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ORIGINAL ARTICLE

Blockade of β_2-adrenoceptor, rather than β_1-adrenoceptor, deteriorates cardiac anaphylaxis in isolated blood-perfused rat hearts

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Background: *Cardiac anaphylaxis is one of the features of anaphylactic hypotension. Patients treated with propranolol, a nonselective β-adrenoceptor (AR) antagonist, develop severe anaphylaxis, but the mechanism remains unknown. Under examination were the effects of β_1- and β_2-AR antagonist on anaphylaxis-induced coronary vasoconstriction and cardiac dysfunction in isolated blood-perfused rat hearts.*

Methods: *Isolated hearts from ovalbumin-sensitized Wistar rats were subjected to coronary perfusion with blood at a constant pressure and measurements were made of coronary blood flow and left ventricular (LV) pressure. Following pretreatment with selective β_2-AR antagonist ICI118,551 or selective β_1-AR antagonist atenolol, cardiac anaphylaxis was induced by intracoronary injections of ovalbumin antigen. LV contractility was evaluated by the maximum increasing rate of systolic LV pressure (dP/dt_{max}).*

Results: *In response to antigen administrations, ICI118,551 pretreated hearts showed a greater decrease in coronary blood flow and consequently a greater increase in coronary vascular resistance than the atenolol pretreated hearts. Pretreatment with ICI118,551 caused a greater decrease in dP/dt_{max} than those with atenolol.*

Conclusions: *Cardiac anaphylaxis-induced contractile dysfunction and coronary spasm are severe in β_2-, rather than β_1-AR antagonist, pretreated isolated blood-perfused rat hearts.* (Cardiol J 2017; 24, 4: 403–408)

Key words: cardiac anaphylaxis, isolated perfused rat heart, β-adrenoceptor antagonist, cardiac contractility

Introduction

Anaphylactic shock is sometimes life-threatening and accompanied by cardiac manifestation, which is clinically characterized by acute myocardial ischemia via coronary artery spasm and contractile dysfunction of left ventricle (LV) [1–5] and is called "Kounis syndrome" [6]. Experimental models of cardiac anaphylaxis were established in excised sensitized rat hearts perfused with crystalloids [7, 8], in which coronary vasoconstriction and cardiac dysfunction, evidenced by a decrease

in the maximum increasing rate of LV pressure (dP/dt_{max}), were induced by administration of the antigen ovalbumin into the coronary artery. Furthermore, new cardiac anaphylaxis models have recently been developed using 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 [9].

Patients with pre-existing cardiovascular diseases and those treated with propranolol, a β-adrenoceptor (AR) antagonist, have increased severity of anaphylaxis [10, 11]. Propranolol is a nonselective β-AR antagonist and can block both β_1-AR and β_2-AR. It has recently been demonstrated that the blockade of β-AR augments the severity of anaphylactic hypotension in anesthetized rats, and that blockade of β_2-AR rather than β_1-AR exerts primarily the detrimental action on systemic anaphylaxis via enhancing the pulmonary vasoconstriction and bronchoconstriction [12]. Therefore, cardiac anaphylactic reactions may also be augmented by blockade of β-AR. If so, it should be clarified whether blockade of β_1-AR or β_2-AR exerts a deleterious effect on cardiac anaphylaxis.

The purpose of this study was to determine the effects of β_1-AR and β_2-AR antagonists on coronary vasoconstriction and cardiac dysfunction during cardiac anaphylaxis in isolated blood-perfused rat hearts [9].

Methods

Animals

Male Wistar rats (n = 14) weighing 374 ± ± 27 g were assigned to one of the two groups: 1) the selective β_2-AR antagonist ICI118,551 (3-(isopropylamino)-1-[(7-methyl-4-indanyl)oxy] butan-2-ol) group; and 2) the selective β_1-AR antagonist atenolol group. Rats were housed in a cage (28 cm × 21 cm × 45 cm; 3 rats/cage) and maintained at 23°C and the humidity of 55% ± ± 15% under pathogen-free conditions on a 12:12-h dark-light cycle, and allowed food (CE-2, CLEA Japan, Tokyo, Japan) and water ad libitum. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University.

Antigen sensitization

Rats 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, USA), as described previously [13].

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 [9]. 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 LV pressure during isometric contractions, with a maximum systolic LV pressure 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 107 ± 15 (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

LV pressure, coronary arterial pressure, and coronary venous pressure were continuously measured with the pressure transducer (Life Kit DX-312, Nihon Kohden) and digitally recorded by PowerLab (AD Instruments, Castle Hill, Australia), which could determine the time derivative of LV pressure and dP/dt_{max} as an index of LV contractility in real time. Total coronary blood flow (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 was determined by the following equation:

Coronary vascular resistance = (Coronary arterial pressure – Coronary venous pressure) / / Coronary blood flow [mm Hg × min × g/mL].

Experimental protocol

Baseline measurements of LV pressure, coronary arterial pressure, coronary venous pressure and coronary blood flow (with determinations of dP/dt_{max} and coronary vascular resistance) were first performed at 10 min after pretreatment with atenolol (0.25 mg) or ICI118,551 (0.06 mg) injected into the coronary artery. The administered doses of atenolol and ICI118,551 were one third of the total doses intravenously injected into the whole body of *in vivo* rats [12],
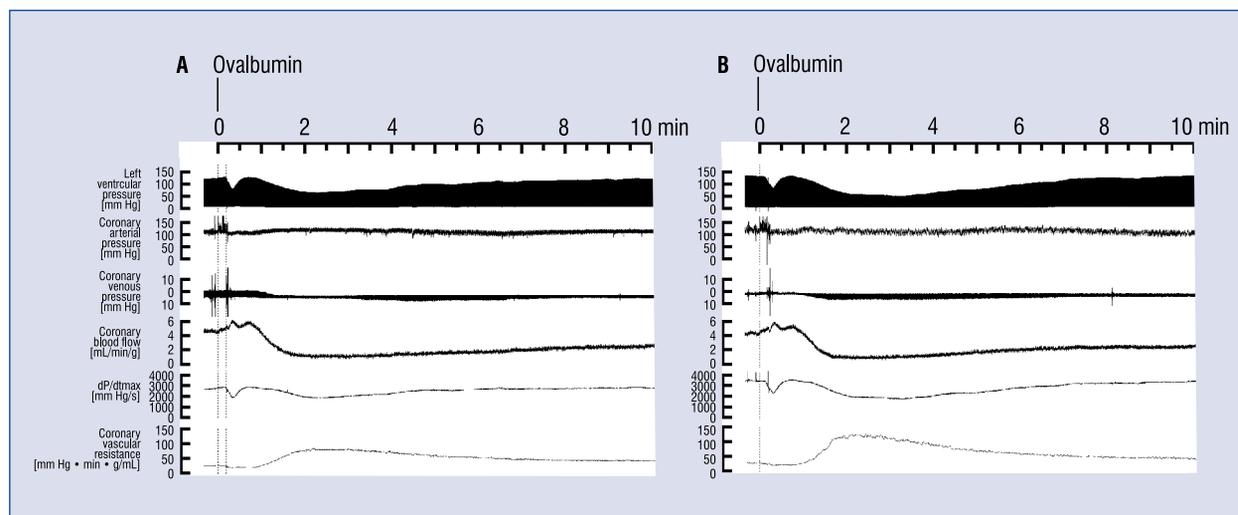
Figure 1. Representative recordings of left ventricular pressure, coronary arterial pressure, coronary venous pressure, coronary blood flow, maximum increasing rate of systolic left ventricular pressure (dP/dt$_{max}$) and coronary vascular resistance in the atenolol (**A**) and ICI118,551 (**B**) groups.

the doses were enough for coronary circulation alone. 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 artery (main trunk). 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 [7, 8]. The variables were continuously measured for 10 min after the antigen administration. We determined the temporal changes in coronary blood flow and LV contractility during cardiac anaphylaxis.

Drugs

All drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA). All drugs were dissolved in saline. The doses of the antagonists were determined according to the previous study [12].

Statistical analysis

All results are expressed as the means $\pm$ standard error of the mean (SEM). Multiple intra-group comparisons were performed using the repeated-measures analysis of variance, followed by the Bonferroni *post hoc* analysis. Comparisons between the atenolol and ICI118,551 groups were performed by Student t-test. The p value of < 0.05 was considered statistically significant. All statistical analyses were performed by StatView (SAS Institute Inc., Cary, NC, USA).

Results

Figure 1 shows representative records of LV pressure, coronary arterial pressure, coronary venous pressure, coronary blood flow, dP/dt$_{max}$ and coronary vascular resistance for 10 min after an antigen challenge from a rat heart pretreated with atenolol (Fig. 1A) and ICI118,551 (Fig. 1B). Averaged values of the measured variables are shown at an interval of 30 s in Figures 2 and 3. In the atenolol pretreated hearts, coronary blood flow (4.4 $\pm$ 0.3 mL/min/g at the baseline) began to decrease as early as 1.5 min after antigen challenge and progressively decreased to the nadir of 1.7 $\pm$ 0.3 mL/min/g at 2.5 min, and then gradually returned toward the baseline level during 10 min measurements (Fig. 2A). Coronary venous pressure slightly but significantly decreased by ~2 mm Hg at 1.5–6.5 min, whereas coronary arterial pressure did not change throughout 10 min recordings (data not shown). The calculated coronary vascular resistance showed significant increases from the baseline value of 25 $\pm$ 3 mm Hg•min•g/mL to the peak of 72 $\pm$ 9 mm Hg • min • g/mL, i.e., 2.9-fold the baseline level, at 2.5 min, followed by subsequent partial recovery toward baseline level (Fig. 2B). In ICI118,551 pretreated hearts, following antigen injection, coronary blood flow decreased from the baseline of 4.0 $\pm$ 0.2 mL/min/g to the nadir of 0.9 $\pm$ 0.2 mL//min/g at 2.5 min, which was significantly smaller than that in atenolol pretreated hearts (Fig. 2A).
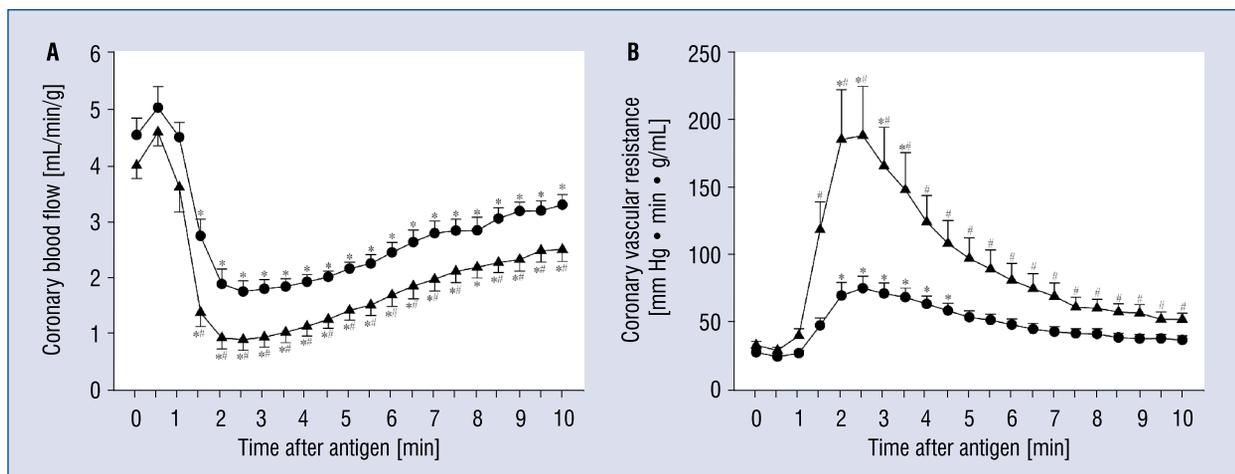
Figure 2. Time course data of coronary blood flow (**A**) and coronary vascular resistance (**B**) after an injection of the ovalbumin antigen. Circle, the atenolol group (n = 7); triangle, the ICI118,551 group (n = 7). Values are means ± SEM; *p < 0.0002 vs. baseline (by the Bonferroni *post hoc* test); #p < 0.05 vs. the atenolol group (by Student t-test); SEM — standard error of the mean.
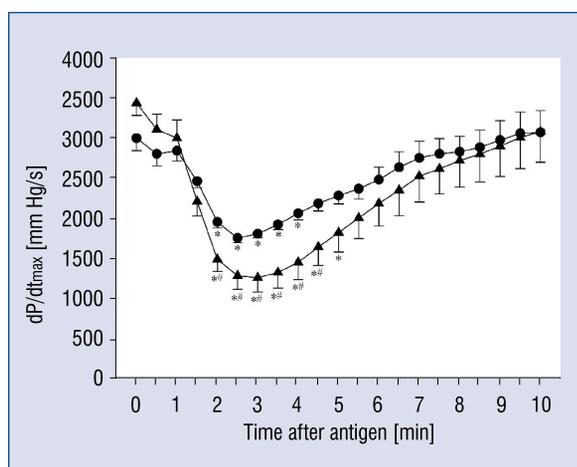

Figure 3. Time course data of maximum increasing rate of systolic left ventricular pressure (dP/dt$_{max}$) after an injection of ovalbumin antigen. Circle, the atenolol group (n = 7); triangle, the ICI118,551 group (n = 7). Values are means ± SEM; *p < 0.0002 vs. baseline (by the Bonferroni *post hoc* test); #p < 0.05 vs. atenolol group (by the Student t-test); SEM — standard error of the mean.

Consequently coronary vascular resistance in ICI118,551 hearts increased 6.2-fold from the baseline at 2.5 min, which was also significantly greater than the 2.9-fold increase in atenolol hearts (Fig. 2B).

In atenolol pretreated hearts, in parallel with the coronary blood flow reduction, the systolic LV pressure (127 ± 2 mm Hg at the baseline) and dP/dt$_{max}$ (2954 ± 166 mm Hg/s at the baseline) began to decrease at 1.5 min after antigen administration, decreased to the nadir of 61 ± 4 mm Hg and 1743 ± 58 mm Hg/s (i.e., to 48% and 59% of the baseline levels), respectively, at 2.5 min, and then gradually went back to the baseline level (Fig. 3). In ICI118,551 pretreated hearts, LV pressure (135 ± ± 4 mm Hg at the baseline) and dP/dt$_{max}$ (3422 ± ± 157 mm Hg/s at the baseline) decreased to the nadir of 39 ± 3 mm Hg and 1250 ± 179 mm Hg/s (i.e., to 29% and 37% of baseline levels) respectively, which were significantly smaller than those in the atenolol pretreated hearts (Fig. 3).

Discussion

In the present study, it was found that pretreatment with the selective β_2-AR blocker ICI118,551 reduced the cardiac contractility index of dP/dt$_{max}$ more dramatically than with the selective β_1-AR blocker atenolol in isolated blood-perfused sensitized rat hearts injected with the antigen. This demonstrates that the blockade of β_2-AR exerted more detrimental effects on anaphylaxis-induced cardiac dysfunction than that of β_1-AR.

In the present study the response of the non--pretreatment sensitized heart (the anaphylaxis group) or non-sensitized heart (the control group)
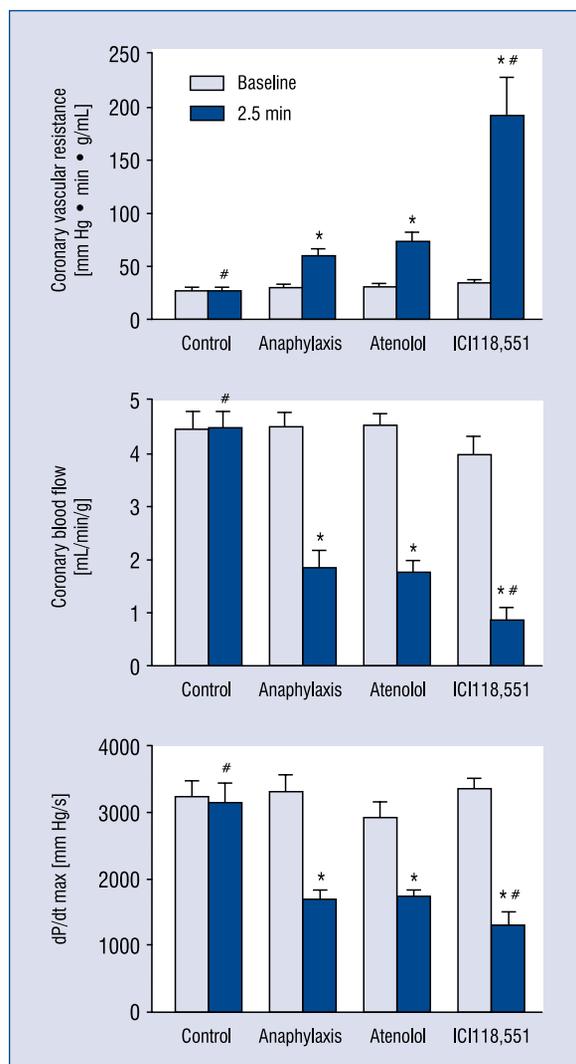
Figure 4. Time data of coronary vascular resistance, coronary blood flow and maximum increasing rate of systolic left ventricular pressure (dP/dt$_{max}$) at baseline and 2.5 min after an injection of the ovalbumin antigen in non-sensitized group (Control; n = 7), sensitized non-pretreatment group (Anaphylaxis; n = 7), atenolol group (n = 7) and ICI118,551 group (n = 7). Values are mean ± SEM; *p < 0.05 vs. baseline (by the Student t-test); #p < 0.05 vs. the Anaphylaxis group (by the Fisher's *post hoc* test); SEM — standard error of the mean.

to the antigen was not examined because they had already been reported in a previous article [9]. Using the previously reported data, herein provided (Fig. 4) shows all four groups data of coronary vascular resistance, coronary blood flow and dP//dt$_{max}$ at baseline and 2.5 min after antigen injec-

tion when the peak changes of the variables were observed. It should be noted that the magnitudes of increased coronary vascular resistance, reduced coronary blood flow and reduced dP/dt$_{max}$ in the atenolol pretreated hearts of the present study were comparable to those of the non-pretreated hearts of the previous study, and were significantly different from those of the ICI118,551-pretreated heart and the non-sensitized heart [9]. This evidence indicates that β_2-AR antagonist, but not β_1-AR antagonist, aggravates cardiac anaphylaxis.

As previously demonstrated, β_2-AR antagonist ICI118,551, rather than β_1-AR antagonist atenolol, exerts a deleterious effect on rat anaphylactic shock [14]. The mechanism, however, remains unclear. The present results demonstrate that β_2-AR antagonist causes more severe antigen-induced cardiac dysfunction than β_1-AR antagonist, which may account, at least in part, for the detrimental action of the β_2-AR antagonist on anaphylactic hypotension. Another possibility is that β_2-AR antagonist increased vascular permeability, resulting in a reduction of effective circulating blood volume and then anaphylactic hypotension, since it has been shown that β-AR-mediated signaling contributes to endothelial barrier maintenance under baseline conditions [15, 16]. In addition, β_2-AR antagonists might exert deleterious effects on the antigen-induced pulmonary vasoconstriction and bronchoconstriction, as observed in β_2-AR antagonist-pretreated rats [12].

The mechanism for β_2-AR antagonist-induced deterioration of cardiac contractility during anaphylaxis may be related to the exaggerated coronary vasoconstriction, which causes ischemia of LV, and finally cardiac dysfunction. It was demonstrated that anaphylactic cardiac dysfunction is almost exclusively due to coronary vasoconstriction in the isolated blood-perfused rat hearts [9]: during cardiac anaphylaxis LV contractility transiently decreased in parallel with coronary blood flow, and the forcible coronary blood flow reduction mimicking the temporal changes in coronary blood flow during anaphylaxis caused a reduction in LV contractility similar to that during anaphylaxis. On the other hand, activation of β_2-AR in the heart causes coronary vasodilatation [17]. Furthermore, Watson et al. [18] have recently reported that the β_2-AR agonist formoterol increases coronary blood flow and LV contractility most likely via β_2-AR-mediated coronary vasodilation in isolated perfused rat hearts. Elimination

of this beneficial action of β_2-AR may lead to aggravation of anaphylactic cardiac dysfunction in the present study.

Limitations of the study

There are limitations of the present study. This study examined the acute effects of β-AR antagonists. However, patients usually take chronically β-AR antagonists; all the adaptive mechanisms, such as desensitization, that are likely to occur were not captured by the present study design. Furthermore the patients with β-AR antagonists may not have a healthy intact heart, as was examined in the present study, but a coronary sclerotic or failing heart. Thus, care should be taken when extrapolating the present findings to clinical situations. Further study may be required using animals with chronic administration of β-AR antagonists or with diseased hearts.

Conclusions

In summary, we determined the pretreatment effects of β_1-AR and β_2-AR antagonists on cardiac contractility, dP/dt_{max}, in isolated blood-perfused rat hearts suffering from anaphylaxis. It was concluded that the blockade of β_2-AR causes greater coronary vasoconstriction, and consequently severer cardiac dysfunction than that of β_1-AR.

Acknowledgements

Conflict of interest: None declared

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