The Protective Effect of H₂-Receptor Activation Against the Duration of Myocardial Hypoxia/Reoxygenation-Induced Ventricular Fibrillation in Sensitized Guinea-Pig Hearts

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Abstract. Patients with high serum immunoglobulin E levels were reported to be protected against sudden death during acute myocardial infarction. The protection mechanism might be attributed to the facilitation of histamine release from sensitized mast cells; however, this remains to be clarified. In this study, we examined the influence of sensitization on ventricular fibrillation (VF) induced by myocardial hypoxia/reoxygenation (H/R). Guinea pigs were actively sensitized by subcutaneous injection of ovalbumin in Bordetella pertussis vaccine. Hearts isolated from non-sensitized and sensitized guinea pigs were subjected to 30-min hypoxia/30-min reoxygenation using a Langendorff apparatus. The amount of histamine released in the sensitized guinea-pig hearts was elevated, and the duration of VF was found to be reduced. The treatment with a histamine H₂-receptor antagonist inhibited the reduction of VF duration. Treatment of the non-sensitized hearts with the histamine H₂-receptor agonist resulted in the decrease of VF duration to the same level as that in the sensitized hearts. In conclusion, these results suggest that the risk of sudden death during myocardial H/R may be attenuated in the sensitized hearts and that histamine H₂-receptor activation due to the released histamine may be involved in the protective effect.

Keywords: histamine, histamine H₂-receptor, hypoxia/reoxygenation, ventricular fibrillation, sensitization

Introduction

Ischemic heart disease (IHD) is the major cause of death due to cardiovascular disease. The death rate of IHD is the highest among all the causes of death in Europe (1) and the United States of America (2), and it was 56.7 for 100,000 people in Japan in 2002 (3). It is assumed that the number of patients with IHD will increase globally in the next decade. On the other hand, a large number of people suffer from allergic diseases, such as asthma and atopic dermatitis, particularly in the urban areas. Many of these patients commonly have high serum immunoglobulin E (IgE) levels. The estimated patients under medical consultation by allergic diseases has already reached one million (4), and hereafter, the number of patients with allergic diseases will also increase globally similar to the number of IHD patients.

As mentioned above, it can be assumed that IHD patients with high IgE level will increase in the future. Szczeklik et al. performed a study focused on the relationship between serum IgE level and sudden cardiac arrest in patients with acute myocardial infarction (5, 6). The report indicated that the death rate from sudden cardiac arrest in patients with high serum IgE levels was lower than that in patients with low serum IgE levels. They speculate that IgE may be bound to the surface of mast cells, which are located adjacent to the coronary arteries, and that the IgE-sensitization might facilitate the release of chemical mediators such as histamine, prostaglandins, and leukotrienes from mast cells (6). This hypothetical mechanism might account for the reduction in death rate in the patients with high serum IgE level. However, to date, the assumed mechanisms...
have rarely been investigated.
Mast cells that localize in dermis, mucosa, and heart contain a large amount of histamine in the intracellular granules, and they induce histamine release in response to immunological and non-immunological stimuli (7, 8). Some studies have reported that during myocardial ischemia and reperfusion, histamine is released from the heart of rats (9), dogs (10, 11), guinea pigs (12, 13), and humans (14, 15). Histamine is known to be one of the chemical mediators with inotropic and chronotropic actions and is also a vasoactive and arrhythmogenic substance (16–18). Activation of histamine H₁-receptors results in a slowing atrioventricular (AV) conduction (19). On the other hand, histamine H₂-receptor stimulation causes sinus tachycardia, AV node automaticity, and ventricular arrhythmias such as extrasystole and ventricular tachycardia (19). However, Levi et al. recently reported that histamine H₂-receptors may regulate cardiac function by negatively modulating the release of norepinephrine from sympathetic nerve endings, indicating that histamine may play an important role in reperfusion arrhythmias (20, 21).

Based on these evidences and speculations, we hypothesized that the increase in released histamine from cardiac mast cells by sensitization may have a protective effect against ventricular fibrillation (VF) induced by myocardial ischemia/reperfusion (I/R). In the present study, we used a hypoxia/reoxygenation (H/R) model in isolated guinea-pig hearts to test this hypothesis. Moreover, we used atenolol, a β₁-selective adrenergic antagonist, and SKF-91488, a histamine N-methyltransferase inhibitor, to observe the effect of histamine clearly. Here, we have examined the role of histamine on the myocardial H/R-induced VF and discussed the effect of sensitization on the myocardial H/R-induced VF.

Materials and Methods

Animals
Female Hartley guinea pigs (Japan SLC, Inc., Hamamatsu) weighing 380–540 g were used in the experiments. They were quarantined for 7 days at a constant temperature (25 ± 1°C) and a constant relative humidity (60 ± 5%) under a 12-h light, 12-h dark cycle before use. Standard laboratory chow and water were available ad libitum. All experiments were approved by the Institute Animal Care and Use Committee, Tokushima Bunri University.

Sensitization of animals
Guinea pigs were actively sensitized by subcutaneous injection of 1 mg ovalbumin in a 0.5-ml (2 × 10¹⁰ bacilli/ml) Bordetella pertussis vaccine. After 14 days, the animals were used for myocardial H/R studies by means of the Langendorff apparatus. A subgroup of animals was used for determination of the serum total-IgE levels. The non-sensitized control group received no adjuvant and antigen.

Determination of serum total-IgE levels
Blood was collected prior to sensitization and on 7, 14, and 21 days after sensitization (non-sensitized guinea pigs, n = 4; sensitized guinea pigs, n = 9). The serum was isolated, and serum total-IgE levels were determined with a commercial kit.

Isolated heart perfusion
Guinea pigs were anesthetized by the administration of an intraperitoneal injection of 30 mg kg⁻¹ of pentobarbital sodium. The hearts were rapidly excised from the thoracic cavity, the aorta was cannulated, and the hearts were transferred to a Langendorff apparatus. The hearts were retrogradely perfused at a constant pressure of 40 mm Hg with Krebs-Henseleit (KH) buffer containing 118.1 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.1 mM glucose. The KH buffer was maintained at 37°C and equilibrated with 95% O₂ and 5% CO₂ throughout the experiment. Bipolar surface electrocardiograms (ECG) were continuously recorded by electrodes attached to the right auricle as well as the apex of the left ventricle of the heart. Coronary effluent was collected at 10-min intervals. The volume of the effluent was measured in order to determine coronary flow; histamine contents in the effluent were also measured.

Experimental protocol
The hearts were subjected to H/R following a 30-min stabilization period. Normothermic hypoxia was induced by perfusing for 30 min with glucose-free KH buffer equilibrated with 95% N₂ and 5% CO₂. Following the hypoxia period, reoxygenation was performed for 30 min by using KH buffer equilibrated with 95% O₂ and 5% CO₂. The hearts were divided into the following groups: Group 1: Non-sensitized guinea-pig hearts (n = 7); Group 2: Sensitized guinea-pig hearts (n = 8); Group 3: Sensitized guinea-pig hearts under treatment with 300 nM pyrilamine (22), a histamine H₁-receptor antagonist (n = 5); Group 4: Sensitized guinea-pig hearts under treatment with 300 nM ranitidine, a histamine H₂-receptor antagonist (n = 5); Group 5: Sensitized guinea-pig hearts under treatment with 300 nM famotidine, a histamine H₂-receptor antagonist (n = 6); Group 6: Sensitized guinea-pig hearts under treatment...
with 300 nM thioperamide (23), a histamine H_{3}-receptor antagonist (n = 5); and Group 7: Non-sensitized guinea-pig hearts under treatment with 100 nM dimaprit (24), a histamine H_{2}-receptor agonist (n = 9).

The experiments were first performed on groups 1 and 2. After completion of these experiments, they were performed on groups 3 – 7. In order to observe the effect of histamine clearly, all the groups were treated with 50 μM atenolol and 10 μM SKF-91488. All the drugs were dissolved in KH buffer, and perfusion of the hearts was performed throughout the experiment. Hearts were weighed at the end of the experiment.

**Visualization of myocardial H/R-induced mast cell degranulation**

In groups 1 and 2, following myocardial H/R, the hearts were fixed in Carnoy’s fixative and then embedded in paraffin. Sections (4 μm) were prepared by microtomy and deparaffinized in accordance with the standard protocol, followed by staining with 0.5% toluidine blue. Subsequently, coverslips were placed on the sections after they were rapidly dehydrated. Myocardial H/R-induced degranulation of mast cells was examined under a light microscope at ×400.

**Measurement of histamine contents**

Ten milliliters of the coronary effluent in groups 1 and 2 was collected and lyophilized. Histamine contents were determined by a commercial kit.

**Evaluation of VF**

VF, associated with myocardial H/R, was recognized as an irregular modulating baseline in the ECG tracing. The incidence and total duration of VF during reoxygenation were calculated in each group.

**Materials**

Ovalbumin, pyrilamine, ranitidine, famotidine, thioperamide, and atenolol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimaprit was purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA, USA). SKF-91488 was purchased from Tocris Cookson, Inc. (Ellisville, MO, USA). Toluidine blue was obtained from Waldeck-Gmbh & Co., KG (Muenster, Germany). Bordetella pertussis vaccine was kindly provided by the Kitasato Institute (Tokyo). The guinea-pig IgE ELISA kit was kindly provided by Dainippon Pharmaceutical Co., Ltd. (Osaka). The histamine ELISA kit was obtained from Immunotech A Beckman Coulter Co. (Marseille, France).

**Statistical analyses**

Results are expressed as the mean ± S.E.M. Statistical comparison was performed by one-way analysis of variance (ANOVA) followed by Dunnett’s test or the unpaired Student’s t-test. The incidence of VF during reoxygenation was compared by Fisher’s exact test. P values less than 0.05 were considered significant.

**Results**

**Serum total-IgE levels**

Basal serum total-IgE level was 7.5 ± 0.9 and 20.9 ± 8.6 ng/ml in the non-sensitized and sensitized guinea pigs, respectively. No significant difference was observed in the basal serum total-IgE level between non-sensitized and sensitized guinea pigs. Serum total-IgE level in the sensitized guinea pigs was markedly elevated to 238.4 ± 55.5 and 234.6 ± 32.0 ng/ml at days 14 and 21, respectively (Fig. 1) (P<0.01); and the values were significantly higher than those in the non-sensitized guinea pigs (Fig. 1) (P<0.01). Serum total-IgE level in the non-sensitized guinea pigs was maintained at a basal level at days 7, 14, and 21.

**Histamine release induced by myocardial H/R**

A large number of mast cells were found in the thin sections from both non-sensitized and sensitized guinea-pig hearts subjected to myocardial H/R, and some of those mast cells showed significant degranulation (Fig. 2: A and B). The number of mast cells was higher in the sensitized guinea-pig hearts than in the non-sensitized hearts. In particular, many degranulated mast cells that surrounded the coronary artery were observed in the sections obtained from the sensitized hearts. At all the time points, histamine levels in the coronary

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**Fig. 1.** Time course of total-IgE level in the serum of non-sensitized and sensitized guinea pigs. Each value is represented as the mean ± S.E.M. (non-sensitized guinea pigs, n = 4; sensitized guinea pigs, n = 9). **P<0.01 vs non-sensitized guinea pigs by the unpaired Student’s t-test, ***P<0.01 vs basal level by ANOVA with Dunnett’s test.
Protective Mechanisms of Sensitization

Effluent were observed to be elevated approximately 2-fold in the sensitized guinea-pig hearts as compared to the non-sensitized hearts (Fig. 3A). Basal histamine level in the coronary effluent (0 min) was 0.7 ± 0.1 and 1.9 ± 0.8 ng/g tissue in the non-sensitized and sensitized guinea-pig hearts, respectively. The histamine level in both groups increased by approximately 2-fold at 10 min following hypoxia; they reverted to the basal level at 30 min following hypoxia.

Coronary flow in the sensitized guinea-pig hearts showed no difference as compared with that in the non-sensitized hearts (Fig. 3B). The basal coronary flow (0 min) was approximately 8 ml/min in the non-sensitized and in the sensitized guinea-pig hearts. Following hypoxia, coronary flow in both groups increased by approximately 1.3-fold at 10 min, and then, it gradually decreased until 60 min.

VF induced by myocardial H/R

ECG tracings in the non-sensitized and sensitized guinea-pig hearts showed a regular sinus rhythm during stabilization. Hypoxia evoked an AV block in both guinea-pig hearts. Furthermore, in both guinea-pig hearts, following reoxygenation, VF occurred within

Fig. 2. Histological evidence of mast cell degranulation. Samples were obtained from non-sensitized (A) and sensitized (B) guinea-pig hearts subjected to 30-min hypoxia following 30-min reoxygenation. Sections were stained with 0.5% toluidine blue to identify mast cells (×400). Arrows show significant degranulation of mast cells.

Fig. 3. Time courses of histamine release into the coronary effluents (A) and coronary flow (B) in the non-sensitized and sensitized guinea-pig hearts subjected to 30-min hypoxia following 30-min reoxygenation. Each value is represented as the mean ± S.E.M. (non-sensitized guinea-pig hearts, n = 7; sensitized guinea-pig hearts, n = 8). A: **P<0.01 vs non-sensitized guinea-pig hearts by the unpaired Student’s t-test. B: No significant differences were observed between non-sensitized and sensitized guinea-pig hearts.
5 min. Following reoxygenation, VF in the non-sensitized guinea-pig hearts did not return to the regular sinus rhythm at 10 min. In contrast, following reoxygenation, VF in the sensitized guinea-pig hearts recovered to a regular sinus rhythm within 10 min (Fig. 4). The incidence of VF during reoxygenation was 57% (4/7) and 38% (3/8) in the non-sensitized and sensitized guinea-pig hearts, respectively. No significant difference was observed in the incidence of VF during reoxygenation between the two groups. However, the total duration of VF during reoxygenation was markedly shortened in the sensitized guinea-pig hearts as compared with the non-sensitized hearts; each duration was 10.7 ± 3.0 min in the non-sensitized group and 1.3 ± 0.8 min in the sensitized group (Fig. 5) (P<0.05).

**Effects of histamine receptor antagonists on VF during reoxygenation in the sensitized guinea-pig hearts**

The incidence of VF during reoxygenation was 38% (3/8), 0% (0/5), 60% (3/5), 50% (3/6), and 0% (0/5) in the non-treated, pyrilamine-treated, ranitidine-treated, famotidine-treated, and thioperamide-treated guinea-pig hearts, respectively. Pyrilamine-treated and thioperamide-treated guinea-pig hearts did not have any VF during reoxygenation. No significant difference was observed in the incidence of VF during reoxygenation between the non-treated and the ranitidine- or famotidine-treated guinea-pig hearts. The total duration of VF

![Fig. 4](image1.png)

**Fig. 4.** Typical ECG tracings obtained from one non-sensitized and one sensitized guinea-pig heart during stabilization, hypoxia, and reoxygenation. VF: ventricular fibrillation.

![Fig. 5](image2.png)

**Fig. 5.** The total duration of VF during reoxygenation in the non-sensitized and sensitized guinea-pig hearts subjected to 30-min hypoxia following 30-min reoxygenation. Each value is calculated based on the results obtained from hearts with VF, represented as the mean ± S.E.M. of three experiments. **P<0.01 vs non-treated guinea-pig hearts by the unpaired Student’s t-test.

![Fig. 6](image3.png)

**Fig. 6.** Effects of ranitidine and famotidine on the total duration of VF during reoxygenation in the sensitized guinea-pig hearts. Each value is calculated based on the results obtained from hearts with VF, represented as the mean ± S.E.M. of three experiments. **P<0.01 vs non-treated guinea-pig hearts by the unpaired Student’s t-test.

![Fig. 7](image4.png)

**Fig. 7.** Effect of dimaprit on the total duration of VF during reoxygenation in the non-sensitized guinea-pig hearts. Each value is calculated based on the results obtained from hearts with VF, represented as the mean ± S.E.M. (non-treated guinea-pig hearts, n = 4; dimaprit-treated guinea-pig hearts, n = 6). *P<0.05 vs non-treated guinea-pig hearts by the unpaired Student’s t-test.
during reoxygenation was 1.3 ± 0.8, 6.4 ± 3.6, and 10.4 ± 1.0 min in the non-treated, ranitidine-treated, and famotidine-treated guinea-pig hearts, respectively. The value was significantly higher in the famotidine-treated guinea-pig hearts as compared to the non-treated hearts (Fig. 6) (P<0.01). Further, the value tended to increase in the ranitidine-treated guinea-pig hearts as compared to the non-treated hearts (Fig. 6).

Effect of dimaprit on VF during reoxygenation in the non-sensitized guinea-pig hearts

The incidence of VF during reoxygenation was 57% (4/7) and 67% (6/9) in the non-treated and dimaprit-treated guinea-pig hearts, respectively. No significant difference was observed with regard to the incidence of VF during reoxygenation between the two groups. However, the total duration of VF during reoxygenation markedly shortened in the dimaprit-treated guinea-pig hearts as compared with the non-treated hearts; the duration was 10.7 ± 3.0 min in the non-treated group and it was 1.7 ± 0.7 min in the dimaprit-treated group (Fig. 7) (P<0.05).

Discussion

This work provides further evidence for the histaminergic effect on duration of VF during reoxygenation and is the first report on the protective effect of histamine via its H₂-receptor.

Serum total-IgE level was measured to assess sensitization of guinea pigs at 7, 14, and 21 days. The result shows that serum total-IgE level was markedly elevated on day 14, thereby indicating that the sensitization of guinea pigs is established on day 14. Therefore, following sensitization, the guinea pigs were used at day 14 so that we could examine the effect of sensitization on VF induced by myocardial H/R.

In this study, many degranulated mast cells were observed in the sensitized guinea-pig hearts subjected to myocardial H/R. The released histamine from sensitized guinea-pig hearts was 2 times higher than that from non-sensitized hearts. The number of cardiac mast cells increased through sensitization, and a large amount of degranulation was observed in the sensitized hearts as compared to that in the non-sensitized hearts. These results indicate that the source of released histamine is cardiac mast cells and suggest that the increase in released histamine after sensitization is strongly related with the increase in the number of mast cells and degranulated mast cells.

VF has been known to occur within seconds to minutes of reperfusion after brief periods of ischemia (25) and has been known as the major cause of sudden death in patients with acute myocardial ischemia (26, 27). Reducing the incidence or duration of VF is pivotal for reduction in the death rate due to VF (28–30). In this study, following reoxygenation, VF occurred within 5 min in the sensitized as well as the non-sensitized hearts. The total duration of VF had significantly decreased in the sensitized heart although the incidence of VF during reoxygenation exhibited no change. Therefore, we hypothesized that the augmentation of released histamine by IgE-sensitization would reduce the incidences of sudden death at least by reducing VF duration. This hypothesis is supported by the report by Szczeklik et al. (5, 6).

We pharmacologically investigated the detailed mechanism of the histaminergic effect on the reduction in the total duration of VF during reoxygenation in the sensitized guinea-pig hearts. Ranitidine, which is an exemplary histamine H₂-receptor antagonist, prolonged VF duration in the sensitized hearts. Famotidine, which is a potent histamine H₂-receptor antagonist with a high binding constant to the histamine H₂-receptor as compared to ranitidine (31), also markedly prolonged the VF duration as compared to ranitidine. Furthermore, dimaprit, a typical histamine H₂-receptor agonist, was observed to significantly shorten VF duration in the non-sensitized hearts as well as in the non-treated hearts obtained from sensitized guinea pigs. These results indicate that histamine H₂-receptor stimulation is related to the reduction of the total duration of VF during reoxygenation in the sensitized guinea-pig hearts. Complex dysfunction at the level of ion channel homeostasis is responsible for regional depolarization and variable changes in refractoriness and conduction velocity, and this results in a predisposition to focal and re-entrant syncytial mechanisms of arrhythmogenesis (27). Previous analysis showed that the ionic determinant of refractoriness is related to the reduced density and abnormal sodium current (I Na) kinetics in myocytes dispersed from the 5-day infarcted canine heart (32). Furthermore, a recent study indicates that a protein kinase A (PKA) activator cocktail does augment I Na density in myocytes dispersed from the 5-day infarcted canine heart (33). In addition, β-adrenergic stimulation has been shown to increase I Na density (34) and has been suggested to be antiarrhythmic in the infarcted heart (33). Histamine H₂-receptors coupled with adenyl cyclase via the GTP-binding protein G, and histamine H₂-receptor stimulation activates PKA via cAMP accumulation (17). This suggests that histamine H₂-receptor stimulation may remit the major cause of sudden death during H/R via PKA-dependent augmentation of I Na density. However, the detailed mechanism underlying this beneficial effect is unclear and further
research would be needed to elucidate the mechanisms. On the other hand, VF due to H/R was cancelled by the treatment of pyrilamine, a histamine H1-receptor antagonist. Valen et al. also has observed that chlorpheniramine inhibits reperfusion-induced arrhythmias (35). Therefore, it was suggested that the reduction of the total duration of VF during reoxygenation in the sensitized guinea-pig hearts is not due to histamine H1-receptor stimulation. In addition, the treatment of thioperaamide did not provoke H/R-induced VF. Liu et al. have revealed thioperaamide to be a histamine H2- and H1-receptor antagonist (36). Therefore, histamine H1-receptor blockade also might be involved in the effect of thioperaamide on VF due to H/R in this study. Our result suggests that the reduction of the total duration of VF during reoxygenation in the sensitized guinea-pig hearts is not due to histamine H2- and H3-receptor stimulation. Levi et al. previously reported that histamine H1-receptor plays an antiarrhythmic role in VF during I/R by attenuating the release of norepinephrine (20, 37). Our results indicate that the duration of VF was affected via histamine H2-receptor and are inconsistent with their reports. However, we used atenolol, a β1-selective adrenergic antagonist, in this study to focus upon the absolute histaminergic effect on H/R. Therefore, we believe that the histaminergic effect itself might have such an effect on H/R provided that it is unaffected by other factors. In summary, these results indicate that cardiac histamine H2-receptors, but not histamine H1-, H3-, and H4-receptors, appear to play a crucial role in the reduction of the total duration of VF during reoxygenation in the sensitized guinea-pig hearts. Therefore, the major cause of sudden death during H/R may be remitted by histamine which is released by H/R. This type of remission via the histamine H2-receptor would be observed particularly in the case of patients with allergic diseases.

In conclusion, the results of this study suggest that the risk of sudden death during reoxygenation is attenuated in the sensitized hearts and that the protective effect involves the histamine H2-receptor activation by the increase in released histamine from sensitized cardiac mast cells. Therefore, the method of assessment of sensitization, such as monitoring serum IgE level, may represent a new advance in estimating the risk of sudden death during reoxygenation.

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