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Cardiac Effects of Novel Histamine H_2 Receptor Agonists[S]

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ABSTRACT

In an integrative approach, we studied cardiac effects of recently published novel H_2 receptor agonists in the heart of mice that overexpress the human H_2 receptor (H_2-TG mice) and littermate wild type (WT) control mice and in isolated electrically driven muscle preparations from patients undergoing cardiac surgery. Under our experimental conditions, the H_2 receptor agonists UR-Po563, UR-MB-158, and UR-MB-159 increased force of contraction in left atrium from H_2-TG mice with pEC_{50} values of 8.27, 9.38, and 8.28, respectively, but not in WT mice. Likewise, UR-Po563, UR-MB-158, and UR-MB-159 increased the beating rate in right atrium from H_2-TG mice with pEC_{50} values of 9.01, 9.24, and 7.91, respectively, but not from WT mice. These effects could be antagonized by famotidine, a H_2 receptor antagonist. UR-Po563 (1 μM) increased force of contraction in Langendorff-perfused hearts from H_2-TG but not WT mice. Similarly, UR-Po563, UR-MB-158, or UR-MB-159 increased the left ventricular ejection fraction in echocardiography of H_2-TG mice. Finally, UR-Po563 increased force of contraction in isolated human right atrial muscle strips. The contractile effects of UR-Po563 in H_2-TG mice were accompanied by an increase in the phosphorylation state of phospholamban. In summary, we report here three recently developed agonists functionally stimulating human cardiac H_2 receptors in vitro and in vivo. We speculate that these compounds might be of some merit to treat neurologic disorders if their cardiac effects are blocked by concomitantly applied receptor antagonists that cannot pass through the blood-brain barrier or might be useful to treat congestive heart failure in patients.

SIGNIFICANCE STATEMENT

Recently, a new generation of histamine H_2 receptor (H_2R) agonists has been developed as possible treatment option for Alzheimer's disease. Here, possible cardiac (side) effects of these novel H_2R agonists have been evaluated.

Introduction

Histamine is a mediator of many physiologic processes like inflammation, allergy, gastric acid secretion, hematopoiesis, cell proliferation, and wound healing (Parsons and Ganellin, 2006). In the central nervous system, histamine is involved in awakening and sleep, regulation of body weight, learning, perception of pain, and memory (Jørgensen et al., 2007). Moreover, histamine dysfunction is probably involved in diseases like narcolepsy, depression, and Alzheimer's disease (Mehta et al., 2021). If one wants to treat, for instance, Alzheimer's disease in the central nervous system with a peroral H_2 receptor (H_2R) agonist, one also treats the patient's heart. In the heart, histaminergic cardiac effects were initially described by Dale and Laidlaw (1910) and Ackermann and Kutscher (1910). In the human heart, H_1 and H_2 receptors have been identified using antibodies (Matsuda et al., 2004). There are many more H_1 receptors in human atrial samples than H_2 receptors (Baumann et al., 1983; Matsuda et al., 2004). Nevertheless, the H_2 receptors currently thought to be responsible for the histaminergic positive inotropic and positive chronotropic effects in the human atrium (Levi et al., 1981; Sanders et al., 1996). These effects are elicited by H_2 receptor agonists and not H_1 receptor agonists because the positive inotropic effects of histamine are blocked by H_2 receptor antagonists like cimetidine and famotidine (Seifert et al., 2013; Panula et al., 2015). In human atrial samples, the stimulation of H_2 receptors led to an increase in cAMP content, an elevated activity of the cAMP dependent protein kinase (Sanders et al., 1996) (scheme in Fig. 1), and subsequently to an increase in the phosphorylation state of phospholamban (PLB) (scheme in Fig. 1) and the inhibitory subunit of troponin (Neumann et al., 2021b). In isolated human atrial or ventricular preparations, besides histamine, other histamine receptor agonists like amthamine (pD_2 = 5.38) or the more potent agonist

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ABBREVIATIONS: H_2R, histamine H_2 receptor; H_2-TG, transgenic and overexpressing human H_2R; pD_2, negative logarithm of the EC_{50} that is the molar concentration of an agonist that produces 50% of the maximal possible effect; PIE, positive inotropic effect; PLB, phospholamban; PS16-PLB, phospholamban phosphorylated at serine 16; WT, wild type.

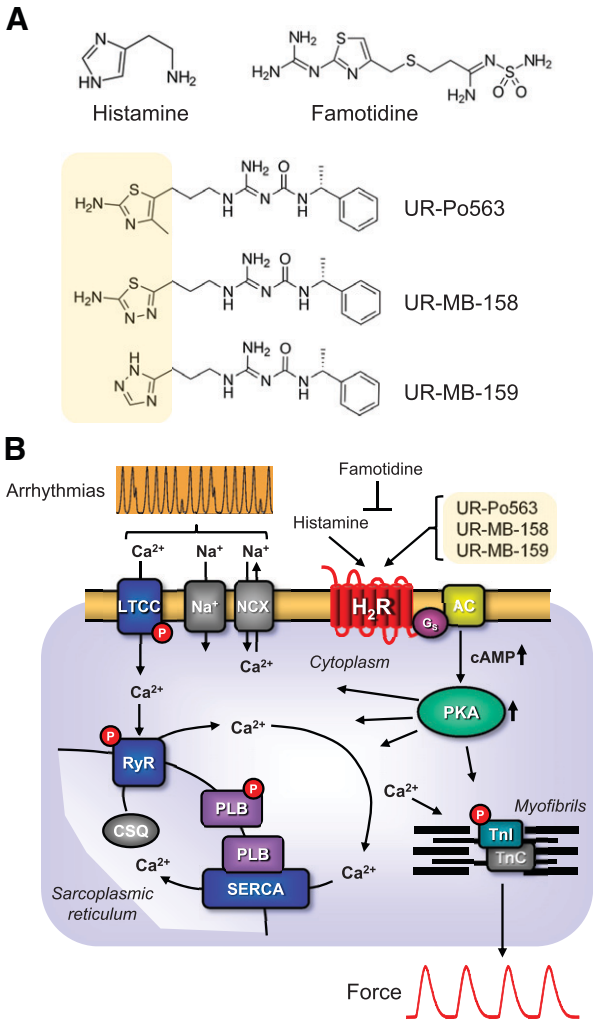

Fig. 1. (A) Structural formulas of histamine, of the H_2R antagonist famotidine and of the novel H_2R agonists (Biselli et al., 2021, Tropmann et al., 2021). The differences between the three agonists are highlighted. (B) Schematic illustration of the putative signaling of the H_2R agonists in the mammalian heart. Activation of the cAMP-dependent protein kinase (PKA) via stimulation of the H_2R increases the cytosolic Ca^{2+} concentration. In this way the force of contraction can be increased (positive inotropic effect) and arrhythmias may be triggered (proarrhythmic effect). The effects can be reversed by the H_2R antagonist famotidine. AC, adenylyl cyclase; CSQ, calsequestrin; Gs, stimulatory G-protein; LTCC, L-type Ca^{2+} channel; NCX, sodium calcium exchanger; P, phosphorylation; RYR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; TnC, troponin C; TnI, troponin inhibitor.

impromidine (pD_2 = 6.59) have been studied (Bristow et al., 1982; Poli et al., 1994; Coruzzi et al., 1995). More recently, we have generated a transgenic mouse model that overexpresses the human H_2 receptor in the murine heart (Gergs et al., 2019). In the hearts of this model (H_2-TG mice) but not in the wild type (WT) littermates, we could identify the human H_2 receptor mRNA and the protein by immunohistology of ventricular slices as well as by autoradiography of hearts with a radioactively labeled H_2R agonist but not in Western blotting experiments (Gergs et al., 2019; Gergs et al., 2020; Neumann et al., 2021b). Moreover, contractile effects to histamine were only present in living H_2-TG mice, isolated perfused hearts from H_2-TG mice, isolated cells from H_2-TG mice, and isolated atrial preparations from H_2-TG mice, but not those from WT mice (Gergs et al., 2019; Gergs et al., 2020; Neumann et al., 2021a; Neumann et al., 2021b). Therefore, we had suggested that this H_2-TG model might be a useful model to study human H_2R in all regions of the heart. It is important to point out that we used an alpha myosin heavy chain promoter for the human H_2R transgene, meaning that we overexpressed the human H_2R only in the cardiomyocytes of the heart (Gergs et al., 2019).

There are studies reporting that stimulation of postsynaptic H_2Rs in the brain might be beneficial for learning and memory and could therefore be interesting in the treatment of, e.g., Alzheimer's disease (Khan et al., 2016). As these effects have only been shown with dual-acting acetylcholinesterase inhibitors and H_3R antagonists initiating this process by inhibiting presynaptic H_3-autoreceptors (Darras et al., 2014; Khan et al., 2016; Sadek et al., 2016) the use of central nervous system–penetrating H_2R agonists is of great interest. In the present work we wanted to test whether the recently published H_2R agonists UR-Po563 (Biselli et al., 2021), UR-MB-158, and UR-MB-159 (Tropmann et al., 2021) (Fig. 1) act on the human cardiac H_2R.

Materials and Methods

Transgenic Mice. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Animals were maintained and handled according to approved protocols of the animal welfare committee of the University of Halle-Wittenberg, Germany.

The plasmid, containing the human H_2 receptor cDNA (GenBank accession number AY136744), was kindly provided by R. Seifert (Institute of Pharmacology, Hannover Medical School, Hannover, Germany). Generation of the transgenic mice with cardiomyocyte-specific expression of the human H_2R was performed by the TRAM unit of the Westfälische Wilhelms-Universität, Münster, Germany, and has been described before (Gergs et al., 2019). Genotypes were identified by polymerase chain reaction analyses of tail tip DNA using the following primers: 5′-ACCCTTACCCCACATAGACC-3′ and 5′-AGCAGGTCAGTGATAGCCAA-3′. The polymerase chain reaction was performed using the Ampliqon Taq DNA polymerase (Biomol, Hamburg, Germany) according to the manufacturer's instructions. For the experiments, 3–6-month-old H_2-TG mice and WT littermates (age-matched) of both sexes (evenly distributed) were used.

Western Blot Analysis. Homogenates from ventricular tissue samples were prepared in 300 µl of 10 mM $NaHCO_3$ and 100 µl 20% SDS. Crude extracts were incubated at 25°C for 30 minutes before centrifugation to remove debris, and 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). Briefly, aliquots of 100 µg of protein were loaded per lane, and bands were detected using enhanced chemifluorescence (GE Healthcare Europe, Freiburg, Germany) together with a Typhoon 9410 Variable Mode Imager (GE Healthcare Europe, Freiburg, Germany). The following primary antibodies were used in this study: polyclonal rabbit anti-calsequestrin (SP5340P, Acris Antibodies, Herford, Germany) and polyclonal rabbit anti–phospho-PLB [antibodies were raised against PLB-peptide phosphorylated at serine 16 (PS16-PLB), A010-12, Badrilla, Leeds, UK]. The characteristics and use of these antibodies have been reported repeatedly by our group (Kirchhefer et al., 2002). The specificity of the anti-calsequestrin antibody was controlled by running in parallel cardiac samples from mice with calsequestrin deletion. In these knockout mice the antibody detected no signal at 58 kDa, the size of calsequestrin (Gergs et al., 2017).

Specificity of anti-phosphorylated PLB antibodies was ascertained by running next to each other boiled and unboiled samples from hearts treated with isoproterenol (to increase PLB phosphorylation). Boiling reduces the apparent molecular weight of PLB from about 27 kDa (pentameric form) to values around 10 kDa (monomeric form) under these experimental conditions (Neumann et al., 2021a).

Echocardiography. Echocardiography in spontaneously breathing mice was performed under anesthesia with 1.5% isoflurane (Gergs et al., 2010). We injected the dihydrochloride salts of UR-Po563, UR-MB-158, or UR-MB-159 (dissolved in water) or famotidine as 100 µl of a 1 mM stock solution into the peritoneum of H_2-TG or WT mice. First famotidine was injected, and five minutes later UR-Po563, UR-MB-158, or UR-MB-159. This was done to offer enough time for the H_2R antagonist famotidine to occupy the cardiac H_2R. After five additional minutes the left ventricle was assessed using B-mode to obtain an overall view. The recording was then changed to M-mode to quantify the function of the left ventricle by measuring the ejection fraction of the left ventricle using the software supplied by the manufacturer (Vevo 2100, Visual Sonic, Toronto, Canada).

Contractile Function. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg kg^{-1}), and hearts were excised. Right and left atria were dissected from isolated H_2-TG and WT mice hearts and mounted in an organ bath. Left atrial preparations were continuously electrically stimulated (field stimulation) with each impulse consisting of 1 Hz, with a voltage of 10–15% above threshold and 5 ms duration. Right atrial preparations were allowed to contract spontaneously. The bathing solution contained (in mM) NaCl 119.8, KCI 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2P0_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, and glucose 5.0, continuously gassed with 95% O_2 and 5% CO_2 and maintained at 35°C resulting in a pH of 7.4. Signals detected via an isometric force transducer were amplified and continuously recorded. UR-Po563, UR-MB-158, or UR-MB-159 was cumulatively applied to the organ bath. After three changes of the buffer in the organ bath of the indicated experiments, famotidine was applied (1 µM in the organ bath), and then cumulative addition of UR-Po563, UR-MB-158, or UR-MB-159 to the organ bath was repeated. This was done to assess the ability of famotidine to antagonize the contractile effects of the respective agonist in the organ bath.

Langendorff-Perfused Hearts. Heart preparations were used as described previously (Kirchhefer et al., 2014). Mice were anesthetized intraperitoneally with pentobarbital sodium (50 mg kg^{-1}) and treated with 1.5 units of heparin. The hearts were removed from the opened chest, immediately attached by the aorta to a 20-gauge cannula, and perfused retrogradely under constant flow of 2 ml min^{-1} with oxygenized buffer solution (37°C) containing (in mM) NaCl 119.8, KCI 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2P0_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, and glucose 5.0 in an isolated heart system. The heart preparations were allowed to equilibrate for 30 minutes before measurements. The developed force was measured with a hook applied to the apex cordis that was connected via a return pulley to a force transducer. The data were recorded using a PowerLab system (ADInstruments, Oxford, UK). Ventricular contractions were measured and monitored continuously. The beating rate and the first derivative of the developed force (+dF/dt and −dF/dt) were calculated electronically using the chart software (ADInstruments, Oxford, UK).

Human Atrium. Human atrial preparations were used as previously described (Neumann et al., 2021a). Right atrial samples were obtained from patients who underwent cardiac bypass surgery because of a three-vessel coronary artery disease. From these right atrial samples, we cut small trabeculae carneae and handled them exactly like mouse atrial samples. In brief, they were mounted in organ baths, attached to an isometric force transducer, and stimulated electrically at 1 Hz, and the buffer had the same composition as described above. The samples were freeze clamped in liquid nitrogen after the experiment to stop all biochemical reactions and to maintain the phosphorylation state of the proteins of interest. From some atrial samples, small cardiac strips were prepared and incubated in 1.5 ml reaction tubes, drugs were added, and then cardiac strips were rapidly frozen. These procedures have been reported in detail before (Gergs et al., 2009; Neumann et al., 2021c). Here, five atrial preparations from three patients could be used. The patient characteristics are described as follows: age, 52–72 years old; sex, male; New York Heart Association class, III–IV; Canadian Cardiovascular Society angina grading scale, III; left ventricular ejection fraction, 40–60%; further diagnoses were arterial hypertension, hypercholesterolemia, and diabetes (two of three); and medications included anticoagulants, platelet aggregation inhibitors, β-adrenoceptor antagonists, calcium channel blockers (dihydropyridines), angiotensin converting enzyme inhibitors or angiotensin receptor blockers, diuretics, proton-pump inhibitors, metformin, or insulin. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Halle-Wittenberg (hm-bü 04.08.2005). All patients gave informed consent.

Mini-G Protein Recruitment Assay. The mini-G protein recruitment assay was performed as previously described using HEK293T cells stably expressing NlucN-mGs/hH_2R-NlucC or NlucN-mGs/gpH_2R-NlucC and UR-Po563 in various concentrations (Höring et al., 2020; Tropmann et al., 2021).

Data Analysis. Data shown are means ± S.E.M. Statistical significance was estimated by a paired or unpaired Student's t test for the concentration response curves or Langendorff experiments or by a two-way analysis of variance (ANOVA) followed by Bonferroni's post-test for the echocardiographic and Western blot results. A value of $P < 0.05$ was considered significant.

Drugs and Materials. UR-Po563 (Biselli et al., 2021), UR-MB-158, and UR-MB-159 (Tropmann et al., 2021) were synthesized as dihydrochloride salts according to the literature (Fig. 1). All other chemicals were of analytical grade. Demineralized water was used throughout the experiments. Stock solutions were freshly prepared daily.

Results

In isolated electrically (1 Hz) driven left atrial preparations, histamine, cumulatively applied, increased force of contraction in preparations from H_2-TG mice (original recording in Fig. 2A) but failed to affect force of contraction in preparations from WT mice (Fig. 2B). The data are summarized in Fig. 2, B–E. Moreover, histamine concentration dependently increased the velocity of contraction (maximum rate of tension development: Fig. 2C) and decreased the velocity of relaxation (minimum rate of tension development: Fig. 2D). Similarly, in spontaneously contracting right atrial preparations from WT mice, histamine, cumulatively given, did not increase the beating rate. However, in H_2-TG right atria, histamine augmented the beating rate in a time- and concentration-dependent fashion (Fig. 2E). These findings are in line with our previous reports (Gergs et al., 2019; Gergs et al., 2020; Neumann et al., 2021a; Neumann et al., 2021b). The effects of histamine in H_2-TG on force of contraction and beating rate were antagonized by H_2R antagonists applied 30 minutes before application of histamine as shown in previous reports where we used either cimetidine (Gergs et al., 2019) or famotidine (Neumann et al., 2021a) to antagonize the histamine effects.

Similar to histamine (Fig. 2), we noted that UR-Po563 increased force of contraction in a time- and concentration-dependent manner in atrial preparations only from H_2-TG. This and the antagonistic effect of famotidine can be seen in the original recordings in Fig. 3A. The data are summarized in Fig. 3, B–E. The respective EC_{50} values of UR-Po563 are provided in Supplemental Table 1 and can be compared with values from UR-MB-158, UR-MB-159, histamine, and those of

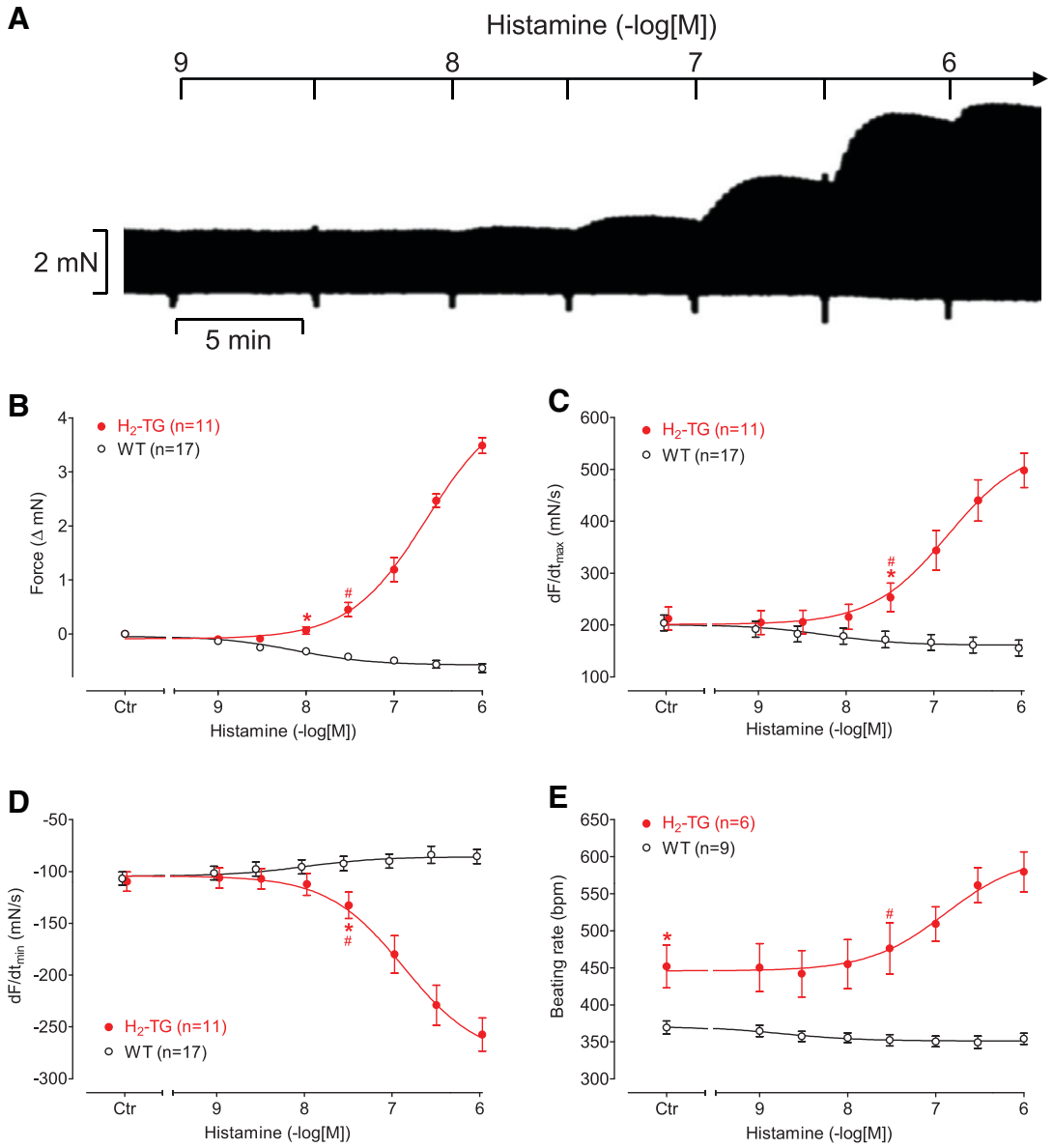

Fig. 2. Histamine increases contractility in H_2-TG but not WT. (A) Original recordings of the effect of cumulatively applied histamine on force of contraction in isolated electrically driven (1 Hz) left atrial preparations. Horizontal bar indicates time in minutes; vertical bar indicates developed force in milli-Newtons. (B) Effects of histamine in H_2-TG (n = 11) and WT (n = 17) left atrium on developed force (ordinate in milli-Newtons). Effects of histamine in H_2-TG (n = 11) and WT (n = 17) left atrium on time to maximum rate of tension development dF/dt in mN/s (ordinate) (C) and on time to minimum rate of tension development dF/dt in mN/s (ordinate) (D). (E) Effects of histamine in H_2-TG (n = 6) and WT (n = 9) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm). *First $P < 0.05$ versus WT; #first $P < 0.05$ versus Ctr. Abscissae: negative logarithmic concentrations of histamine in moles.

some typical older histamine H_2R ligands. UR-Po563 increased the beating rate in preparations from H_2-TG but not WT mice (Fig. 3C). In the left atrial samples, UR-Po563 concentration- and time-dependently increased the first derivative of force versus time dF/dt in absolute terms (Fig. 3, D and E). The effects of UR-Po563 on force of contraction, its first derivative, or the beating rate in H_2-TG were antagonized by famotidine (Fig. 3).

In a more potent way (Supplemental Table 1), UR-MB-158 increased the force of contraction and the beating rate in atrial preparations from H_2-TG but not WT mice in a concentration- and time-dependent manner. This can be seen in an original recording (Fig. 4A) and is summarized in Fig. 4, B–E. UR-MB-158 increased the beating rate in right atrial preparations from H_2-TG but not WT mice (Fig. 4C). Moreover, in the left atrial samples, UR-MB-158 increased the first derivative of force versus time dF/dt in absolute terms (Fig. 4, D and E). The effects of UR-MB-158 on force of contraction or beating rate were antagonized by famotidine (Fig. 4).

UR-MB-159 was also more potent than histamine under the present experimental conditions (Supplemental Table 1): UR-MB-159 increased force of contraction (original tracing: Fig. 5A and summary: Fig. 5B) and beating rate (Fig. 5C) in a time- and concentration-dependent manner in atrial preparations from H_2-TG but not WT mice. In the left atrial samples, UR-MB-159 increased the maximum and minimum first

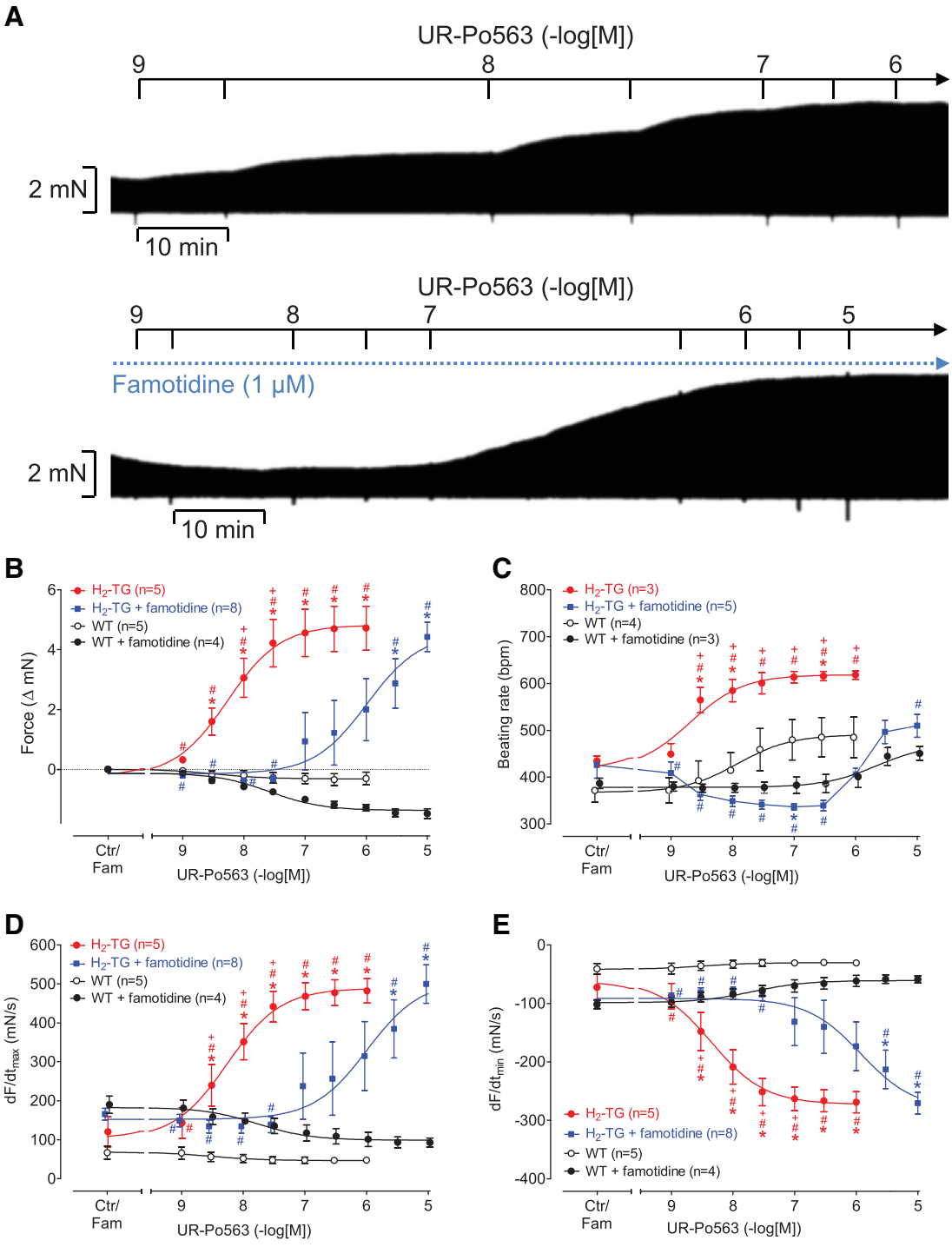

Fig. 3. Contractile effects of UR-Po563. (A) Original recordings of the effect of cumulatively applied UR-Po563 on force of contraction in isolated electrically driven (1 Hz) left atrial preparations. Horizontal bar indicates time in minutes; vertical bar indicates developed force in milli-Newtons. (B) Effects of UR-Po563 in H_2-TG ($n = 5$) and WT ($n = 5$) left atrium on developed force (ordinate in milli-Newtons) versus H_2-TG ($n = 8$) and WT ($n = 4$) in presence of famotidine. (C) Effects of UR-Po563 in H_2-TG ($n = 3$) and WT ($n = 4$) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm) versus H_2-TG ($n = 5$) and WT ($n = 3$) in presence of famotidine. (D) Effects of UR-Po563 in H_2-TG ($n = 5$) and WT ($n = 5$) left atrium on time to maximum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG ($n = 8$) and WT ($n = 4$) in presence of famotidine. (E) Effects of UR-Po563 in H_2-TG ($n = 5$) and WT ($n = 5$) left atrium on time to minimum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG ($n = 8$) and WT ($n = 4$) in presence of famotidine. Abscissae: negative logarithmic concentrations of UR-Po563 in moles. #Significant effects of UR-Po563 compared with predrug values (Ctr./Fam); *significant effects of UR-Po563 in H_2-TG compared with WT; +significant effects of Po563 in H_2-TG compared with H_2-TG in presence of famotidine.

derivative of force versus time (dF/dt, Fig. 5, D and E). The effects of UR-MB-159 in left and right atria were antagonized by famotidine (Fig. 5).

Next, it was of interest to investigate ventricular effects of histamine. To that end, we used isolated retrogradely perfused hearts (Langendorff preparations). These preparations were

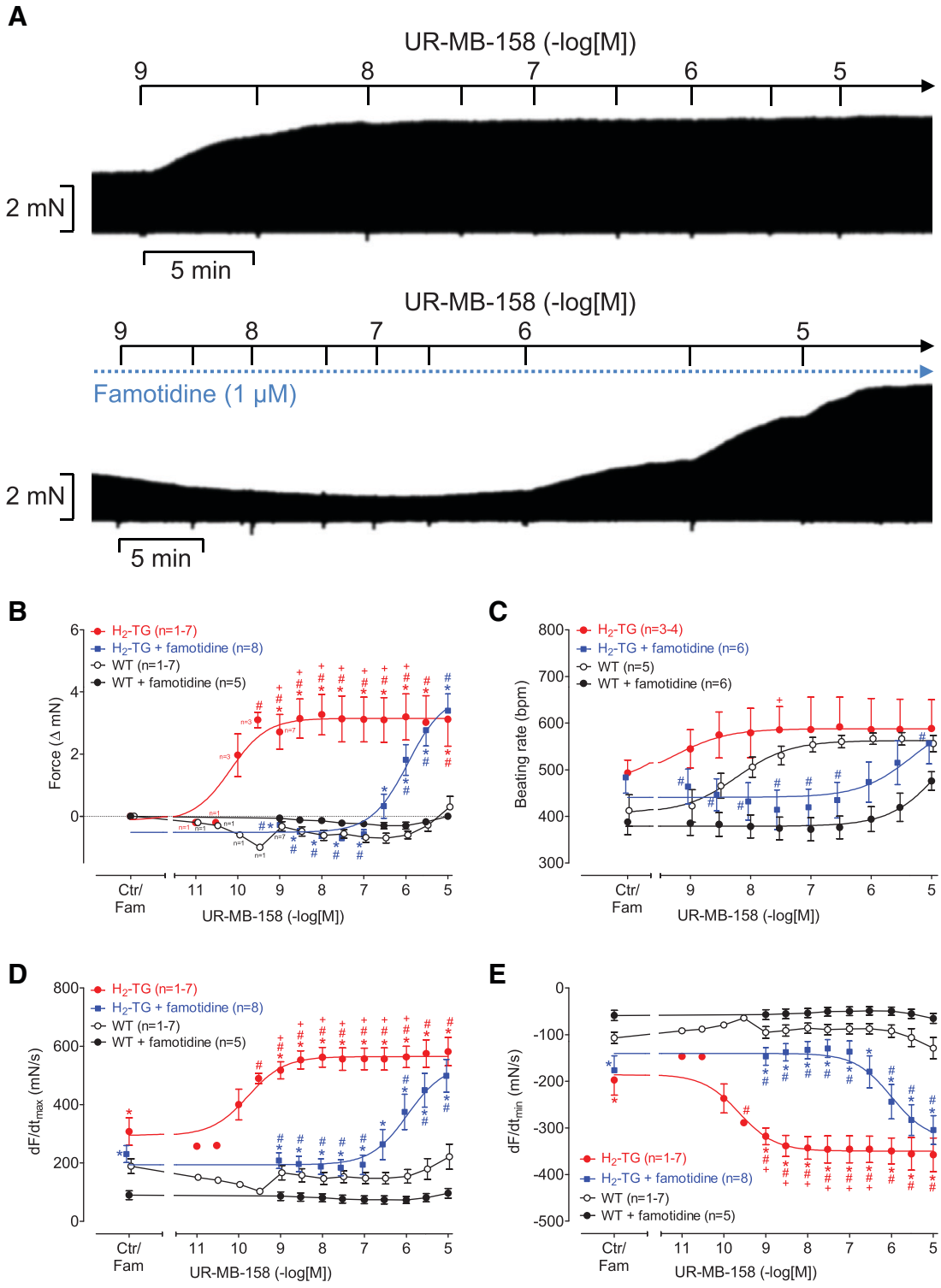

Fig. 4. Contractile effects of UR-MB-158. (A) Original recordings of the effect of cumulatively applied UR-MB-158 on force of contraction in isolated electrically driven (1 Hz) left atrial preparations. Horizontal bar indicates time in minutes; vertical bar indicates developed force in milli-Newtons. (B) Effects of UR-MB-158 in H_2-TG (n = 6) and WT (n = 6) left atrium on developed force (ordinate in milli-Newtons) versus H_2-TG (n = 8) and WT (n = 5) in presence of famotidine. (C) Effects of UR-MB-158 in H_2-TG (n = 4) and WT (n = 5) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm) versus H_2-TG (n = 6) and WT (n = 6) in presence of famotidine. (D) Effects of UR-MB-158 in H_2-TG (n = 6) and WT (n = 6) left atrium on time to maximum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG (n = 8) and WT (n = 5) in presence of famotidine. (E) Effects of UR-MB-158 in H_2-TG (n = 6) and WT (n = 6) left atrium on time to minimum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG (n = 8) and WT (n = 5) in presence of famotidine. Abscissae: negative logarithmic concentrations of UR-MB-158 in moles. #Significant effects of UR-MB-158 compared with predrug values (Ctr.); *significant effects of UR-MB-158 in H_2-TG compared with WT; +significant effects of UR-MB-158 in H_2-TG compared with H_2-TG in presence of famotidine.

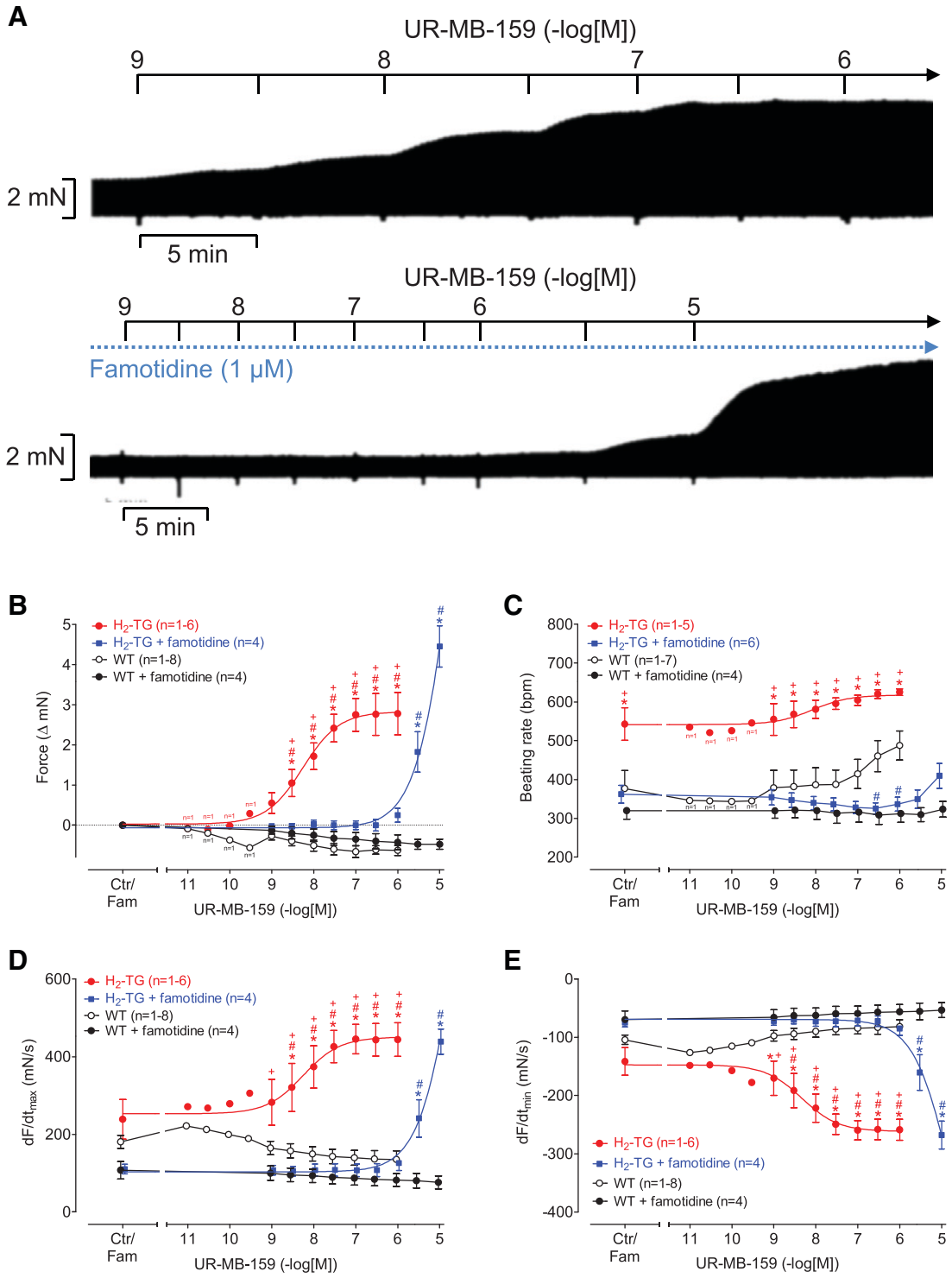

Fig. 5. Contractile effects of UR-MB-159. (A) Original recordings of the effect of cumulatively applied UR-MB-159 on force of contraction in isolated electrically driven (1 Hz) left atrial preparations. Horizontal bar indicates time in minutes; vertical bar indicates developed force in milli-Newtons. (B) Effects of UR-MB-159 in H_2-TG (n = 6) and WT (n = 8) left atrium on developed force (ordinate in milli-Newtons) versus H_2-TG (n = 4) and WT (n = 4) in presence of famotidine. (C) Effects of UR-MB-159 in H_2-TG (n = 5) and WT (n = 7) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm) versus H_2-TG (n = 6) and WT (n = 4) in presence of famotidine. (D) Effects of UR-MB-159 in H_2-TG (n = 6) and WT (n = 8) left atrium on time to maximum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG (n = 4) and WT (n = 4) in presence of famotidine. (E) Effects of UR-MB-159 in H_2-TG (n = 6) and WT (n = 8) left atrium on time to minimum rate of tension development dF/dt in mN/s (ordinate) versus H_2-TG (n = 4) and WT (n = 4) in presence of famotidine. Abscissae: negative logarithmic concentrations of UR-MB-159 in moles. $^{\#}$Significant effects of UR-MB-159 compared with predrug values (Ctr.); *significant effects of UR-MB-159 in H_2-TG compared with WT; $^{+}$significant effects of UR-MB-159 in H_2-TG compared with H_2-TG in presence of famotidine.

TABLE 1
Effect of UR-Po563 (1 μM) on force of contraction and beating rate in isolated perfused Langendorff hearts from H_2-TG and WT (n=3–4, each). Hearts were perfused initially with buffer for about 30 minutes for stabilization and allowed to beat spontaneously. Then with a syringe, driven by an electric pump, UR-Po563 solution was added to the perfusion buffer. Force was measured with a hook applied to the apex cordis and fed into a computer. After 5 minutes of drug perfusion the whole heart was rapidly frozen with Wollenberger clamps precooled in liquid nitrogen.

	WT		H_2-TG	
	Basal	UR-Po563	Basal	UR-Po563
Force (mN)	7.89 ± 1.28	8.95 ± 1.95	11.49 ± 2.07	20.46 ± 2.81*
Beating rate (bpm)	308 ± 8.60	321 ± 13.2	351 ± 37.3	391 ± 16.2
dF/dt max (mN/s)	204.6 ± 43.8	220.6 ± 36.1	296 ± 42.5	718 ± 90.9*
dF/dt min (mN/s)	−151 ± 53.2	−172 ± 56.5	−230 ± 39.3	−566 ± 52.7*

bpm, beats per minute.
*$P < 0.05$ versus basal.

allowed to beat spontaneously. We recorded force of contraction from the apex; therefore, we measured left ventricular force under these conditions. We noted that 1 μM of UR-Po563 increased force of contraction in hearts from H_2-TG but not WT mice (Table 1). This was in line with our previous reports on histamine in Langendorff-perfused hearts (Gergs et al., 2019; Gergs et al., 2021; Neumann et al., 2021a; Neumann et al., 2021b).

To investigate the effects of the novel H_2 receptor agonists on cardiovascular performance in vivo, we performed echocardiographic measurements in H_2-TG and WT mice under anesthesia (original M-mode recordings: Fig. 6A). We noted that intraperitoneally injected UR-Po563 (100 μl of a 1 mM solution) increased left ventricular ejection fraction and beating rate in H_2-TG mice (Table 2; Fig. 6B). Furthermore, using echocardiography, we studied typical dimensions of the heart like left ventricular systolic and diastolic diameters under basal and UR-Po563-stimulated conditions. We noted substantial alterations in these parameters after injection of UR-Po563 in H_2-TG but not in WT mice (Fig. 6). These results are also reminiscent of our previous studies with histamine itself under these conditions (Gergs et al., 2019). Effects of UR-Po563 were antagonized by famotidine (Table 2; Fig. 6B). Interestingly, we had the opportunity to study atrial preparations of three animals in which UR-Po563 was injected and from which the heart could be harvested (under general anesthesia) after performing echocardiography. To our surprise in left atrial preparations from H_2-TG, where UR-Po563 exerted a profound increase in ejection fraction, UR-Po563 was unable to increase force of contraction under the conditions described in Fig. 2 (data not shown), despite the fact that we exchanged the organ bath buffer (10 ml volume) three times before we started the contraction experiment. Thus, one can speculate that UR-Po563 sticks quite tight to cardiac tissue, but the exact reasons remain to be elucidated.

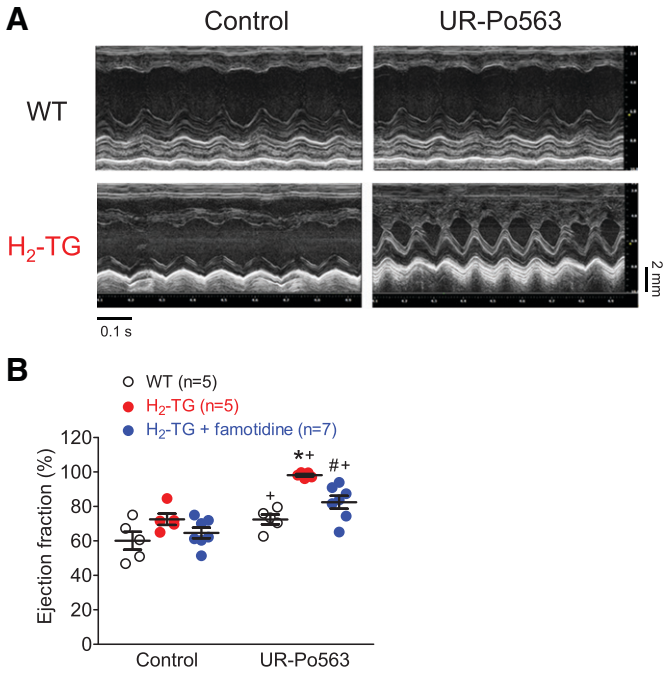

Fig. 6. Echocardiography. (A) M-mode pictures of H_2-TG or WT injected into the peritoneum with 100 μl of a 1 mM solution of UR-Po563. The left ventricle is visible. UR-Po563 led to an increase in systolic wall motion in H_2-TG but not in WT. Pictures were taken before (control = baseline values) and 5 minutes after injection of UR-Po563 solution. Vertical bar indicates the size marker in millimeters, and horizontal bar indicates the time marker in seconds. Data are summarized in (B) as well as in Table 2.

Furthermore, to better understand the underlying signal transduction mechanism (Fig. 1), we assessed the phosphorylation state of PLB in atrial and ventricular preparations by Western blotting of the frozen samples with a phosphorylation state sensitive antibody. As expected from our previous reports with histamine (Gergs et al., 2019; Gergs et al., 2021; Neumann et al., 2021a; Neumann et al., 2021b), UR-Po563 increased the phosphorylation of PLB at serine 16 in left and right atrial preparations from H_2-TG mice (freeze clamped at the maximum of the positive inotropic effect) (Fig. 7). In contrast, in atrial preparations from WT mice, UR-Po563 failed to increase the phosphorylation state of PLB (Fig. 7). The data are summarized as scatter plots. Similar results were obtained in freeze clamped isolated perfused heart: UR-Po563 (1 μM) increased the phosphorylation state of PLB in preparations from H_2-TG [freeze clamped at the maximum of the positive inotropic effect (PIE)] but not WT mice (Fig. 8).

Finally, it was of interest whether UR-Po563, like histamine itself, could increase force of contraction in the human heart. As a model we used electrically driven (1 Hz) isolated right atrial strips obtained from the operating theater in routine bypass surgery. Here, we noted that UR-Po563 concentration-dependently could increase force of contraction in atrial preparations of human hearts (Fig. 9A: original tracings; Fig. 9, B–E: summary) and increased dF/dt (Fig. 9, D and E).

Discussion

In our integrative approach, we have functionally shown that UR-Po563, UR-MB-158 and UR-MB-159 can stimulate

TABLE 2

Effects of UR-Po563, UR-MB-158, and UR-MB-159 in echocardiography. Mice were studied under isoflurane narcosis. Drugs were applied via injection into the peritoneum. Transthoracic ultrasound was performed in supine mice. B-mode and M-mode pictures were taken before and 5 minutes after injection of UR-Po563 into H_2-TG and WT. Data for ejection fraction and beating rate are listed in the table.

	Heart rate (bpm)		Ejection fraction (%)	
	Basal	Stimulated	Basal	Stimulated
UR-Po563				
WT (n = 5)	436.8 ± 23.2	511.9 ± 113.6	60.14 ± 11.58	72.44 ± 6.40
H_2-TG (n = 5)	499.0 ± 83.3	626.8 ± 64.5*	74.31 ± 7.84	98.21 ± 9.97*
H_2-TG + famotidine (n = 7)	469.8 ± 99.8	517.4 ± 131.2	64.61 ± 8.19	82.47 ± 9.97*
UR-MB-158				
WT (n = 1)	447.5	596.5	55.83	83.2
H_2-TG (n = 2)	526.3	656.2	62.34	98.15
UR-MB-159				
WT (n = 1)	417.8	513.0	51.62	82.42
H_2-TG (n = 2)	380.9	686.3	54.04	94.21

*P < 0.05 versus basal (= predrug values).

human cardiac H_2 receptors. The substances described have already been characterized in two detailed studies (Biselli et al., 2021; Tropmann et al., 2021) with regard to receptor binding (Supplemental Table 2) and functionality (G protein recruitment, β-arrestin2 recruitment, and guinea pig right atrium; Supplemental Tables 3 and 4). The published data were supplemented in this study for UR-Po563 (Supplemental Tables 3 and 4) and summarized in Supplemental Tables 2, 3 and 4 for a better comparability. In addition to the detailed characterization of H_2R orthologs, statements on selectivity within the family of histamine receptors can also be found in Supplemental Table 2.

Previously, dimaprit has been developed as a H_2R agonist, devoid of action at H_1 receptors (Panula et al., 2015). However, later (when H_3 and H_4 receptors were identified, cloned, and characterized), dimaprit was found also to be an agonist at H_3 and H_4 receptors. Amthamine was the next step to find a selective agonist at the H_2R (Panula et al., 2015). In some systems, histamine and dimaprit were equipotent [U937 cells (Smit et al., 1994)]; in other cells, histamine was more potent than dimaprit [H_2-transfected CHO cells (Smit et al., 1994)]. In the human heart, dimaprit was a full agonist for its PIE with an EC_{50} value of 43 μM (Poli et al., 1994). However, in

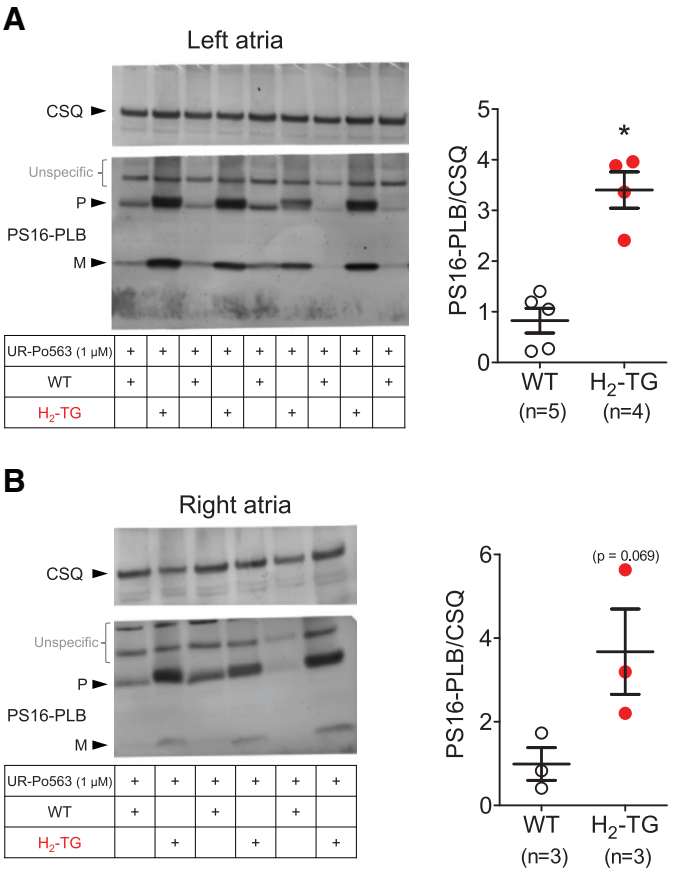

Fig. 7. Effect of UR-Po563 on protein phosphorylation in the atrium. Concentration response curves as depicted in Fig. 2 were generated for UR-Po563 in isolated left atrium or right atrium of WT and H_2-TG mice. At the end of the concentration response curves, the atria were frozen in the presence of 1 μM UR-Po563 and analyzed by Western blotting. Antibodies for PS16-PLB were used for incubation and quantification. In the same lanes but at higher molecular weight (58 kDa) calsequestrin (CSQ; compare Fig. 1) was detected as a loading control. Signals were quantified and the ratio of phosphorylated phospholamban to calsequestrin in arbitrary units was plotted in the ordinates. Number in bars give number of mice studied. (A) Left atria. (B) Right atria. *P < 0.05 versus WT.

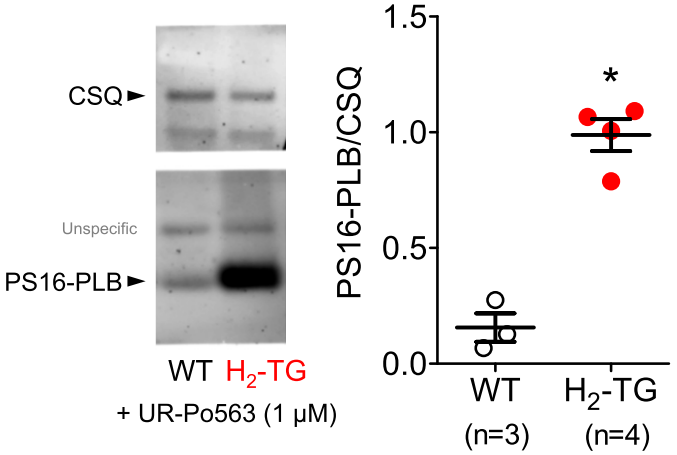

Fig. 8. Effect of UR-Po563 on protein phosphorylation in the ventricle. In isolated Langendorff-perfused hearts, UR-Po563 increased PS16-PLB. Expression of calsequestrin (CSQ) served as loading control. On top a typical Western blot is depicted. The bar diagram summarizes the results. The ordinate is given in the bar diagram. + indicates significant effects of UR-Po563 in H_2-TG compared with WT. Ratio of phosphorylated phospholamban to calsequestrin in arbitrary units was plotted in the ordinates. Numbers in bars give number of mice studied. *P < 0.05 versus WT.

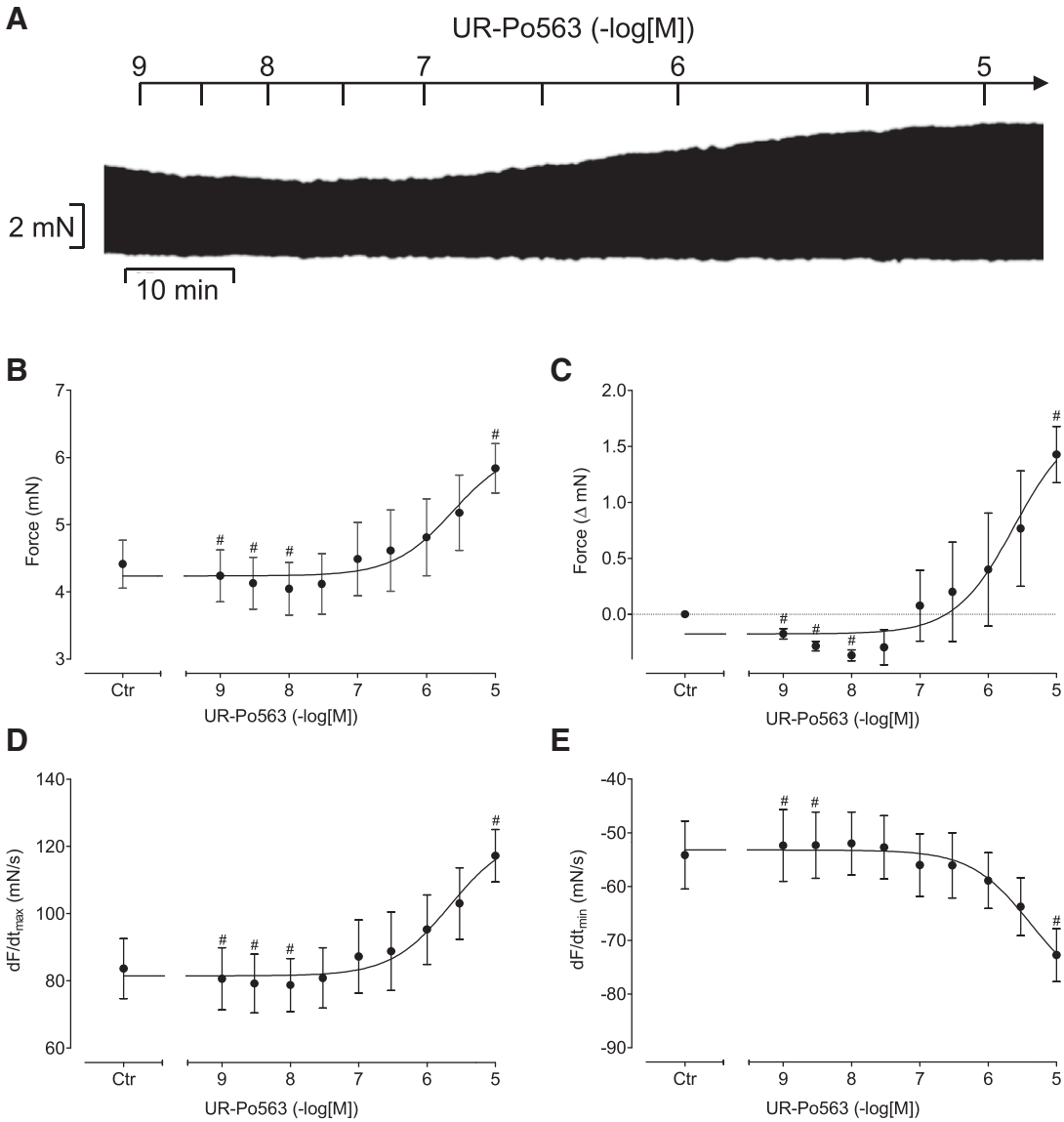

Fig. 9. Human atrial preparations. (A) Original recording of the effect of cumulatively applied UR-Po563 on force of contraction in an isolated electrically driven (1 Hz) human right atrial preparation. Horizontal bar indicates time in minutes; vertical bar indicates developed force in milli-Newtons. (B) Effects of UR-Po563 in human right atrial preparations ($n = 5$) on developed force (ordinate in milli-Newtons). (C) Effects of UR-Po563 in human right atrial preparations ($n = 5$) on developed force presented as delta milli-Newtons. (D) Effects of UR-Po563 in human right atrial preparations ($n = 5$) on the maximum rate of tension development dF/dt in mN/s (ordinate). (E) Effects of UR-Po563 in human right atrial preparations ($n = 5$) on the minimum rate of tension development dF/dt in mN/s (ordinate). $^{\#}P < 0.05$ versus Ctr. Abscissae: negative logarithmic concentrations of UR-Po563 in moles.

other laboratories, dimaprit was more potent for its PIE (human left atrium, right atrium, papillary muscle) with an EC_{50} around 3 μM (Bristow et al., 1982; Eckel et al., 1982; Brown et al., 1986). In the guinea pig papillary muscle, amthamine was more potent and equieffective to histamine with respect to the PIE (Poli et al., 1993). Amthamine, compared with histamine, acts as a full agonist with respect to PIE in human right atrial preparations with an EC_{50} value of 4.2 μM (Poli et al., 1994). In contrast, in the guinea pig right atrium, amthamine was more potent than histamine with respect to the positive chronotropic effect (Poli et al., 1993). Interestingly, histamine was more potent in the H_2-TG mouse atrium with respect to the PIE than in human atrium, although the receptor was the same (Supplemental Table 1). This clearly demonstrates the limitation of model systems (animal models or cell cultures). Species differences and differences in, e.g., receptor density must always be considered. Unfortunately, it was not possible to quantify the expression level of the H_2R in the H_2-TG mice by either Western blotting or radioligand binding in heart homogenates or membrane preparations (Gergs et al., 2019). In this context, it is not surprising that UR-Po563 is more potent in H_2-TG atrial preparations compared with human atrial preparations.

PLB is only expressed in cardiomyocytes and not in noncardiomyocytes. The fact that we measured an increase in PLB phosphorylation in atrial and ventricular preparations is strongly indicative that UR-Po563 acts on H_2 receptors in cardiomyocytes.

The fact that we measured an increase in serine 16 phosphorylation of PLB is easily interpreted as a result from cAMP

generation after UR-Po563 application in H_2-TG mice. H_2R activation is expected to activate protein kinase A (Fig. 1), which only phosphorylates PLB on serine 16 and which is thought to augment cardiac relaxation (Simmerman et al., 1986). More recently, we have shown that H_2R stimulation leads to PLB phosphorylation in isolated atrial preparations from H_2-TG but not WT mice (Gergs et al., 2019). In addition, we could measure that histamine increases the phosphorylation in the human heart, more specifically in isolated electrically stimulated (1 Hz) right atrial preparations from patients undergoing heart surgery (Neumann et al., 2021a).

Clinical Relevance. In contrast to β_1 adrenoceptors, the density of H_2 receptors in the failing human hearts were not changed compared with nonfailing controls (ventricular samples from transplants in Munich, Germany); Baumann et al. (1984) used tritiated tiotidine for binding studies). In isolated ventricular preparations from failing human hearts, Bauman et al. did not detect a reduced PIE to histamine compared with nonfailing controls (Baumann et al., 1982; Baumann et al., 1983; Baumann et al., 1984) and suggested that H_2R agonists might be clinical useful for the treatment of heart failure. They even tested a H_2R agonist (impromidine) in the intensive care unit in patients and described beneficial acute effects but also serious side effects like increased gastric acid (Baumann et al., 1984; Felix et al., 1995). In contrast, others noted a reduced PIE to histamine in samples from failing human hearts (Brown et al., 1986; Böhm et al., 1988).

There are interesting data that H_2 blockers reduce the propensity for heart failure in humans. Apparently, the first data in this regard were Japanese register data (Kim et al., 2004); this prompted a better controlled but still retrospective study in the United States. Leary et al. (2014, 2016b) provided the first evidence for a beneficial role of H_2R blockers by delaying the onset of heart failure and alterations of the morphology of the heart: the development of left ventricular dilation, a hallmark of imminent heart failure, was delayed. They suggested furthermore that this might be an example of repurposing of drugs (antihistamines) for heart failure (Leary et al., 2016a; Leary and Bristow, 2016). In a subsequent analysis, they suggested that it might be possible to identify a subgroup of patients with risk of heart failure, which might benefit particularly from treatment with H_2R antagonists (Leary et al., 2018).

Hence, one could argue for a twofold clinical utility of the present data. If stimulation of H_2 receptors is a valid concept to increase force of contraction in patients, UR-MB-158 and UR-MB-159 are more potent than histamine and older H_2R agonists in functional studies on human H_2 receptors. Thus, they may be tested for patients with end stage heart failure to sustain contractility under clinical conditions. Alternatively, if it turns out that H_2R agonists are useful for the treatment of patients with Alzheimer's disease, it might be conceivable that drugs like UR-Po563, UR-MB-158, or UR-MB-159 might be tested. Here, the cardiac effects might be unwanted side effects, and one might block these peripheral effects by treating the patients concomitantly with a H_2R antagonist that does not pass through the blood-brain barrier. That was why we used famotidine in this experimental study. It is known that famotidine in contrast to cimetidine does not pass the human blood-brain barrier. On the other hand, the preliminary experiments with human atrial preparations presented here indicate a relatively low potency of the new H_2R agonist UR-Po563 with respect to the PIE in the human atrium. Therefore, the therapeutic dose necessary to treat Alzheimer's disease may be at least in part safe with respect to cardiac side effects. However, the cardiac response to H_2R agonists may vary in a wide range from patient to patient, in contrast to a genetically homogenous mouse model. Moreover, patients differ widely in their diseases and medications, which make comparisons very difficult. Therefore, these aspects have to be studied in more detail in future work. Studies are ongoing in our groups to measure the concentrations of applied UR-Po563, UR-MB-158, and UR-MB-159 in the heart and brain in mice. Moreover, we have started to measure famotidine concentrations in heart and brain of mice. These data might support or repudiate our suggestions.

In summary, we describe that three novel H_2R agonists named UR-Po563, UR-MB-158, and UR-MB-159 behave as functional full agonists on human cardiac H_2 receptors. They are functionally more potent than histamine in the heart at H_2 receptors. They stimulate H_2 receptors in the right and left atrium, the ventricle in vitro, and the ventricle in vivo of H_2-TG but not WT mice. Finally, UR-Po563 increase force of contraction in the human heart in vitro. These novel agents could now be clinically tested to treat heart failure or Alzheimer's disease.

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Authorship Contributions

Participated in research design: Gergs, Neumann.

Conducted experiments: Büxel, Bresinsky, Kirchhefer, Fehse, Höring, Hofmann, Marušáková, Čináková, Schwarz, Pockes.

Contributed new reagents or analytic tools: Bresinsky, Höring, Pockes.

Performed data analysis: Büxel, Bresinsky, Fehse, Höring, Marušáková, Čináková, Schwarz, Pockes.

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

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