Cardiac effects of novel histamine H₂ receptor agonists

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Non-standard abbreviations:
H₂R histamine H₂ receptor
MHC myosin heavy chain
PIE positive inotropic effect
PLB phospholamban
TG transgenic
WT wild type

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Abstract

In an integrative approach, we studied cardiac effects of recently published novel H₂ receptor agonists in the heart of mice that overexpress the human H₂ receptor (H₂-TG), littermate wild type control mice (WT) and in isolated electrically driven muscle preparations from patients undergoing cardiac surgery. Under our experimental conditions, the H₂ receptor agonists UR-Po563, UR-MB-158 and UR-MB-159 increased force of contraction in left atrium from H₂-TG with pEC₅₀ values of 8.27, 9.38, and 8.28, respectively, but not in WT. Likewise, UR-Po563, UR-MB-158 and UR-MB-159 increased the beating rate in right atrium from H₂-TG with pEC₅₀ values of 9.01, 9.24, and 7.91, respectively, but not in WT. These effects could be antagonized by famotidine, a H₂ receptor antagonist. UR-Po563 (1 µM) increased force of contraction in Langendorff perfused hearts from H₂-TG but not WT. Similarly, UR-Po563, UR-MB-158 or UR-MB-159 increased the left ventricular ejection fraction in echocardiography of H₂-TG. Finally, UR-Po563 increased force of contraction in isolated human right atrial muscle strips. The contractile effects of UR-Po563 in H₂-TG were accompanied by an increase in the phosphorylation state of phospholamban. In summary, we report here three recently developed agonists functionally stimulating human cardiac H₂ receptors in vitro and in vivo.

We speculate that these compounds might be of some merit to treat neurological 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.

Keywords: Histamine; H₂ receptor agonists; atrium; ventricle; mouse; human

Significance Statement:

Recently, a new generation of H₂R agonists has been developed as possible treatment option for Alzheimer's disease. Here, possible cardiac (side) effects of these novel H₂R agonists have been evaluated.
Introduction

Histamine is a mediator of many physiological 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 H₂ receptor (H₂R) agonist, one also treats the patient’s heart. In the heart, histaminergic cardiac effects were initially described by Dale and Laidlaw (Dale and Laidlaw, 1910) and Ackermann and Kutscher (Ackermann and Kutscher, 1910). In the human heart, H₁ and H₂ receptors have been identified using antibodies (Matsuda et al., 2004). There are much more H₁ receptors in human atrial samples than H₂ receptors (Baumann et al., 1983; Matsuda et al., 2004). Nevertheless, the H₂ 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 H₂ receptor agonists and not H₁ receptor agonists because the positive inotropic effects of histamine are blocked by H₂ receptor antagonists like cimetidine and famotidine (Seifert et al., 2013; Panula et al., 2015). In human atrial samples, the stimulation of H₂ receptors led to an increase in cAMP content, an elevated activity of the cAMP dependent protein kinase (Sanders et al., 1996) (see scheme in Fig. 1) and subsequently to an increase in the phosphorylation state of phospholamban (PLB, compare 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 (pD₂ = 5.38) or the more potent agonist impromidine (pD₂ = 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 H₂ receptor in the murine heart (Gergs et al., 2019). In the hearts of this model (H₂-TG) but not in the wild type (WT) littermates, we could identify the human H₂ receptor
mRNA and the protein by immunohistology of ventricular slices as well as by autoradiography of hearts with a radioactively labeled H₂R 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 H₂-TG, isolated perfused hearts from H₂-TG, isolated cells from H₂-TG, isolated atrial preparations from H₂-TG but not from WT (Gergs et al., 2019; Gergs et al., 2020; Neumann et al., 2021a; Neumann et al., 2021b). Therefore, we had suggested that this H₂-TG might be a useful model to study human H₂R in all regions of the heart. It is important to point out that we used an alpha myosin heavy chain promoter for the human H₂R transgene, meaning that we overexpressed the human H₂R only in the cardiomyocytes of the heart (Gergs et al., 2019).

There are studies reporting that stimulation of postsynaptic H₂Rs 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 H₂R antagonists initiating this process by inhibiting presynaptic H₃-autoreceptors (Darras et al., 2014; Khan et al., 2016; Sadek et al., 2016) the use of central nervous system-penetrating H₂R agonists is of great interest. In the present work we wanted to test whether the recently published H₂R 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 H₂R.
Materials and Methods

Transgenic Mice

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council (National Academies Press (US), 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 (Gene bank 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$_2$R was performed by the TRAM unit of the Westfälische Wilhelms-Universität, Münster, Germany and have been described before (Gergs et al., 2019). Genotypes were identified by PCR analyses of tail tip DNA using the following primers: 5’-ACCCTTACCCCACATAGACC-3’ and 5’-AGCAGGTCAGTGATAGCCAA-3’. The PCR reaction was performed using the Ampliqon Taq DNA polymerase (Biomol, Hamburg, Germany) according to the manufacturer’s instructions. For the experiments, 3 to 6 months 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 min 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 finally, bands were detected using enhanced chemifluorescence (ECF, 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), 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 knock out 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 un-boiled 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 H₂-TG or WT. 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 H₂R antagonist famotidine to occupy the cardiac H₂R. 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 H₂-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, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 1.05, NaH$_2$P0$_4$ 0.42, NaHCO$_3$ 22.6, Na$_2$EDTA 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 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 utilized 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, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 1.05, NaH$_2$P0$_4$ 0.42, NaHCO$_3$ 22.6, Na$_2$EDTA 0.05, ascorbic acid 0.28 and glucose 5.0 in an isolated heart system. The heart preparations were allowed to equilibrate for 30 min 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 described (Neumann et al., 2021a). Right atrial samples were obtained from patients that underwent cardiac bypass surgery because of a 3-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, 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 utilized. The patient characteristics are described below. Age: 52-72 years old; sex: male; NYHA class: III-IV; angina grading scale (CCS): III; left ventricular EF: 40-60%; further diagnoses were arterial hypertension, hypercholesterolemia, and diabetes (two of three); the 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/hH₂R-NlucC or NlucN-mGs/gpH₂R-NlucC and UR-Po563 in various concentrations (Höring et al., 2020; Tropmann et al., 2021).

**Data Analysis**
Data shown are means ± SEM. 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 a Bonferroni’s posttest for the echocardiographic and Western blot results. A p-value < 0.05 was considered as 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 H₂-TG (original recording in Fig. 2A) but failed to affect force of contraction in WT (Fig. 2B). The data are summarized in Fig. 2B-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 H₂-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 H₂-TG on force of contraction and beating rate were antagonized by H₂R antagonists applied 30 min 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 H₂-TG. This and the antagonistic effect of famotidine can be seen in the original recordings in Fig. 3A. The data are summarized in Fig. 3B-E. The respective EC₅₀ values of UR-Po563 are provided in the Supplemental Table S1 and can be compared with values from UR-MB-158, UR-MB-159, histamine and those of some typical older histamine H₂R ligands. UR-Po563 increased the beating rate in H₂-TG, but not in WT (Fig. 3C). In the left atrial samples, UR-Po563 concentration- and time-dependently increased the first derivate of force versus time dF/dt in absolute terms (Fig. 3D and 3E). The effects of UR-Po563 on force of contraction, its first derivative or on the beating rate in H₂-TG were antagonized by famotidine (Fig. 3).

In a more potent way (Supplemental Table S1), UR-MB-158 increased the force of contraction and the beating rate in atrial preparations from H₂-TG but not in WT in a concentration- and time-dependent manner. This can be seen in an original recording (Fig. 4A) and is summarized in Fig. 4B-E. UR-MB-158 increased the beating rate in right atrial preparations from H₂-TG, but not WT (Fig. 4C). Moreover, in the left atrial samples, UR-MB-158 increased the first derivate of force versus time dF/dt in absolute terms (Fig. 4D and 4E). 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 S1): 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 H₂-TG, but not in WT. In the left atrial samples, UR-MB-159 increased the maximum and minimum first derivate of force versus time (dF/dt, Fig. 5D and 5E). 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 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 H₂-TG, but not WT (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 H₂ receptor agonists on cardiovascular performance in vivo, we performed echocardiographic measurements in H₂-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 TG (Table 2 and 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 TG, but not in WT (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 and 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 H₂-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.
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 (freeze clamped at the maximum of the positive inotropic effect) (Fig. 7). In contrast, in atrial preparations from WT, 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 (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 theatre 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. 9B-E: summary) and increased dF/dt (Fig. 9D and 9E).
Discussion

In our integrative approach, we have functionally shown that UR-Po563, UR-MB-158 and UR-MB-159 can stimulate human cardiac H₂ 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 S2) and functionality (G protein recruitment, β-arrestin2 recruitment and guinea pig right atrium; Supplemental Table S3 and S4). The published data were supplemented in this study for UR-Po563 (Supplemental Table S3 and S4) and summarized in Supplemental Table S2, S3 and S4 for a better comparability.

In addition to the detailed characterization of H₂R orthologs, statements on selectivity within the family of histamine receptors can also be found (Supplemental Table S2).

Previously, dimaprit has been developed as a H₂R agonist, devoid of action at H₁ receptors (Panula et al., 2015). However, later (when H₃ and H₄ receptors were identified, cloned and characterized), dimaprit was found also to be an agonist at H₃ and H₄ receptors. Amthamine was the next step to find a selective agonist at the H₂R (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 (H₂-transfected CHO cells (Smit et al., 1994)).

In the human heart, dimaprit was a full agonist for its PIE with an EC₅₀ value of 43 µM (Poli et al., 1994). However, in other laboratories, dimaprit was more potent for its PIE (human left atrium, right atrium, papillary muscle) with an EC₅₀ 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 to histamine, acts as a full agonist with respect to PIE in human right atrial preparations with an EC₅₀ 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 H₂-TG atrium with respect to the PIE than in human atrium, although the receptor was the same (Supplemental table S1). 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$_2$R in the H$_2$-TG mice neither by Western blotting nor by 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 to human atrial preparations.

PLB is only expressed in cardiomyocytes and not in non-cardiomyocytes. 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. H$_2$R activation is expected to activate PKA (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$_2$R stimulation leads to PLB phosphorylation in isolated atrial preparations from H$_2$-TG but not WT (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 to non-failing controls (ventricular samples from transplants in Munich, Germany: (Baumann et al., 1984), they used tritiated tiotidine for binding studies). In isolated ventricular preparations from failing human hearts, they did not detect a reduced PIE to histamine compared to non-failing controls (Baumann et al., 1982; Baumann et al., 1983; Baumann et al., 1984) and suggested that H$_2$R agonists might be clinical useful for the treatment of heart failure. They even tested a H$_2$R 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 H₂ 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 US (Leary et al., 2014; Leary et al., 2016b). The authors provided first evidence for a beneficial role of H₂R 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 H₂R antagonists (Leary et al., 2018).

Hence, one could argue for a twofold clinical utility of the present data. If stimulation of H₂ 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 H₂R agonists in functional studies on human H₂ 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 H₂R agonists are useful for the treatment of Alzheimer patients, 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 H₂R 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 H₂R 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 H₂R 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 H₂R agonists named UR-Po563, UR-MB-158 and UR-MB-159 behave as functional full agonists on human cardiac H₂ receptors. They are functionally more potent than histamine in the heart at H₂ receptors. They stimulate H₂ receptors in the right and left atrium, the ventricle in vitro and the ventricle in vivo of H₂-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: Neumann and Gergs Conducted experiments: Büxel, Bresinsky, Kirchhefer, Fehse, Höring, Hofmann, Marušáková, Čínáková, Schwarz, and Pockes Contributed new reagents or analytic tools: Bresinsky, Höring, and Pockes Performed data analysis: Büxel, Bresinsky, Fehse, Höring, Marušáková, Čínáková, Schwarz, and Pockes Wrote or contributed to the writing of the manuscript: Neumann, Pockes, and Gergs
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gene and protein levels are differentially expressed in the hearts of rodents and humans.

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Legends to figures

Fig. 1. (A) Structural formulas of histamine, of the H₂R antagonist famotidine and of the novel H₂R 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 H₂-receptor (H₂R) agonists in the mammalian heart. Activation of the cAMP-dependent protein kinase (PKA) via stimulation of the H₂R increases the cytosolic Ca²⁺ concentration. By this way the force of contraction can be increased (positive inotropic effect) and arrhythmias may be triggered (pro-arrhythmic effect). The effects can be reversed by the H₂R antagonist famotidine. AC, adenylyl cyclase; CSQ, calsequestrin; Gs, stimulatory G-protein; LTCC, L-type Ca²⁺ channel; NCX, sodium calcium exchanger; P, phosphorylation; PLB, phospholamban; RYR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase; TnC, troponin C; TnI, troponin inhibitor.

Fig. 2. Histamine increases contractility in H₂-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 (min); vertical bar indicates developed force in milli-Newton (mN). (B) Effects of histamine in H₂-TG (n=11) and WT (n=17) left atrium on developed force (ordinate in mN). (C) Effects of histamine in H₂-TG (n=11) and WT (n=17) left atrium on time to maximum rate of tension development dF/dt in milli-Newton per second (mN/s, ordinate) and (D) on time to minimum rate of tension development dF/dt in milli-Newton per second (mN/s, ordinate). (E) Effects of histamine in H₂-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 vs. WT. #first p < 0.05 vs. Ctr. Abscissae: negative logarithmic concentrations of histamine in mole (M).
**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 (min); vertical bar indicates developed force in milli-Newton (mN). (B) Effects of UR-Po563 in H2-TG (n=5) and WT (n=5) left atrium on developed force (ordinate in mN) versus H2-TG (n=8) and WT (n=4) in presence of famotidine. (C) Effects of UR-Po563 in H2-TG (n=3) and WT (n=4) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm) versus H2-TG (n=5) and WT (n=3) in presence of famotidine. (D) Effects of UR-Po563 in H2-TG (n=5) and WT (n=5) left atrium on time to maximum rate of tension development dF/dt in milli-Newton per second (mN/s, ordinate) versus H2-TG (n=8) and WT (n=4) in presence of famotidine. (E) Effects of UR-Po563 in H2-TG (n=5) and WT (n=5) left atrium on (ordinate = time to minimum rate of tension development dF/dt in milli-Newton per second (mN/s, ordinate) versus H2-TG (n=8) and WT (n=4) in presence of famotidine. Abscissae: negative logarithmic concentrations of UR-Po563 in mole (M). *Indicates the significant effects of UR-Po563 compared to pre-drug values (Ctr./Fam); †Indicates the significant effects of UR-Po563 in H2-TG compared to WT; ‡Indicates the significant effects of Po563 in H2-TG compared to H2-TG in presence of famotidine.

**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 (min); vertical bar indicates developed force in milli-Newton (mN). (B) Effects of UR-MB-158 in H2-TG (n=6) and WT (n=6) left atrium on developed force (ordinate in mN) versus H2-TG (n=8) and WT (n=5) in presence of famotidine. (C) Effects of UR-MB-158 in H2-TG (n=4) and WT (n=5) in spontaneously beating right atrium on beating rate (ordinate) in beats per minute (bpm) versus H2-TG (n=6) and WT (n=6) in presence of famotidine. (D) Effects of UR-MB-158 in H2-TG (n=6) and WT (n=6) left atrium on time to maximum rate of tension development dF/dt in milli-Newton per second
(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 (ordinate= time to minimum rate of tension development $dF/dt$ in milli-Newton per second (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 mole (M). # Indicates the significant effects of UR-MB-158 compared to pre-drug values (Ctr.): * Indicates the significant effects of UR-MB-158 in $H_2$-TG compared to WT. + Indicates the significant effects of UR-MB-158 in $H_2$-TG compared to $H_2$-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 (min); vertical bar indicates developed force in milli-Newton (mN). (B) Effects of UR-MB-159 in $H_2$-TG ($n=6$) and WT ($n=8$) left atrium on developed force (ordinate in mN) 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 milli-Newton per second (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 (ordinate= time to minimum rate of tension development $dF/dt$ in milli-Newton per second (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 mole (M). # Indicates the significant effects of UR-MB-159 compared to pre-drug values (Ctr.): * Indicates the significant effects of UR-MB-159 in $H_2$-TG compared to WT. + Indicates the significant effects of UR-MB-159 in $H_2$-TG compared to $H_2$-TG in presence of famotidine.
**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 min after injection of UR-Po563 solution. Vertical bar indicates the size marker in milli-Meter (mm) and horizontal bar indicates the time marker in seconds (s). Data are summarized in (B) as well as in Table 2.

**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 wild type (WT) and H$_2$R transgenic mice (H$_2$-TG). 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 serine-16-phosphorylated phospholamban (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 vs. WT.

**Fig. 8.** Effect of UR-Po563 on protein phosphorylation in the ventricle. In isolated Langendorff-perfused hearts, UR-Po563 increased the phosphorylation state of phospholamban at the amino acid serine-16 (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 to 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 vs. 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 (min); vertical bar indicates developed force in milli-Newton (mN). (B) Effects of UR-Po563 in human right atrial preparations (n=5) on developed force (ordinate in mN). (C) Effects of UR-Po563 in human right atrial preparations (n=5) on developed force presented as delta mN. (D) Effects of UR-Po563 in human right atrial preparations (n=5) on the maximum rate of tension development dF/dt in milli-Newton per second (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 milli-Newton per second (mN/s, ordinate). *p < 0.05 vs. Ctr. Abscissae: negative logarithmic concentrations of UR-Po563 in mole (M).
Tables

TABLE 1

Effect of UR-Po563 (1 µM) on force of contraction and beating rate in isolated perfused Langendorff hearts from H₂-TG and WT (n=3-4, each). Hearts were perfused initially with buffer for about 30 min 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 min of drug perfusion the whole heart was rapidly frozen with Wollenberger clamps pre-cooled in liquid nitrogen.

<table>
<thead>
<tr>
<th></th>
<th>WT Basal</th>
<th>UR-Po563</th>
<th>H₂-TG Basal</th>
<th>UR-Po563</th>
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</thead>
<tbody>
<tr>
<td>Force (mN)</td>
<td>7.89 ± 1.28</td>
<td>8.95 ± 1.95</td>
<td>11.49 ± 2.07</td>
<td>20.46 ± 2.81*</td>
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<tr>
<td>Beating rate (bpm)</td>
<td>308 ± 8.60</td>
<td>321 ± 13.2</td>
<td>351 ± 37.3</td>
<td>391 ± 16.2</td>
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<tr>
<td>dF/dt max (mN/s)</td>
<td>204.6 ± 43.8</td>
<td>220.6 ± 36.1</td>
<td>296 ± 42.5</td>
<td>718 ± 90.9*</td>
</tr>
<tr>
<td>dF/dt min (mN/s)</td>
<td>-151 ± 53.2</td>
<td>-172 ± 56.5</td>
<td>-230 ± 39.3</td>
<td>-566 ± 52.7*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. basal
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 min after injection of UR-Po563 into H₂-TG and WT. Data for ejection fraction and beating rate are listed in the table.

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (bpm)</th>
<th>Ejection fraction (%)</th>
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<tbody>
<tr>
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<td>Basal</td>
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<tr>
<td><strong>UR-Po563</strong></td>
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<tr>
<td>WT (n = 5)</td>
<td>436.8 ± 23.2</td>
<td>511.9 ± 113.6</td>
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<tr>
<td>H₂-TG (n = 5)</td>
<td>499.0 ± 83.3</td>
<td>626.8 ± 64.5 *</td>
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<tr>
<td>H₂-TG + Famotidine (n = 7)</td>
<td>469.8 ± 99.8</td>
<td>517.4 ± 131.2</td>
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<td>WT (n = 1)</td>
<td>447.5</td>
<td>596.5</td>
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<tr>
<td>H₂-TG (n = 2)</td>
<td>526.3</td>
<td>656.2</td>
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<td><strong>UR-MB-159</strong></td>
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<tr>
<td>WT (n = 1)</td>
<td>417.8</td>
<td>513.0</td>
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<td>H₂-TG (n = 2)</td>
<td>380.9</td>
<td>686.3</td>
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*P < 0.05 vs. basal (= pre-drug values)
Figure 1

A

Histamine

Famotidine

UR-Po563

UR-MB-158

UR-MB-159

B

Arrhythmias

Histamine

Famotidine

UR-Po563

UR-MB-158

UR-MB-159

LTCC

HCX

AC

PKA

RyR

PLB

SERCA

Myofibrils

Sarcoplasmic reticulum

Cytoplasm

Ca^{2+}

Na^{+}

P

Force

Ca^{2+}

cAMP

Ca^{2+}

Myofibrils

Ca^{2+}

P

Tnl

TnC
Figure 5

A

B

C

D

E
**Figure 7**

**A**  
**Left atria**

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<th>UR-Pα563 (1 μM)</th>
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<tr>
<td>H₂-TG</td>
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<td>+</td>
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<td>+</td>
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**B**  
**Right atria**

<table>
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<th>UR-Pα563 (1 μM)</th>
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<tr>
<td>H₂-TG</td>
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<td>+</td>
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</table>

(p = 0.069)
Supplemental Table S1. EC\textsubscript{50} values of drugs agonistic at cardiac H\textsubscript{2} receptors

Potency of histamine and the indicated histamine receptor agonist (first column), the cardiac tissue and species studied (second column), the potencies of the agonists (fourth column) were calculated from the negative decadic logarithm of the EC\textsubscript{50}-values. The EC\textsubscript{50}-values indicate the concentration of a drug, where 50% of the maximum effect on force or frequency is noted. In right atrial preparations from animals in this table the beating frequency was measured to assess potency whereas in electrically stimulated strips from the right atrium of the human heart and the left atrium of animals the force of contraction was studied (third column). Typical reports of the histamine effects on the heart are listed in the references (fifth column).

<table>
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<tr>
<th>Drug</th>
<th>Tissue</th>
<th>Parameter</th>
<th>Potency</th>
<th>Reference</th>
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<td>Histamine</td>
<td>Human right atrium</td>
<td>Increase in force</td>
<td>pD\textsubscript{2}=5.54 ± 0.09</td>
<td>(Zerkowski et al., 1993; Gergs et al., 2019)</td>
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<tr>
<td>Histamine</td>
<td>Human right atrium</td>
<td>Increase in force</td>
<td>pD\textsubscript{2}=5.19 ± 0.30</td>
<td>(Poli et al., 1994)</td>
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<tr>
<td>Amthamine</td>
<td>Human right atrium</td>
<td>Increase in force</td>
<td>pD\textsubscript{2}=5.38 ± 0.36</td>
<td>(Poli et al., 1994)</td>
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<tr>
<td>Impromidine</td>
<td>Human right atrium</td>
<td>Increase in force</td>
<td>pD\textsubscript{2}=6.59 ± 0.35</td>
<td>(Poli et al., 1994)</td>
</tr>
<tr>
<td>Dimaprit</td>
<td>Human right atrium</td>
<td>Increase in force</td>
<td>pD\textsubscript{2}=4.37 ± 0.59</td>
<td>(Poli et al., 1994)</td>
</tr>
<tr>
<td>Histamine</td>
<td>Guinea pig right atrium</td>
<td>frequency</td>
<td>pD\textsubscript{2}=6.01 ± 0.24</td>
<td>(Poli et al., 1993)</td>
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<td>Amthamine</td>
<td>Guinea pig right atrium</td>
<td>frequency</td>
<td>pD\textsubscript{2}=6.72 ± 0.11</td>
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<td>Guinea pig right atrium</td>
<td>frequency</td>
<td>pD\textsubscript{2}=5.32 ± 0.22</td>
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<td>Effect Measure</td>
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<tr>
<td>Histamine</td>
<td>Rabbit left atrium</td>
<td>Increase in force</td>
<td>5.53 ± 0.06</td>
<td>(Hattori et al., 1988)</td>
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<td>Impromidine</td>
<td>Rabbit left atrium</td>
<td>Increase in force</td>
<td>8.69 ± 0.16</td>
<td>(Hattori et al., 1988)</td>
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<td>Increase in force</td>
<td>pD&lt;sub&gt;2&lt;/sub&gt;=6.73 ± 0.16</td>
<td>(Gergs et al., 2019)</td>
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<td>pD&lt;sub&gt;2&lt;/sub&gt;=6.39 ± 0.17</td>
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<td>pD&lt;sub&gt;2&lt;/sub&gt;=6.59 ±</td>
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<td>pD&lt;sub&gt;2&lt;/sub&gt;=8.27 ± 0.18 n=5</td>
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<td>time to maximum rate of tension development dF/dt</td>
<td>pD&lt;sub&gt;2&lt;/sub&gt;=8.26 ± 0.18 n=5</td>
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<td>pD&lt;sub&gt;2&lt;/sub&gt;=8.35 ± 0.2 n=5</td>
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<td>frequency</td>
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<td>Increase in force</td>
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<td>pD&lt;sub&gt;2&lt;/sub&gt;=9.52 ± 0.27 n=6</td>
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<td>time to of relaxation</td>
<td>pD&lt;sub&gt;2&lt;/sub&gt;=9.37 ± 0.46 n=6</td>
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<td>H₂-TG left atrium</td>
<td>time to minimum rate of tension development dF/dt</td>
<td>pD₂=8.34 ± 0.3 n=6</td>
<td>This report</td>
</tr>
<tr>
<td>UR-MB-159</td>
<td>H₂-TG right atrium</td>
<td>frequency</td>
<td>pD₂=8.07 ± 0.55 n=5</td>
<td>This report</td>
</tr>
</tbody>
</table>

**References**


Supplemental Table S2. Binding affinities and selectivity ratios of the \(N^{\text{G}}\)-carbamoylated guanidines UR-Po563, UR-MB-158, and UR-MB159 at the hH\(_1\)-R.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(pK_i)</th>
<th>Selectivity ratios of (K_i)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(hH_1R)</td>
<td>(N)</td>
</tr>
<tr>
<td>UR-Po563</td>
<td>5.06 ± 0.05</td>
<td>3</td>
</tr>
<tr>
<td>UR-MB-158</td>
<td>&lt; 5 (^b)</td>
<td>3</td>
</tr>
<tr>
<td>UR-MB-159</td>
<td>5.10 ± 0.05</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) Data cf. (Biselli et al., 2021); \(^b\) Data cf. (Tropmann et al., 2021).

References


**Supplemental Table S3.** Potencies and efficacies of the \(N^2\)-carbamoylated guanidines UR-Po563, UR-MB-158, and UR-MB159 in the \(\beta\)-arrestin2 and mini-\(G\) protein recruitment assays at the \(hH_2R\).

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\beta)-arrestin2 recruitment (^a)</th>
<th>mGs recruitment (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC\textsubscript {50}</td>
<td>(E_{\text{max}})</td>
</tr>
<tr>
<td>UR-Po563</td>
<td>7.34 ± 0.11(^a)</td>
<td>0.34 ± 0.03(^a)</td>
</tr>
<tr>
<td>UR-MB-158</td>
<td>6.55 ± 0.09(^c)</td>
<td>0.87 ± 0.02(^c)</td>
</tr>
<tr>
<td>UR-MB-159</td>
<td>6.49 ± 0.10(^c)</td>
<td>0.37 ± 0.02(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Data and procedure cf. (Biselli et al., 2021); \(^b\)Procedure cf. (Höring et al., 2020). \(^c\)Data cf. (Tropmann et al., 2021).

**References**


Supplemental Table S4. Potencies and efficacies of the \(N^\text{G}\)-carbamoylated guanidines UR-Po563, UR-MB-158, and UR-MB159 in the mini-G protein recruitment assays at the gpH2R or by organ bath studies at the spontaneously beating guinea pig right atrium (gpH2R).

<table>
<thead>
<tr>
<th>Compound</th>
<th>mGs recruitment (^{a})</th>
<th>Atrium (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{pEC}_{50})</td>
<td>(E_{\text{max}})</td>
</tr>
<tr>
<td>UR-Po563</td>
<td>8.19 ± 0.05</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>UR-MB-158</td>
<td>7.60 ± 0.03(^{c})</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>UR-MB-159</td>
<td>7.79 ± 0.02(^{c})</td>
<td>0.86 ± 0.03</td>
</tr>
</tbody>
</table>

\(^{a}\)Procedure cf. (Höring et al., 2020). \(^{b}\)Data and procedure cf. (Biselli et al., 2021); \(^{c}\)Data cf. (Tropmann et al., 2021).

References

