Characterization of Stressed Transgenic Mice Overexpressing H₂-Cardiovascular Receptors in the Heart

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ABSTRACT

We studied transgenic mice with cardiac-specific overexpression of H₂-histamine receptors (H₂-TG) by using the α -myosin heavy-chain promoter. We wanted to address whether this overexpression would protect the heart against paradigmatic stressors. To this end, we studied isolated atrial preparations in an organ bath under normoxic and hypoxic conditions and after prolonged exposure to high histamine concentrations. Moreover, we assessed cardiac function using echocardiography in mice with cardiac hypertrophy due to overexpression of the catalytic subunit of PP2A (PP2A-TG) in the heart [H₂-TG $\times$ PP2A-TG = double transgenic (DT)] or H₂-TG with cardiac systolic failure due to treatment of mice with lipopolysaccharides (LPSs). Furthermore, the effect of ischemia and reperfusion was studied in isolated perfused hearts (Langendorff mode) of H₂-TG. We detected evidence for the protective role of the overexpressed H₂-histamine receptors in the contractile dysfunction of DT and isolated atrial preparations subjected to hypoxia. In contrast, we noted the detrimental role of

H₂-histamine receptor overexpression against ischemia (Langendorff perfusion) and LPS-induced systolic heart failure. Hence, the role of H₂-histamine receptors in the heart is context-sensitive: the results differ between hypoxia (in atrium) and ischemia (perfused whole heart), as well as between genetically induced hypertrophy (DT) and toxin-induced heart failure (LPS). The underlying molecular mechanisms for the protective or detrimental roles of H₂-histamine receptor overexpression in the mammalian heart remain to be elucidated.

SIGNIFICANCE STATEMENT

The beneficial and detrimental effects of the cardiac effects of H₂-histamine receptors in the heart under stressful conditions, here intended to mimic clinical situations, were studied. The data suggest that depending on the clinically underlying cardiac pathophysiological mechanisms, H₂-histamine agonists or H₂-histamine antagonists might merit further research efforts to improve clinical drug therapy.

Introduction

An important monoamine, histamine [imidazolyl-ethylamine or 2-(1H-imidazol-4-yl)ethanamine; review: Parsons and Ganelin (2006)], can mediate many pathophysiological processes, including inflammation, allergies, and responses to stress in the widest sense (Jutel et al., 2009). Histamine was first synthesized by Windaus and Vogt (1907) from imidazolylpropionic acid in vitro. Ackermann (1910) showed that bacteria could convert histidine to β -imidazolyl-ethylamine, which was later called histamine (Fühner, 1912).

Dale and Laidlaw (1911) and subsequent papers from Sir Dale's group in Oxford (England) were the first to describe the presence, synthesis, and degradation of histamine in mammalian tissue and a possible role of histamine as a transmitter in the cardiovascular system and the putative role of histamine in cardiac anaphylaxis; they noted that histamine exerted positive chronotropic effects and positive inotropic effects not only in

the spontaneously beating Langendorff heart of rabbits but also in rabbits in vivo (Dale and Laidlaw, 1911).

We recently overexpressed the H₂-histamine receptor in the heart using the α -myosin promoter. This promoter drives the expression in all cells that physiologically contain α -myosin. In other words, we overexpressed the H₂-histamine receptor in cardiomyocytes specifically. We (Gergs et al., 2019) published an entire extensive report on the cardiac phenotype of this H₂-histamine receptor-overexpressing mouse (H₂-TG) compared with its wild-type (WT) littermates. In brief, only in H₂-TG, but not in WT, did histamine increase the force of contraction and the beating rate (Gergs et al., 2019). This was seen in isolated atrial preparations, isolated electrically driven cardiomyocytes, Langendorff perfused hearts, and in vivo using echocardiography (Gergs et al., 2019). Using autoradiography with a ligand for the H₂-histamine receptors, we could localize the overexpressed receptor to the atrium and the ventricle of H₂-TG (Gergs et al., 2019).

In the present study, we used this model to address the possible protective or detrimental role of H₂-histamine receptors

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ABBREVIATIONS: DT, double transgenic; HDC, histidine decarboxylase; H₁, histamine receptor type 1; H₂, histamine receptor type 2; LAD, left descending coronary artery; LPS, lipopolysaccharide; PLB, phospholamban; PP2A, protein phosphatase 2A; TG, transgenic; WT, wild-type.

in the mammalian heart under stressful conditions. In the present context, it is important to keep in mind that histamine has failed to exert any cardiac effects in isolated tissue or living WT mice (Gergs et al., 2019). Hence, the present model offers a unique opportunity to study the human H₂-histamine receptor in a mammalian cardiac model using WT mice as a negative control.

During myocardial infarction, the plasma levels of histamine greatly increase in men and mice [Chen et al., 2017, review; Reid et al. (2011)]. Histamine is also released from isolated saline buffer-perfused ischemic hearts (Genovese et al., 1988). In various models of sepsis, an increase of histamine in plasma has been found; for instance, lipopolysaccharide (LPS, a model of Gram negative bacteria-mediated sepsis) was intraperitoneally injected in rabbits (Matsuda et al., 2002) and in mice (Yokoyama et al., 2004) and led to elevated histamine levels in the plasma.

Elevated histamine levels might be protective because histamine injections can increase the survival rate of LPS-treated mice (Yokoyama et al., 2004). These high levels of histamine, on the other hand, might lead to receptor desensitization: one of the earliest reports on desensitization of H₂-histamine receptors was in human T-lymphocytes (Schreurs et al., 1984), with later evidence suggesting that this desensitization of the H₂-histamine receptor involves protein kinases (Fernández et al., 2002, 2011).

Firm evidence indicates that H₂-histamine receptors can play a role in cardiomyopathy and heart failure; for instance, in a dog model, cardiomyopathy (pacing-induced tachycardia leading to cardiac hypertrophy over time) was ameliorated by giving an H₂-histamine receptor antagonist (Takahama et al., 2010). In the H₂-histamine receptor knockout mice, aortic banding led to less cardiac fibrosis than in sham-operated mice, which may mean that under these conditions, H₂-histamine receptors are detrimental for cardiac function by facilitating cardiac hypertrophy (Zeng et al., 2014).

Generally, the density of mast cells is increased in the hypertrophied heart (Francis and Tang, 2006). At the same time, histamine is highly concentrated in mast cells (Jutel et al., 2009); this may mean that, via mast cells, cardiomyopathy leads to an increase in the availability of putative detrimental histamine in heart tissue.

Hitherto, in the current study, we hypothesized that in H₂-TG mice, the H₂-histamine receptors are detrimental for cardiac function under pathologic conditions and answered the following questions: Do H₂-histamine receptors in the heart protect the heart against ischemic, reperfusion, or hypoxic injury? Do high cardiac levels of histamine (which can occur under pathologic conditions) functionally impair H₂-histamine receptor function in the heart? Do H₂-histamine receptors in the heart protect the heart against genetic hypertrophy (PP2A mice) or toxin-induced hypertrophy (LPS) and impaired cardiac function? Progress reports of this work have been published previously as congress abstracts (Künstler et al., 2015; Bergmann et al., 2016; Gergs et al., 2016; Neumann et al., 2016, 2017a,b, 2018a,b; Mißlinger et al., 2017; Au et al., 2018).

Materials and Methods

Experimental Animals. The investigations in the present report conform to the Guide for the Care and Use of Laboratory Animals published by the National Research Council (2011). Animals were

maintained and handled according to the approved protocols of the animal welfare committees of the University of Münster, Germany, and of the University of Halle-Wittenberg, Germany. The generation of H₂-TG has recently been reported (Gergs et al., 2019), and before the PP2A-TG (Gergs et al., 2004), double transgenic (DT) mice were obtained by crossbreeding these lines (H₂-TG × PP2A-TG = DT).

Isolated Atrial Preparations. Jointly, the contractile studies followed our published procedures (e.g., Gergs et al., 2010, 2019). Mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg kg⁻¹), and the hearts were excised. The right and left atria were dissected from hearts of H₂-TG or littermate wild-type mice and mounted in an organ bath. Left atrial preparations were continuously electrically stimulated (field stimulation) with each impulse; these consisted of 1 Hz, with a voltage 10%–15% above the threshold and a 5-millisecond duration. Right atrial preparations were allowed to contract spontaneously. The bathing solution contained (in mM) NaCl 119.8, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 22.6, Na₂EDTA 0.05, ascorbic acid 0.28, and glucose 5.0; this was continuously gassed with 95% O₂ and 5% CO₂ and maintained at 35°C, resulting in a pH of 7.4. Forces of contraction detected via an isometric force transducer were amplified and continuously recorded using a PowerLab system (ADInstruments, Oxford, UK).

The key findings were obtained in isolated left and right atria of mice as reported repeatedly before (e.g., Neumann et al., 1998, Gergs et al., 2019). Briefly, hypoxia was induced by switching the gas supply for 20 minutes to a mixture of 95% N₂ and 5% CO₂ (obtained from Linde Gas supply, Germany). Thereafter, the gas supply was switched again to carbogen. At the indicated times, the atrium was rapidly frozen to the temperature of liquid nitrogen (−196°C) and stored at −80°C until biochemical analysis (see below). In some experiments, different protocols were used, including hypoxic precondition. Details can be found in the appropriate legends and figures.

Isolated Perfused Hearts. Langendorff preparations of the mouse hearts were used as described previously (Kirchhefer et al., 2014, Gergs et al., 2019). The mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg kg⁻¹), and the hearts were excised. The hearts were removed from the opened chest, immediately attached by the aorta to a 20-gauge cannula, and perfused retrogradely under a constant flow of 2 ml min⁻¹ with oxygenized buffer solution (37°C) containing (in mM) NaCl 119.8, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 22.6, Na₂EDTA 0.05, ascorbic acid 0.28, and glucose 5.0 in an isolated heart system built in-house. The heart preparations were allowed to equilibrate for 30 minutes before measurements were taken. Hearts beating spontaneously and left ventricular force were measured and monitored continuously. Ischemia was simulated by stopping the peristaltic pump for the buffer for 20 minutes. Reperfusion was simulated by starting the pump again. At the end of the reperfusion time, whole hearts were frozen by freeze clamping and brought to the temperature of liquid nitrogen (−196°C) by using precooled aluminum (Wollenberger) tongs.

Western Blot Analysis. Material from atrial or ventricular tissue was homogenized (as described repeatedly in Neumann et al., 1998, Gergs et al., 2019) in 300 µl of 10 mM NaHCO₃ and 100 µl 20% SDS. Crude extracts were incubated at 25°C for 30 minutes before centrifugation to remove debris; thereafter, the supernatants (homogenates) were separated and stored at −80°C until further use. Western blot analysis was performed as previously described (Gergs et al., 2004, 2010, 2019). Briefly, aliquots of 100 µg of protein were loaded per lane. Finally, bands were detected using enhanced chemifluorescence (GE Healthcare Europe, Freiburg, Germany) together with a Typhoon 9410 Variable Mode Imager (GE Healthcare Europe). The following primary antibodies were used in the current study: polyclonal rabbit anti-calnexin (dilution 1:1000; Acris Antibodies, Hiddenhausen, Germany), monoclonal mouse anti-phospholamban (PLB; A-1, dilution 1:2000; Badrilla, Leeds, UK), polyclonal rabbit anti-phosphoserine-16-PLB (dilution 1:5000; Badrilla), and monoclonal

rabbit anti-troponin inhibitor and polyclonal rabbit anti-phosphoserine-23/24 troponin inhibitor (dilution 1:1000; both from Cell Signaling Technology, Frankfurt am Main, Germany). The characteristics and use of these antibodies have been reported repeatedly by our group (Kirchhefer et al., 2002).

Echocardiography. The echocardiographic measurements in spontaneously breathing mice were performed under anesthesia with 1.5% isoflurane, as described previously (Gergs et al., 2010, 2019).

Sepsis Model. Intraperitoneal injections of LPS in mice were performed with 30 $\mu\text{g/g}$ body weight. LPS was dissolved in isotonic NaCl solution. The control mice were injected with isotonic NaCl solution.

Data Analysis. The data shown are means $\pm$ S.D. Statistical significance was estimated by an ANOVA followed by Bonferroni *t* test using Prism 5 (GraphPad Software, San Diego, CA). A *P* value <0.05 was considered as significant.

Drugs and Materials. Histamine, dimaprit, and LPS [*Escherichia coli* (O55:B5)] were purchased from Sigma-Aldrich (Taufkirchen, Germany). All other chemicals were of analytical grade. Demineralized water was used throughout the experiments. Stock solutions were freshly prepared daily.

Results

The quantification of cardiac weight in PP2A-TG (see Fig. 1) detected cardiac hypertrophy [as described before in Gergs et al. (2004)], whereas H₂-TG was devoid of an increase in cardiac size (Fig. 1). In DT mice (generated by crossbreeding H₂-TG with PP2A-TG), we did not notice a reduction of cardiac weight compared with monotransgenic PP2A-TG mice (Fig. 1).

Regarding cardiac echocardiography, however, the systolic (Fig. 2) and diastolic cardiac functions were less impaired in DT than in PP2A-TG (Fig. 3). Interestingly, the β -adrenoceptor agonist isoproterenol (isoprenaline) increased systolic function in vivo in all four genotypes, as assessed by measuring the ejection fraction (Fig. 2). However, histamine only increased the ejection fraction in mice in H₂-TG or DT (Fig. 2, black bars). Likewise, cardiac dimensions were elevated in PP2A mice and less elevated in DT (Fig. 4, A and B).

Thereafter, we investigated the effect of global ischemia in isolated buffer-perfused hearts (Langendorff mode) and subsequent reperfusion. The protocol used and typical force tracings

Fig. 1. PP2A-TG showed an increased relative heart weight (ordinate). Ordinate: relative heart weight (heart weight divided by body weight in milligrams per gram). WT, open circles; H₂-TG, closed circles; PP2A-TG, squares; DT, triangles. Numbers in brackets indicate the numbers of animals investigated. **P* <0.05 vs. WT.

Fig. 2. Ejection fraction (EF) of left ventricle after intraperitoneal injection of histamine or isoproterenol. Using echocardiography, histamine (100 μl of 1 mM, i.p.) elevated contractility in H₂-TG compared with WT mice. PP2A-TG and DT (H₂-TG $\times$ PP2A-TG) were less reactive to isoproterenol (100 μl of 10 mM, i.p.). Interestingly, in DT the EF was higher than in PP2A-TG at similar heart rates. ANOVA with Bonferroni post hoc test. **P* <0.05 vs. WT, +*P* <0.05 vs. H₂-TG, #*P* <0.05 vs. PP2A-TG, **P* <0.05 vs. corresponding basal level.

can be seen in Fig. 5. Under these conditions, we did not note a protection in H₂-TG (Fig. 6). In contrast, there was evidence that the force of hearts from H₂-TG deteriorated faster than in WT mouse hearts (Fig. 6C). Moreover, after reperfusion, more time was required to regain sinus rhythm in atria from H₂-TG overexpressing mice than in WT (Fig. 6D).

Underexposure of the isolated left or right atrium from H₂-TG to high bath concentrations of histamine (100 μM , 1 hour) reduced the ability of histamine (Fig. 7, A and B) or the H₂-receptor agonist dimaprit (Fig. 7, C and D) to elicit a concentration-dependent positive inotropic effect and a positive chronotropic effect (Fig. 7, E and F), respectively. For comparison, we also found that the pretreatment of whole animals with dimaprit could attenuate the positive inotropic effect and the positive chronotropic effect of dimaprit in vivo (echocardiography: Fig. 8) in H₂-TG.

As shown in Fig. 9A, the atria of H₂-TG are more resistant to hypoxia than the atria from WT (Fig. 9A). This protective effect of transgenicity was abolished by adding the H₂-histamine receptor antagonist cimetidine to the organ bath shortly before the induction of hypoxia (Fig. 9B). A similar protective effect of transgenicity was noted after preconditioning of the atria (Fig. 9C).

When stimulating the atria with histamine, the protective effect of transgenicity against hypoxia was greatly increased: only in H₂-TG, but not in WT, did histamine quickly elicit a pronounced positive inotropic effect (Fig. 9D). During hypoxia, the force of contraction fell in the atrium from both H₂-TG and WT. However, during reoxygenation, the force returned to higher values in the atrium of H₂-TG than in WT, but not to prehypoxic levels (Fig. 9D). The phosphorylation states of PLB at serine 16 and the inhibitory protein of troponin were higher in TG than in WT when measured at the end of 30 minutes of reoxygenation under the conditions shown in Fig. 9D.

However, LPS led to a time-dependent decrease in cardiac function (Fig. 10). In some parameters, a decrease in cardiac function was noted as early as 1 hour after injecting LPS. This decrease was more severe in hearts from H₂-TG than in WT (Fig. 10). LPS induced the mRNA expression of various genes,

Fig. 3. (A) E'/A' ratio in DT mice was increased. (B) Representative pulsed wave (PW) tissue Doppler of WT, which marks the S, E', and A' waves. S, systolic wave; E wave, mitral peak velocity blood flow in early diastole caused by left ventricular relaxation; A wave, peak velocity blood flow in late diastole caused by atrial contraction. By tissue Doppler, the retrograde waves, E' (passive LV filling) and A'-wave (atrial contraction) can be measured and E'/A' can be used as a marker of diastolic dysfunction, $^*P < 0.05$ vs. H₂-TG. WT, open circles; H₂-TG, closed circles; PP2A-TG, squares; DT, triangles.

such as interleukin-6 and interleukin-1 β (data not shown), in these hearts. LPS increased the mRNA expression of tumor necrosis factor α much further in WT than in H₂-TG.

Discussion

As a new contribution to the field, in the present work, we studied the effect of the various stressors of cardiac function in vitro and in vivo in H₂-TG—a novel model system (Fig. 11; Gergs et al., 2019) with cardiac-specific expression of a functional human H₂-histamine receptor—compared with appropriate control conditions.

Fig. 4. Overexpression of the catalytic subunit of PP2A in PP2A-TG led to increased systolic and diastolic diameters of the left ventricle. (A) Quantification of echocardiographically measured systolic or diastolic left ventricular (LV) diameters in parasternal long axis view (PLAX). DT (H₂-TG $\times$ PP2A-TG) seemed to be less affected than PP2A-TG. ANOVA with Bonferroni post hoc test: $^*P < 0.05$ vs. WT, $^*P < 0.05$ vs. H₂-TG, $^{\#}P < 0.05$ vs. PP2A-TG. (B) Representative M-mode pictures for all investigated genotypes. Red, systolic diameter; blue, diastolic diameter.

We have a long-standing interest in cardiac phosphatases, namely serine/threonine phosphatases. We have worked for a long time on how they might contribute to β -adrenergic and muscarinic acetylcholine receptor M₂ signaling in the heart (Neumann et al., 1994). Based on our old studies, we have continuously developed new transgenic models for the main serine/threonine phosphatases in the heart. We were interested in not only how they alter the signals leading from the sarcolemmal receptors to the activation of protein kinases but also how they might play a role in the development of cardiac hypertrophy (Boknik et al., 2000) and even heart failure, which we looked at in some initial studies in human hearts (Neumann et al., 1999). In this line, we quite extensively characterized PP2A in humans (Knapp et al., 1999) and animal hearts (Neumann et al., 1993; Gombosova et al., 1998), overexpressed the catalytic subunit of PP2A (Gergs et al., 2004), and studied its role under stressful conditions, such as hypertension-induced hypertrophy (aortic banding) myocardial infarction (Brüchert et al., 2008; Grote-Wessels et al., 2008; Hoehn et al., 2015). Because the H₂-histamine receptor mediates its effects via cAMP and protein kinase A and phospholamban phosphorylation (Gergs et al., 2019), it seemed logical to test the influence of PP2A on H₂-histamine receptor-mediated effects because PP2A is known to dephosphorylate phospholamban not only in vitro (Neumann et al., 1993) but also in the beating heart (Gergs et al., 2004).

Before, we have reported and confirmed that cardiac-specific overexpression of the catalytic subunit of PP2A leads to cardiac hypertrophy and signs of systolic and diastolic heart failure and impaired cardiac dimensions (Gergs et al., 2004). H₂-histamine receptor overexpression in hearts of transgenic mice alone (H₂-TG) has been found to be devoid of cardiac hypertrophy or impaired cardiac function to β -adrenoceptor stimulation by isoproterenol (Gergs et al., 2019, and the current report). In the present study, at least diastolic heart failure was reduced in DT compared with PP2A mice. We regard this as an example of the putative protective role of H₂-histamine receptors in the heart. One could speculate that this beneficial effect would be seen in patients with elevated histamine levels. The biochemical reasons need to be elucidated: we have shown that overexpression of the catalytic subunit of PP2A leads to dephosphorylation of several cardiac regulatory proteins (PP2A-TG: Gergs et al., 2004), whereas the stimulation of H₂-histamine receptors leads to acute increases in the phosphorylation state of cardiac regulatory proteins (H₂-TG: Gergs et al., 2019). Therefore, it seems reasonable to hypothesize that H₂-histamine receptor overexpression additionally to

Fig. 5. Typical original recordings of the time course of force reduction (no flow ischemia) and force recovery [reperfusion (Rep)] in isolated perfused heart (Langendorff) preparations from WT and H₂-TG. The flow of 2 ml/min was stopped (global ischemia = no flow ischemia) for 20 minutes, as indicated by a horizontal line and restarted at the indicated time point. Ordinate: developed tension in millinewtons.

PP2A overexpression normalizes these phosphorylation states and, thus, cardiac dimensions and function.

Concerning plasma histamine levels, the literature agrees on a substantial increase in this parameter after an experimental induction of sepsis in animal models [such as by LPS used in the present report, reviewed in Matsuda et al. (2002)]. In rats, endotoxin-induced mortality was reduced if (probably by normalizing a decreased blood pressure) H₁-histamine receptor antagonists or H₂-histamine receptor antagonists were applied, implying the role of H₂-histamine receptor overexpression in sepsis (Brackett et al., 1985). Giving an inhibitor of histamine methyltransferase activity (an enzyme that degrades and, thus, inactivates histamine) enhanced the concentrations of histamine in the livers of LPS-treated

mice, and this was accompanied by reduced mortality of the animals, conceivably because of a fall in tumor necrosis factor α levels (Yokoyama et al., 2007). In rabbits, after the induction of sepsis, the plasma histamine level increased 100-fold, whereas catecholamines in plasma of the same animals increased more slowly and to a lesser extent (to 130%; Matsuda et al., 2002). This was accompanied by an increase in heart rate and a fall in systolic and diastolic blood pressure. An H₂-histamine receptor antagonist could attenuate this increase in heart rate (Matsuda et al., 2002). Interestingly, LPS increased the expression (mRNA and protein) of H₁- and H₂-histamine receptors in a rabbit ventricle, as well as the protein levels of the histidine decarboxylase (HDC), the pace-making enzyme for the in vivo synthesis of

Fig. 6. A period of 20 minutes of ischemia (see Fig. 5) did not cause permanent damage because, after reperfusion, force (A) and heart rate (B) of both H₂-TG and WT reached preischemic values again. Time to 50% decline of developed force ($F_{1/2}$) during ischemia was reduced in H₂-TG compared with WT (C), and after start of reperfusion, time to return to sinus rhythm was prolonged in H₂-TG (*two of six preparations did not reach sinus rhythm within 15 minutes) compared with WT (D). Ctr, control perfusion without ischemia. * $P < 0.05$ vs. WT, $n = 6$.

Fig. 7. *In vitro* desensitization of H₂-histamine receptors in isolated electrically driven (1 Hz) left atrial preparations of H₂-TG mice. First (see schematic drawing), initial concentration-response curves for histamine or the H₂-receptor agonist dimaprit were established. The effect of histamine was completely washed out by repeatedly exchanging the buffer in the organ bath. Thereafter, preparations were incubated with a high concentration of histamine or dimaprit (100 μ M) for 1 hour, or no additions were given [nondesensitized (N-DS)]. After complete washout of histamine/dimaprit, a second concentration-response curve for (A) histamine or (C) dimaprit was obtained. (B) EC₅₀ value of histamine; (D) comparison of desensitization (des.) with histamine and dimaprit. Similarly, as in left atria, desensitization was studied in isolated spontaneously beating right atrial preparations comparing histamine (E) and dimaprit (F) as histamine receptor agonists. The positive chronotropic effect (given as beating rate in the ordinates) of H₂-receptor stimulation was subject to desensitization by both histamine and dimaprit. Ctr, control data before drug application. Numbers in brackets indicate animals studied. *First $P < 0.05$ vs. Ctr; # $P < 0.05$ vs. nondesensitized; * $P < 0.05$ vs. desensitization with histamine. DS, desensitization.

histamine (Matsuda et al., 2002). In H₂-histamine receptor knockout mice or HDC knockout mice, LPS greatly reduced life expectancy (Yokoyama et al., 2004).

Because of H₂-histamine receptors and their stimulation via histamine, cardiac tissue can apparently be protected in sepsis under isometric conditions. Fittingly, injection of histamine improved the prognosis (probably by the expression of tumor necrosis factor α) in HDC knockout mice (Yokoyama et al., 2004). On the other hand, LPS injection increased the HDC expression (as tested by Western blot and enzyme activity) in the liver and the amount of histamine and *N*-methylhistamine (indicative of a *de novo* production of histamine; Yokoyama et al., 2004). Histologically, in the liver, an induction of HDC was lacking in mast cells (likewise, the number of mast cells was not increased) but not in Kupffer cells (Yokoyama et al., 2004). However, our data show partial support of a similar way as Yokoyama et al.: H₂-TG mice were functionally more

compromised than WT mice after LPS treatment. We do not know whether this would be accompanied by increased mortality because such studies were not part of our permission from the ethical committee of our institution. We would predict that the mortality in our model would decline when pretreating the animals with H₂-histamine receptor antagonists.

Emanating from ischemic hearts, plasma histamine levels can increase in many laboratory animals [as discussed in Genovese et al. (1988)]. Similar results have been obtained in hypoxia in human hearts [review: Reid et al. (2011)]. In the past, it has been speculated that this released histamine might originate from mast cells or from sympathetic nerve endings [as discussed in He et al. (2012)]. However, when doing these experiments in mast cell-deficient mouse hearts, one can still measure similar levels of histamine in ischemia (10 minutes in Langendorff System) in the eluate

In vivo desensitization protocol with dimaprit:

Fig. 8. *In vivo* desensitization. Living H₂-TG mice were treated intraperitoneally with dimaprit, and echocardiographic responses were studied. After recording basal cardiac parameters and dimaprit-induced cardiac effects, a high dose of dimaprit was injected [saline buffer in control experiments; non-desensitized (N-DS)], and 30 minutes later, dimaprit was injected again. Here, the effects of the third application of dimaprit are shown: (A) changes in heart rate after dimaprit injection and (B) changes in ejection fraction (EF) after dimaprit injection. **P* < 0.05 vs. N-DS. Hence, the contractile and chronotropic effects of dimaprit are attenuated after desensitization. DS, desensitization.

(He et al., 2012). This strongly argues against the role of mast cells in this phenomenon (He et al., 2012). In ischemic human cardiac tissue, histamine can be released (Hatta et al., 1997). Similar results have been reported *in vivo* in an animal system; for example, when the left descending coronary artery (LAD) was closed in dogs, and then in the coronary sinus, during LAD occlusion, the histamine levels in the plasma increased (about 10-fold to 1, 2 ng/ml), reaching a peak after 10 minutes and then declining to preocclusion values (although the occlusion of the LAD persisted) (Wolff and Levi, 1988). In our studies, there were different results that depended on the region of the heart

used and the method to lower partial oxygen pressure in the heart.

For atrial preparations, we observed an inotropic benefit from H₂-histamine receptor overexpression under basal conditions and preconditions, and these beneficial effects were cimetidine-sensitive; hence, in all likelihood, they were mediated by H₂-histamine receptors on cardiomyocytes. For whole perfused hearts, a detrimental effect of H₂-histamine receptor overexpression was apparent. It may be clinically relevant that under these conditions, reperfusion arrhythmias persisted longer: the existence of these arrhythmias can be explained by the elevation of intracellular cytosolic calcium

Fig. 9. Hypoxia in isolated electrically driven (1 Hz) left atrium in the organ bath. (A–D) Representative recordings of WT and H₂-TG left atrial preparations demonstrate the experimental design of the study (left-hand sides of panels, respectively). In the diagrams, the force of contraction in % of control (Ctr = initial force at the beginning of the experiment) during the time of reoxygenation is presented. In H₂-TG left atria, a better recovery was noted after a single hypoxia compared with WT (A). In presence of cimetidine (10 μ M), this effect was absent (B). A preconditioning period (10 minutes of hypoxia) was not beneficial (C). In the presence of histamine (1 μ M), the relative force during reoxygenation was greatly increased in H₂-TG and reached again the initial force (D). Cim, cimetidine; His, histamine. **P* < 0.05 vs. WT.

Fig. 10. Echocardiographic parameters under simulated sepsis. H₂-TG and WT mice were injected with LPS or solvent control (NaCl). Data were collected before [basal (B)] as well as 1, 3, and 7 hours after LPS application. (A) Ejection fraction (EF) measured in the parasternal long-axis (PLAX) view; (B) heart rate; (C) pulmonary arterial flow; (D) cardiac output. Numbers in brackets indicate numbers of animals investigated. ANOVA with Bonferroni post hoc test: * $P < 0.05$ vs. corresponding NaCl; # $P < 0.05$ vs. B.

ions because of histamine addition in isolated cardiomyocytes from H₂-TG. It could be argued that our ischemic/hypoxic conditions were not severe enough to notice more pronounced differences; for instance, we could have used longer time periods of hypoxia and/or ischemia to cause substantial cell death and, thus, mimic cardiac infarction. However, this type of research was beyond the scope of the present work and might be subject to future reports.

Generally, severe sepsis and myocardial infarction (but also allergic processes) might lead to high levels of histamine. Usually, the heart, like other organs, tries to protect itself against continuous stimulation. The textbook example lies in cardiac protection against high amount of circulating β -adrenergic catecholamines in heart failure. A similar mechanism(s) might be active in the heart against histamine levels in the plasma. In vitro, the desensitization of the H₂-histamine receptor has been carefully studied in cell cultures: functional homologous H₂-histamine receptor desensitization has been found to be independent of a reduction of the number of H₂-histamine receptors (Arima et al., 1993; Fernández et al., 2011). Desensitization has been found to be dependent not only on the time of exposure but also on the concentration of the agonist (Schreurs et al., 1984; Emami und Gespach, 1986). This is in line with our present findings. Ginsburg et al. (1980) mentioned (data not shown) that in isolated human cardiac electrically driven preparations, the response curves of repeated concentrations to histamine led to a rightward shift of the curves over time, which is consistent with desensitization;

this is consistent with the present report. To the best of our knowledge, our study is the first to report actual data on H₂-histamine receptor desensitization of cardiac tissue in vitro or in vivo. One could ask why we used histamine and dimaprit. Dimaprit was developed as a specific H₂-histamine receptor agonist, whereas histamine has long been known to act agonistically on all four known H₂-histamine receptors [as discussed in Gergs et al. (2019)]. Hence, we used dimaprit to stimulate more specific H₂-histamine receptors than would be possible using histamine itself.

Hitherto, in register studies of patients, famotidine (a newer H₂-histamine receptor antagonist that is chemically similar to cimetidine) reduced the incidence of heart failure or cardiac remodeling (Kim et al., 2004, 2006). Consistent with the role of histamine in human heart failure, higher concentrations of histamine have been reported in the blood of patients with idiopathic dilated cardiomyopathy (Zdravkovic et al., 2011). Thus, histamine has been suggested as contributing to heart failure, for instance, by augmenting fibrosis in heart failure patients (Patella et al., 1998). A 10-year-long observational study of humans initially free from any heart disease provided evidence for a reduced development of left or right ventricular cardiac hypertrophy in the study participants who took H₂-histamine receptor antagonists (Leary et al., 2016, 2014).

In summary, our studies suggest that, in the heart, beneficial (in atrial hypoxia) and detrimental (e.g., in sepsis, reperfusion arrhythmias) effects of cardiac H₂-histamine receptor expression occur. It is interesting to speculate that the same

Fig. 11. Scheme of a mammalian cardiomyocyte. Histamine in the extracellular space stimulates H₂-histamine receptors (H₂R). This can lead to increased activity of stimulatory GTP-binding proteins (Gs) and hence increased activity of the adenylyl cyclase (AC). This will increase the level of cAMP, which elevates the activity of cAMP-dependent protein kinases A (PKA). The kinases can increase the phosphorylation state (P) of cardiac regulatory proteins like the L-type calcium ion channel (LTCC), the ryanodine receptor (RyR) in the junctional sarcoplasmic reticulum, and PLB in the free sarcoplasmic reticulum. Phosphorylation of PLB increases the uptake of calcium ions (Ca²⁺) into the sarcoplasmic reticulum via sarcoplasmic reticulum ATPase (SERCA), where it binds mainly to calsequestrin (CSQ). Protein phosphatases like the catalytic subunit of PP2A (a serine, threonine phosphatase) reverse these phosphorylations. Increases in free cytosolic calcium ion concentrations due to H₂-histamine receptor stimulation lead to an increase in force of contraction via action on myofibrils. Under pathophysiological conditions like sepsis, histamine levels can increase in the extracellular space and might desensitize histamine receptors. H₂-histamine receptors are typically blocked by cimetidine. LPSs, mediators of bacterial sepsis, can decrease cardiac contractility via action on the toll like receptor 4 complex (CD14/TLR4) on cardiomyocytes and increased levels of cardiac depressant inflammatory mediators.

dichotomous role of H₂-histamine receptors might also exist in humans, which needs to be studied further to decide which patients might benefit from H₂-histamine receptor agonists or antagonists. In future studies, we will try to get better insights into the underlying pathways and pathomechanisms by extending biochemical analyses such as Western blotting and realtime quantitative PCR of already available and new tissue samples. In this context, histamine concentrations in the blood and in the cardiac tissue of our experimental animals will be analyzed to complete the data on the role of histamine in heart failure.

Authorship Contributions

Participated in research design: Gergs, Neumann.

Performed experiments and analyzed data: Kirchhefer, Bergmann, Künstler, Mißlinger, Au, Mahnkopf, Wache.

Wrote or contributed to the writing of the manuscript: Gergs, Neumann.

References

- Ackermann D (1910) Über den bakteriellen Abbau des Histidins. *Zeitschr Physiol Chem* **65**:504–510.
- Arima N, Kinoshita Y, Nakamura A, Yamashita Y, and Chiba T (1993) Homologous desensitization of histamine H₂ receptors in the human gastric carcinoma cell line MKN-45. *Am J Physiol* **265**:G987–G992 Available from: 10.1152/ajpgi.1993.265.5.G987.
- Au B, Gergs U, and Neumann J (2018) Histamine H₂-receptors affect atrial function during hypoxia and reoxygenation in transgenic mice. *Naunyn Schmiedeberg Arch Pharmacol* **391** (Suppl 1):S29.
- Bergmann F, Gergs U, and Neumann J (2016) Histamine H₂-receptor overexpression is beneficial for systolic dysfunction in PP2A overexpressing mice. *Naunyn Schmiedeberg Arch Pharmacol* **389** (Suppl 1):S27.
- Boknik P, Vahlensieck U, Huke S, Knapp J, Linck B, Lüss H, Müller FU, Neumann J, and Schmitz W (2000) On the cardiac contractile, electrophysiological and biochemical effects of endothall, a protein phosphatase inhibitor. *Pharmacology* **61**: 43–50.
- Brackett DJ, Schaefer CF, and Wilson MF (1985) The effects of H₁ and H₂ histamine receptor antagonists on the development of endotoxemia in the conscious, unrestrained rat. *Circ Shock* **16**:141–153.

- Brüchert N, Mavila N, Boknik P, Baba HA, Fabritz L, Gergs U, Kirchhefer U, Kirchhof P, Matus M, Schmitz W, et al. (2008) Inhibitor-2 prevents protein phosphatase 1-induced cardiac hypertrophy and mortality. *Am J Physiol Heart Circ Physiol* **295**:H1539–H1546.
- Chen J, Hong T, Ding S, Deng L, Abudupataer M, Zhang W, Tong M, Jia J, Gong H, Zou Y, et al. (2017) Aggravated myocardial infarction-induced cardiac remodeling and heart failure in histamine-deficient mice. *Sci Rep* **7**:44007 Available from: 10.1038/srep44007.
- Dale HH and Laidlaw PP (1911) Further observations on the action of beta-iminazolyethylamine. *J Physiol* **43**:182–195.
- Emami S and Gespach C (1986) Desensitization by histamine of H₂ receptor activity in HGT-1 human cancerous gastric cells. *Agents Actions* **18**:129–133.
- Fernández N, Gottardo FL, Alonso MN, Monczor F, Shayo C, and Davio C (2011) Roles of phosphorylation-dependent and -independent mechanisms in the regulation of histamine H₂ receptor by G protein-coupled receptor kinase 2. *J Biol Chem* **286**:28697–28706 Available from: 10.1074/jbc.M111.269613.
- Fernández N, Monczor F, Lemos B, Notovich C, Baldi A, Davio C, and Shayo C (2002) Reduction of G protein-coupled receptor kinase 2 expression in U-937 cells attenuates H₂ histamine receptor desensitization and induces cell maturation. *Mol Pharmacol* **62**:1506–1514 Available from: 10.1124/mol.62.6.1506.
- Francis GS and Tang WH (2006) Histamine, mast cells, and heart failure: is there a connection? *J Am Coll Cardiol* **48**:1385–1386 Available from: 10.1016/j.jacc.2006.07.014.
- Fühner H (1912) Das Pituitrin und seine wirksamen Bestandteile. *Munch Med Wochenschr* **16**:852–853.
- Genovese A, Gross SS, Sakuma I, and Levi R (1988) Adenosine promotes histamine H₁-mediated negative chronotropic and inotropic effects on human atrial myocardium. *J Pharmacol Exp Ther* **247**:844–849.
- Gergs U, Baumann M, Böckler A, Buchwalow IB, Ebelt H, Fabritz L, Hauptmann S, Keller N, Kirchhof P, Klöckner U, et al. (2010) Cardiac overexpression of the human 5-HT₄ receptor in mice. *Am J Physiol Heart Circ Physiol* **299**:H788–H798 Available from: 10.1152/ajpheart.00691.2009.
- Gergs U, Boknik P, Buchwalow I, Fabritz L, Matus M, Justus I, Hanske G, Schmitz W, and Neumann J (2004) Overexpression of the catalytic subunit of protein phosphatase 2A impairs cardiac function. *J Biol Chem* **279**:40827–40834 Available from: 10.1074/jbc.M405770200.
- Gergs U, Bernhardt G, Buchwalow IB, Edler H, Fröba J, Keller M, Kirchhefer U, Köhler F, Mißlinger N, Wache H, et al. (2019) Initial characterization of transgenic mice overexpressing human histamine H₂ receptors. *J Pharmacol Exp Ther* **369**: 129–141 Available from: 10.1124/jpet.118.255711.
- Gergs U, Bergmann F, Wache H, and Neumann J (2016) Co-overexpression of Gs coupled receptors is beneficial for cardiac dysfunction in PP2A overexpressing mice (Abstract). In: *Phosphatases and Signalling in Health and Disease*; 2016 Jun 20–26, Bath, UK: Biochemical Society, pp 14.
- Ginsburg R, Bristow MR, Stinson EB, and Harrison DC (1980) Histamine receptors in the human heart. *Life Sci* **26**:2245–2249.
- Gombosova I, Boknik P, Kirchhefer U, Knapp J, Luss H, Müller FU, Müller T, Vahlensieck U, Schmitz W, Bodor GS, et al. (1998) Postnatal changes in contractile

- time parameters, calcium regulatory proteins, and phosphatases. *Am J Physiol* **274**:H2123–H2132.
- Grote-Wessels S, Baba HA, Boknik P, El-Armouche A, Fabritz L, Gillmann HJ, Kucerova D, Matus M, Müller FU, Neumann J, et al. (2008) Inhibition of protein phosphatase 1 by inhibitor-2 exacerbates progression of cardiac failure in a model with pressure overload. *Cardiovasc Res* **79**:464–471.
- Hatta E, Yasuda K, and Levi R (1997) Activation of histamine H₃ receptors inhibits carrier-mediated norepinephrine release in a human model of protracted myocardial ischemia. *J Pharmacol Exp Ther* **283**:494–500.
- He G, Hu J, Li T, Ma X, Meng J, Jia M, Lu J, Ohtsu H, Chen Z, and Luo X (2012) Arrhythmogenic effect of sympathetic histamine in mouse hearts subjected to acute ischemia. *Mol Med* **18**:1–9 Available from: 10.2119/molmed.2011.00225.
- Hoehn M, Zhang Y, Xu J, Gergs U, Boknik P, Werdan K, Neumann J, and Ebel H (2015) Overexpression of protein phosphatase 2A in a murine model of chronic myocardial infarction leads to increased adverse remodeling but restores the regulation of β -catenin by glycogen synthase kinase β . *Int J Cardiol* **183**:39–46.
- Jutel M, Akdis M, and Akdis CA (2009) Histamine, histamine receptors and their role in immune pathology. *Clin Exp Allergy* **39**:1786–1800 Available from: 10.1111/j.1365-2222.2009.03374.x.
- Kim J, Ogai A, Nakatani S, Hashimura K, Kanzaki H, Komamura K, Asakura M, Asanuma H, Kitamura S, Tomoike H, et al. (2006) Impact of blockade of histamine H₂ receptors on chronic heart failure revealed by retrospective and prospective randomized studies. *J Am Coll Cardiol* **48**:1378–1384 Available from: 10.1016/j.jacc.2006.05.069.
- Kim J, Washio T, Yamagishi M, Yasumura Y, Nakatani S, Hashimura K, Hanatani A, Komamura K, Miyatake K, Kitamura S, et al. (2004) A novel data mining approach to the identification of effective drugs or combinations for targeted endpoints—application to chronic heart failure as a new form of evidence-based medicine. *Cardiovasc Drugs Ther* **18**:483–489 Available from: 10.1007/s10557-004-6226-y.
- Kirchhefer U, Baba HA, Kobayashi YM, Jones LR, Schmitz W, and Neumann J (2002) Altered function in atrium of transgenic mice overexpressing triadin 1. *Am J Physiol Heart Circ Physiol* **283**:H1334–H1343 Available from: 10.1152/ajpheart.00937.2001.
- Kirchhefer U, Brekle C, Eskandar J, Isensee G, Kučerová D, Müller FU, Pinet F, Schulte JS, Seidl MD, and Boknik P (2014) Cardiac function is regulated by B56 α -mediated targeting of protein phosphatase 2A (PP2A) to contractile relevant substrates. *J Biol Chem* **289**:33862–33873 Available from: 10.1074/jbc.M114.598938.
- Knapp J, Boknik P, Deng MC, Huke S, Lüss I, Klein-Wiele O, Linck B, Lüss H, Müller FU, Nacke P, et al. (1999) On the contractile function of protein phosphatases in isolated human coronary arteries. *Naunyn Schmiedeberg Arch Pharmacol* **360**:464–472.
- Künstler B, Gergs U, and Neumann J (2015) Desensitization of the human cardiac H₂ receptor. *Naunyn Schmiedeberg Arch Pharmacol* **388** (Suppl 1):S6.
- Leary PJ, Barr RG, Blumke DA, Bristow MR, Kronmal RA, Lima JA, Ralph DD, Ventetuolo CE, and Kawut SM (2014) H₂ receptor antagonists and right ventricular morphology: the MESA right ventricle study. *Ann Am Thorac Soc* **11**:1379–1386 Available from: 10.1513/AnnalsATS.201407-344OC.
- Leary PJ, Tedford RJ, Blumke DA, Bristow MR, Heckbert SR, Kawut SM, Krieger EV, Lima JA, Masri CS, Ralph DD, et al. (2016) Histamine H₂ receptor antagonists, left ventricular morphology, and heart failure risk: the MESA study. *J Am Coll Cardiol* **67**:1544–1552 Available from: 10.1016/j.jacc.2016.01.045.
- Matsuda N, Hattori Y, Sakuraya F, Kobayashi M, Zhang XH, Kemmotsu O, and Gando S (2002) Hemodynamic significance of histamine synthesis and histamine H₁- and H₂-receptor gene expression during endotoxemia. *Naunyn Schmiedeberg Arch Pharmacol* **366**:513–521 Available from: 10.1007/s00210-002-0651-x.
- Mißlinger N, Gergs U, and Neumann J (2017) Ischemia and reperfusion in the mouse heart overexpressing the H₂-receptor. *Naunyn Schmiedeberg Arch Pharmacol* **390** (Suppl 1):S33.
- National Research Council (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed., The National Academies Press, Washington, DC.
- Neumann J, Au B, and Gergs U (2018b) H₂ receptors may protect against acute cardiac hypoxia. *Inflamm Res* **67** (Suppl 1):S33.
- Neumann J, Bergmann F, and Gergs U (2016) Cardiac H₂ receptor overexpression: protective against PP2A mediated heart failure. *Inflamm Res* **65** (Suppl 1):S51–S52.
- Neumann J, Boknik P, Bodor GS, Jones LR, Schmitz W, and Scholz H (1994) Effects of adenosine receptor and muscarinic cholinergic receptor agonists on cardiac protein phosphorylation. Influence of pertussis toxin. *J Pharmacol Exp Ther* **269**:1310–1318.
- Neumann J, Boknik P, DePaoli-Roach AA, Field LJ, Rockman HA, Kobayashi YM, Kelley JS, and Jones LR (1998) Targeted overexpression of phospholamban to mouse atrium depresses Ca²⁺ transport and contractility. *J Mol Cell Cardiol* **30**:1991–2002 Available from: 10.1006/jmcc.1998.0760.
- Neumann J, Boknik P, Herzig S, Schmitz W, Scholz H, Gupta RC, and Watanabe AM (1993) Evidence for physiological functions of protein phosphatases in the heart: evaluation with okadaic acid. *Am J Physiol* **265**:H257–H266.
- Neumann J, Gerigk T, Mahnkopf M, Edler H, and Gergs U (2017b) Physiological and biochemical alterations of experimental systolic heart failure in mice overexpressing a serotonin receptor in the heart. *Acta Physiol (Oxf)* **221** (Suppl S731):114.
- Neumann J, Maas R, Boknik P, Jones LR, Zimmermann N, and Scholz H (1999) Pharmacological characterization of protein phosphatase activities in preparations from failing human hearts. *J Pharmacol Exp Ther* **289**:188–193.
- Neumann J, Mahnkopf M, Edler H, Bergmann F, and Gergs U (2017a) Hemodynamic and biochemical effects of immunological stress in mice with heart specific overexpression of the human H₂ receptor. *Inflamm Res* **66** (Suppl 1):S43.
- Neumann J, Mahnkopf M, Misslinger N, Wache H, Bergmann F, and Gergs U (2018a) The human H₂ receptor as a possible target for treatment of acute heart failure. *Eur J Heart Fail* **20** (Suppl S1):623.
- Parsons ME and Ganellin CR (2006) Histamine and its receptors. *Br J Pharmacol* **147** (Suppl 1):S127–S135 Available from: 10.1038/sj.bjp.0706440.
- Patella V, Marinò I, Arbustini E, Lamparter-Schummert B, Verga L, Adt M, and Marone G (1998) Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. *Circulation* **97**:971–978.
- Reid AC, Brazin JA, Morrey C, Silver RB, and Levi R (2011) Targeting cardiac mast cells: pharmacological modulation of the local renin-angiotensin system. *Curr Pharm Des* **17**:3744–3752.
- Schreurs J, Dailey MO, and Schulman H (1984) Pharmacological characterization of histamine H₂ receptors on clonal cytolytic T lymphocytes. Evidence for histamine-induced desensitization. *Biochem Pharmacol* **33**:3375–3382.
- Takahama H, Asanuma H, Sanada S, Fujita M, Sasaki H, Wakeno M, Kim J, Asakura M, Takashima S, Minamino T, et al. (2010) A histamine H₂ receptor blocker ameliorates development of heart failure in dogs independently of β -adrenergic receptor blockade. *Basic Res Cardiol* **105**:787–794 Available from: 10.1007/s00395-010-0119-y.
- Windaus A and Vogt W (1907) Synthese des Imidazolyläthylamins. *Ber Dtsch Chem Ges* **40**:3691–3695.
- Wolff AA and Levi R (1988) Ventricular arrhythmias parallel cardiac histamine efflux after coronary artery occlusion in the dog. *Agents Actions* **25**:296–306.
- Yokoyama A, Mori S, Takahashi HK, Kanke T, Wake H, and Nishibori M (2007) Effect of amodiaquine, a histamine N-methyltransferase inhibitor, on, Propionibacterium acnes and lipopolysaccharide-induced hepatitis in mice. *Eur J Pharmacol* **558**:179–184 Available from: 10.1016/j.ejphar.2006.11.033.
- Yokoyama M, Yokoyama A, Mori S, Takahashi HK, Yoshino T, Watanabe T, Watanabe T, Ohtsu H, and Nishibori M (2004) Inducible histamine protects mice from P. acnes-primed and LPS-induced hepatitis through H₂-receptor stimulation. *Gastroenterology* **127**:892–902.
- Zdravkovic V, Pantovic S, Rosic G, Tomic-Lucic A, Zdravkovic N, Colic M, Obradovic Z, and Rosic M (2011) Histamine blood concentration in ischemic heart disease patients. *J Biomed Biotechnol* **2011**:315709 Available from: 10.1155/2011/315709.
- Zeng Z, Shen L, Li X, Luo T, Wei X, Zhang J, Cao S, Huang X, Fukushima Y, Bin J, et al. (2014) Disruption of histamine H₂ receptor slows heart failure progression through reducing myocardial apoptosis and fibrosis. *Clin Sci (Lond)* **127**:435–448 Available from: 10.1042/CS20130716.

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