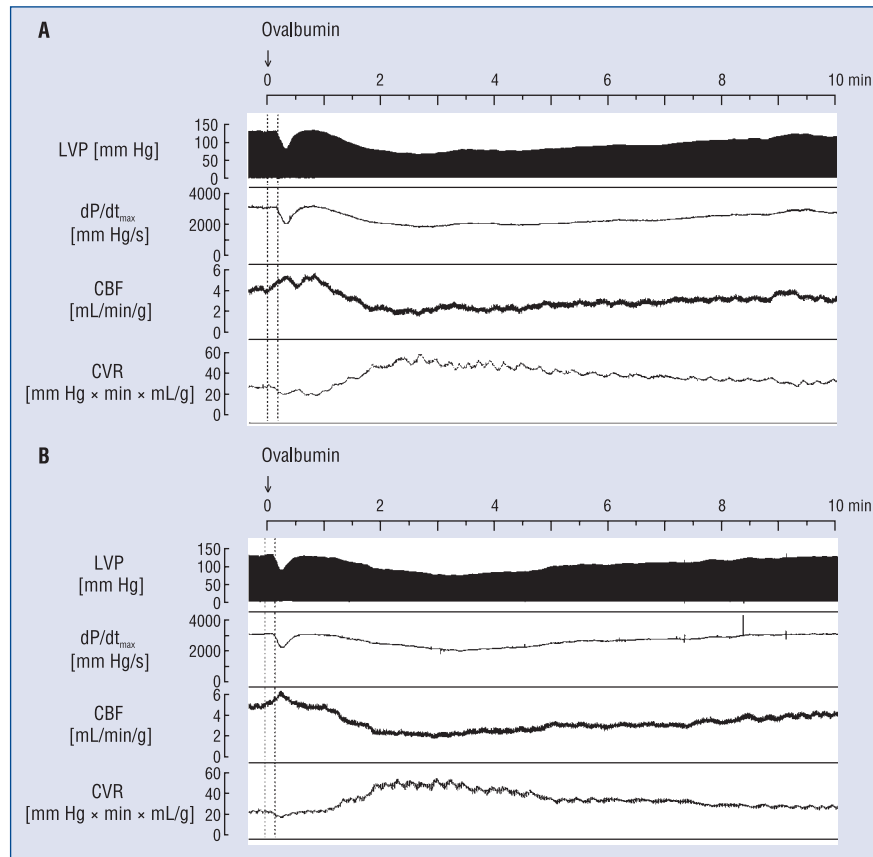
Figure 1. Temporal changes in measured variables during anaphylaxis and forcible flow reduction. Shown are: representative recordings of the left ventricular pressure (LVP), maximum rate of changes in systolic LVP (dP/dt$_{max}$), coronary blood flow (CBF), and coronary vascular resistance (CVR) after an intracoronary injection of ovalbumin (1.2 mg) to a sensitized rat heart from the anaphylaxis group (**A**) and a non-sensitized one from the flow reduction group (**B**).

artery (main trunk) only in the sensitized hearts. Direct injection of the antigen ovalbumin into coronary arteries of isolated sensitized rat hearts is a well-established experimental method to create rat cardiac anaphylaxis models efficiently [10, 11]. The variables were continuously measured for 10 min after the antigen administration.

We determined the temporal changes in CBF and LV contractility during cardiac anaphylaxis, and LV contractility during imposed CBF reduction. For the flow reduction group, the coronary arterial catheter was constricted under monitoring the real-time CBF using a clamp so as to reproduce the temporal CBF changes in the same manner as observed in the anaphylaxis group.

Drugs

All drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA). All drugs were dissolved in saline.

Statistical analysis

All results are expressed as the means ± SEM, unless stated otherwise. Multiple intra-group and between-group comparisons were performed using the repeated-measures analysis of variance, followed by the Bonferroni *post hoc* analysis. The p value of < 0.05 was considered statistically significant. All statistical analyses were performed by StatView (SAS Institute Inc., Cary, NC).

Results

Figure 1 shows the representative records of LVP, CAP, CVP, CBF, dP/dt$_{max}$ and CVR for 10 min after an antigen challenge from an anaphylaxis group rat heart (Fig. 1A) and flow reduction group rat heart (Fig. 1B). Averaged values of the measured variables are shown at an interval of 30 s in Figures 2 and 3. No significant differences in baseline values of the variables were observed
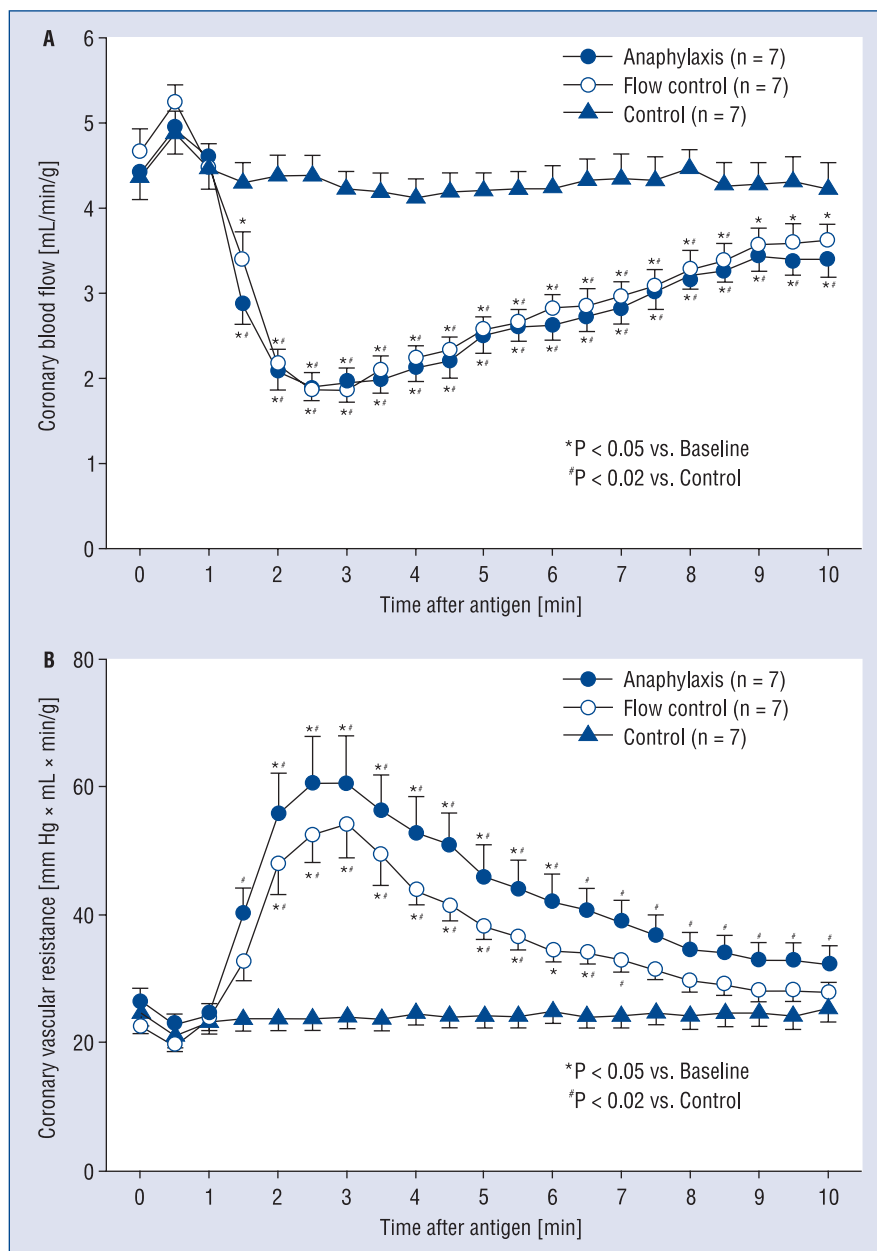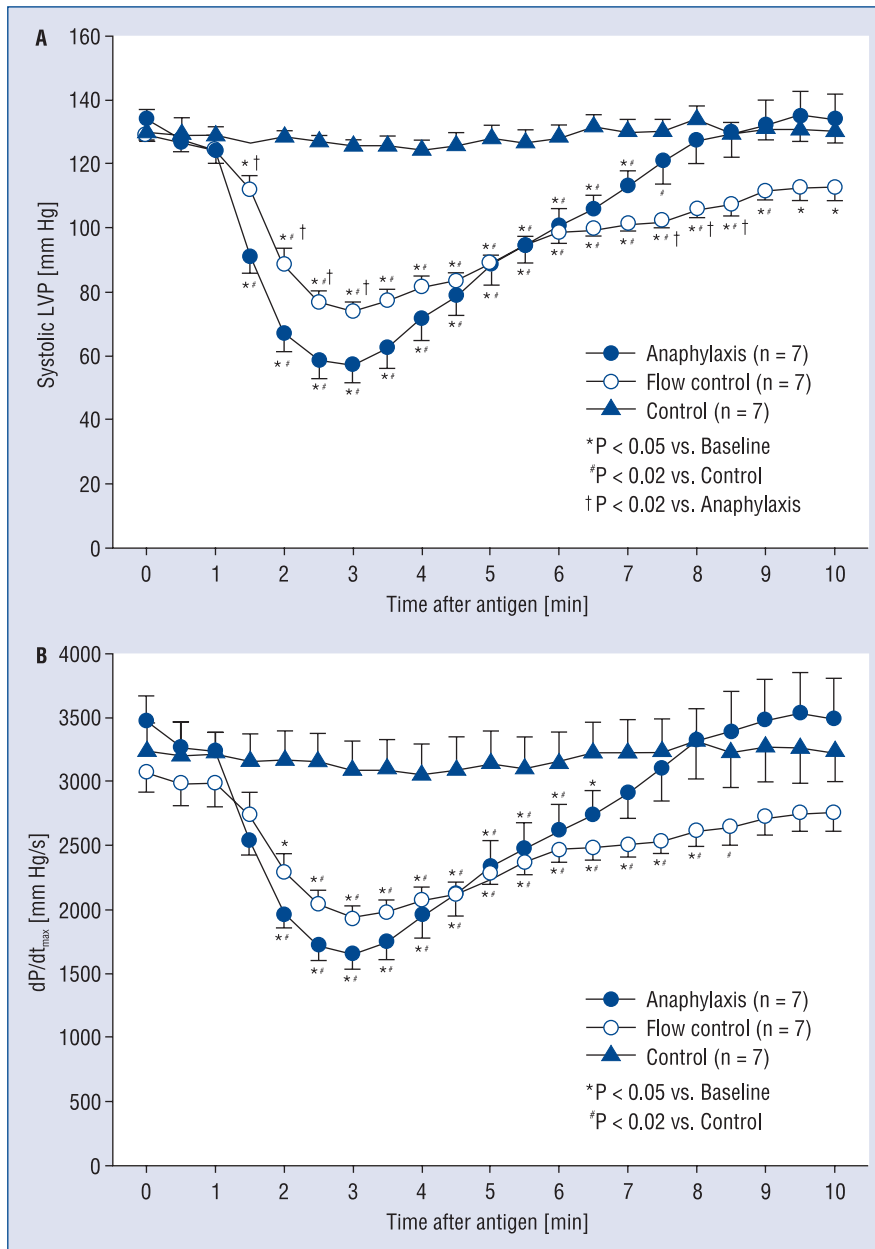
Figure 2. Averaged temporal changes in coronary blood flow (**A**) and calculated coronary vascular resistance (**B**) for 10 min after an injection of the ovalbumin antigen. The measurements were made at an interval of 0.5 min for the anaphylaxis group (closed circles, n = 7) and the flow reduction group (open circles, n = 7), as well as the time control group (triangles, n = 7); *p < 0.05, vs. the baseline; #p < 0.02, vs. the time control group.

between sensitized and non-sensitized hearts. The time control rat hearts showed no significant changes in the variables after antigen challenge.

Anaphylaxis caused coronary vasoconstriction and parallel decreases in CBF and dP/dt$_{max}$

During cardiac anaphylaxis evoked by the antigen, the CBF (4.4 ± 0.2 mL/min/g at the baseline)

began to decrease as early as 1.5 min after antigen challenge, progressively decreased to the nadir of 1.8 ± 0.2 mL/min/g at 2.5 min, and then gradually returned toward the baseline level during 10 min measurements (Fig. 2A). CVP slightly but significantly decreased by ~2 mm Hg at 1.5–6.5 min, whereas CAP did not change throughout 10 min recordings (data not shown). The calculated CVR showed significant increases from the baseline

Figure 3. Averaged temporal changes in the systolic left ventricular pressure (LVP) (**A**) and dP/dt$_{max}$ (**B**) for 10 min after an injection of the ovalbumin antigen. The measurements were made at an interval of 0.5 min for the anaphylaxis group (closed circles, n = 7) and the flow reduction group (open circles, n = 7), as well as the time control group (triangles, n = 7); *p < 0.05, vs. the baseline; #p < 0.02, vs. the control group.

value of 26 ± 2 mm Hg × min × g/mL to the peak of 63 ± 7 mm Hg × min × g/mL, i.e., 2.4-fold the baseline level, at 2.5 min, followed by the subsequent partial recovery toward the baseline level (Fig. 2B).

In parallel with the CBF reduction, the systolic LVP (132 ± 2 mm Hg at the baseline) and dP/dt$_{max}$ (3347 ± 163 mm Hg/s at the baseline) began to decrease at 1.5 min after antigen administration, decreased to the nadir of 60 ± 5 mm Hg and 1722 ±

± 115 mm Hg/s (i.e., to 46% and 51% of the baseline levels), respectively, at 3 min, and then gradually went back to the baseline level (Fig. 3).

Imposed CBF reduction yielded the same degree of LV dysfunction as anaphylactic response

The flow reduction group rat hearts exhibited the decrease in CBF along with the increase in the

CVR, which well mimicked the responses of the cardiac anaphylaxis group (Fig. 2). The decrease in dP/dt_{max} in response to imposed CBF reduction was very similar to that of the anaphylaxis group, not significantly different from the anaphylactic response (Fig. 3B). Thus, the anaphylactic and forcible reductions in CBF caused coequal negative inotropic responses of LV contractility.

Discussion

In the current study, for the first time, we determined the role of reduced CBF in the LV dysfunction induced by cardiac anaphylaxis using isolated blood-perfused rat heart preparations. Distinct features of this study as compared with previous studies on cardiac anaphylaxis are 1) more physiological coronary perfusion with blood instead of crystalloid, and 2) the use of the flow reduction maneuver to determine the contribution of coronary vasoconstriction (reduced CBF) to LV dysfunction during cardiac anaphylaxis. There are two major findings: 1) after antigen administration to sensitized rat hearts, LV contractility (dP/dt_{max}) transiently decreased in parallel with CBF decrease via coronary vasoconstriction with complete recovery within 10 min; 2) the attenuation of LV contractility during the imposed CBF reduction to reproduce the anaphylactic CBF reduction was very similar to that in the anaphylactic response. These findings suggest that anaphylactic LV dysfunction is attributed primarily to reduced CBF due to coronary vasoconstriction and resulting myocardial ischemia.

Previous experimental and clinical studies suggested the involvements of coronary vasoconstriction, myocardial ischemia, and LV contractile failure in pathogenesis of anaphylactic hypotension [3–6, 8–11, 15]. However, it was not properly determined whether impaired CBF was primarily responsible for the detrimental effects of anaphylaxis on cardiac contractility, because of inappropriate conditions of the studies for assessment of cardiac functions (e.g., concomitant changes in uncontrolled preload and/or heart rates, non-physiological coronary perfusion with crystalloid). Using the flow control and blood perfusion methods, we clearly demonstrated the major role of coronary vasoconstriction and CBF reduction in anaphylactic LV dysfunction.

It is not clearly known to what extent the direct negative inotropic effect of chemical mediators such as histamine and leukotrienes is implicated in their detrimental action on cardiac contractility

during anaphylaxis. The maximum reductions in LV contractility (dP/dt_{max}) were not significantly different between the anaphylaxis and CBF reduction groups, indicating that the direct negative inotropic effect of anaphylactic mediators, if any, would be relatively small. However, our results also suggest small positive inotropic action of chemical mediators during the recovery phase of reduced cardiac contractility: the restoration of LV contractility was more complete during anaphylaxis than in the forcible CBF reduction (Fig. 3B), while the recovery of CBF was nearly identical (Fig. 2A). The complete recovery during anaphylaxis may be due to the direct positive inotropic effect of chemical mediators and/or modulators including histamine (via the H_2 receptor) [16], calcitonin gene-related peptide [17, 18], and anaphylatoxins [19, 20]. Further studies are required on direct modifications of cardiac contractility by anaphylactic mediators and modulators.

As mentioned above, anaphylactic LV dysfunction was not persistent but only transient, with LV function completely recovering within 10 min along with CBF recovery, whereas the previous experimental studies using crystalloid perfusion reported incomplete recovery of LV contractility during cardiac anaphylaxis for longer periods (~60 min) of measurements [10, 11]. This inconsistency may be at least in part related to the difference in perfusate, i.e., blood vs. crystalloid: the use of more physiological perfusate of blood might enhance the release of chemical mediators or modulators to yield coronary vasodilation and/or positive inotropic effects. Thus, LV dysfunction may not strongly affect prolonged anaphylactic hypotension; in vivo, however, anaphylactic LV dysfunction may be protracted by mediators which are released systemically and reach the heart.

Conclusions

In summary, we determined the coronary vascular and LV contractility responses to anaphylaxis in isolated blood-perfused rat hearts, with special references to the relationship of CBF and LV contractility. LV contractility transiently decreased in parallel with CBF and the CBF control mimicking the temporal changes in CBF during anaphylaxis caused a reduction in LV contractility similar to that during anaphylaxis. These results suggest that LV dysfunction during anaphylaxis is attributed mainly to coronary vasoconstriction and resulting myocardial ischemia.

References

1. Kemp SF, Lockey RF. Anaphylaxis: A review of causes and mechanisms. J Allergy Clin Immunol, 2002; 110: 341–348.
2. Triggiani M, Patella V, Staiano RI, Granata F, Marone G. Allergy and the cardiovascular system. Clin Experimental Immunol, 2008; 153 (suppl. 1): 7–11.
3. Golden DB. What is anaphylaxis? Curr Opin Allergy Clin Immunol, 2007; 7: 331–336.
4. Hirsch SA. Acute allergic reaction with coronary vasospasm. Am Heart J, 1982; 193: 928–932.
5. Lombardi A, Vandelli R, Cere E, Di Pasquale G. Silent acute myocardial infarction following a wasp sting. Ital Heart J, 2003; 4: 638–641.
6. Mueller UR. Cardiovascular disease and anaphylaxis. Curr Opin Allergy Clin Immunol, 2007; 7: 337–341.
7. Simons FE, Frew AJ, Ansotegui IJ et al. Risk assessment in anaphylaxis: current and future approaches. J Allergy Clin Immunol, 2007; 120: S2–24.
8. Wagdi P, Mehan VK, Burgi H, Salzmann C. Acute myocardial infarction after wasp stings in a patient with normal coronary arteries. Am Heart J, 1994; 128: 820–823.
9. Kounis NG. Kounis syndrome (allergic angina and allergic myocardial infarction): A natural paradigm? Int J Cardiol, 2006; 110: 7–14.
10. Sun S, Weil MH, Tang W et al. Cardiac anaphylaxis in the Sprague-Dawley rat. J Lab Clin Med, 1992; 120: 589–596.
11. Vleeming W, van Rooij HH, Wemer J, Porsius AJ. Characterization and modulation of antigen-induced effects in isolated rat heart. J Cardiovasc Pharmacol, 1991; 18: 556–565.
12. Takaki M. Left ventricular mechanoenergetics in small animals. Jpn J Physiol, 2004; 54: 175–207.
13. Yoshikawa Y, Zhang GX, Obata K et al. Cardioprotective effects of a novel calpain inhibitor, SNJ-1945 for reperfusion injury after cardioplegic cardiac arrest. Am J Physiol Heart Circ Physiol, 2010; 298: H643–H651.
14. Shibamoto T, Cui S, Ruan Z, Liu W, Takano H, Kurata Y. Hepatic venoconstriction is involved in anaphylactic hypotension in rats. Am J Physiol Heart Circ Physiol, 2005; 289: H1436–H1441.
15. Low I, Stables S. Anaphylactic deaths in Auckland, New Zealand: A review of coronial autopsies from 1985 to 2005. Path, 2006; 38: 328–332.
16. Felix SB, Baumann G, Helmus S, Sattelberger U. The role of histamine in cardiac anaphylaxis; characterization of histaminergic H1- and H2-receptor effects. Basic Res Cardiol, 1988; 83: 531–539.
17. Schuligoi R, Amann R, Donnerer J, Peskar BA. Release of calcitonin gene-related peptide in cardiac anaphylaxis. Naunyn-Schmiedeberg's Arch Pharmacol, 1997; 355: 224–229.
18. Dai W, Zhou F-W, Song Q-J, Li Y-J, Deng H-W, Xiong X-M. Protective effects of calcitonin gene-related peptide on guinea-pig cardiac anaphylaxis. Naunyn-Schmiedeberg's Arch Pharmacol, 2000; 361: 161–165.
19. del Balzo U, Polley MJ, Levi R. Activation of the third complement component (C3) and C3a generation in cardiac anaphylaxis: histamine release and associated inotropic and chronotropic effects. J Pharmacol Exp Ther, 1988; 246: 911–916.
20. del Balzo U, Polley MJ, Levi R. Cardiac anaphylaxis. Complement activation as an amplification system. Circ Res, 1989; 65: 847–857.