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Effects of psilocin and psilocybin on human 5-HT$_4$ serotonin receptors in atrial preparations of transgenic mice and humans

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ABSTRACT

Several fungi belonging to the genus *Psilocybe*, also called "magic mushrooms", contain the hallucinogenic drugs psilocybin and psilocin. They are chemically related to serotonin (5-HT). In addition to being abused as drugs, they are now also being discussed or used as a treatment option for depression. Here, we hypothesized that psilocybin and psilocin may act also on cardiac serotonin receptors and studied them in vitro in atrial preparations of our transgenic mouse model with cardiac myocytes-specific overexpression of the human 5-HT$_4$ receptor (5-HT$_4$-TG) as well as in human atrial preparations. Both psilocybin and psilocin enhanced the force of contraction in isolated left atrial preparations from 5-HT$_4$-TG, increased the beating rate in isolated spontaneously beating right atrial preparations from 5-HT$_4$-TG and augmented the force of contraction in the human atrial preparations. The inotropic and chronotropic effects of psilocybin and psilocin at 10 μM were smaller than that of 1 μM 5-HT on the left and right atria from 5-HT$_4$-TG, respectively. Psilocybin and psilocin were inactive in WT. In the human atrial preparations, inhibition of the phosphodiesterase III by cilostamide was necessary to unmask the positive inotropic effects of psilocybin or psilocin. The effects of 10 μM psilocybin and psilocin were abrogated by 10 μM tropisetron or by 1 μM GR125487, a more selective 5-HT$_4$ receptor antagonist. In summary, we demonstrated that psilocin and psilocybin act as agonists on cardiac 5-HT$_4$ receptors.

1. Introduction

Psilocybin and psilocin have hallucinogenic properties and are chemically related to serotonin (5-HT, Fig. 1) (Hofmann et al., 1958; Hofmann et al., 1959). Chemically, they are indole derivatives named [3-(2-dimethylaminoethyl)-1 H-indol-4-yl] dihydrogen phosphate and 4-hydroxy-N,N-dimethyltryptamine, respectively (Hofmann et al., 1958; Hofmann et al., 1959) (Fig. 1). At least psilocin binds with high affinity to several G-protein coupled receptors including 5-HT$_{2A,B,C}$ (Halberstadt and Geyer, 2011). The hallucinogenic effects of psilocin are usually explained by its binding to neuronal 5-HT$_{2A}$ receptors (Ki = 81 nM) (Nichols, 2020). It is currently not clear whether psilocin also binds to 5-HT$_4$ receptors (McKenna et al., 1990). Nonetheless, the FDA has approved psilocybin for the treatment of depression (Hesselgrave et al., 2021). The antidepressant effect of psilocin could be due to an inhibition of SERT activity, as this transporter is inhibited by psilocin with a Ki value of 3.8 μM (Poulie et al., 2020).

Psilocybin is considered as prodrug and its dephosphorylation by alkaline phosphatases leads to the active metabolite psilocin (in vitro: (Horita and Weber, 1962); in humans: (Hasler et al., 1997). Psilocin is a structural isomer of bufotenin, which is also hallucinogenic and an agonist on human 5-HT$_4$ receptors (Neumann et al., 2023). Psilocybin and psilocin are natural ingredients of many mushrooms of the *Psilocybe* genus (review: (Nichols, 2020). The name comes from ancient Greek and was coined by botanists based on the appearance of the mushrooms: psilos (ψιλος, naked) kube (κυβη, head). The mushrooms have been used in religious ceremonies in some parts of the world since prehistoric times. Because they can cause mind-altering experiences such as hallucinations, they are also commonly known as "magic mushrooms". The Swiss organic chemist Albert Hofmann, known as the inventor of LSD, firstly identified the active ingredients in mushrooms from central Mexico. He also succeeded to synthesize psilocin and psilocybin in vitro. (Hofmann et al., 1958; Hofmann et al., 1959). In the United States of America, but not only there, magic mushrooms and their active

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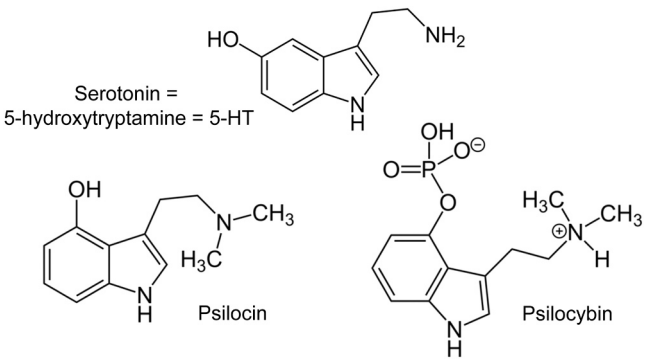


Fig. 1. Structural formulas of serotonin, psilocin and psilocybin.

ingredients are popular as recreational drugs. In recent years, there has been a renaissance of psilocybin in people suffering from depression and terminally ill cancer patients. In these more recent studies, side effects of psilocybin also on cardiovascular parameters such as tachycardia were observed in patients (Ross et al., 2016). However, the underlying receptor mechanism has not been investigated (Ross et al., 2016). Thus, the aim of the present work was to find out whether or not, psilocybin and psilocin act on isolated mammalian cardiac preparations, and if they increase cardiac force or beating rate via cardiac 5-HT_4 receptors in our established transgenic 5-HT_4-TG mouse model (Gergs et al., 2010) and if they also increase cardiac force in human atrial preparations. In brief, our hypothesis was that psilocybin and psilocin are agonists on human cardiac 5-HT_4 receptors, and for this purpose, we used functional methods. Parts of these studies have been presented on congresses and were therefore published as abstracts (Dimov et al., 2023; Jacob et al., 2023).

2. Materials and methods

2.1. Transgenic mice

The mouse model with cardiomyocyte-specific expression of the human 5-HT_{4a} receptor has been generated and characterized recently (Gergs et al., 2010; Gergs et al., 2013). The cardiac myocyte-specific expression of the receptor was driven by the mouse α-myosin heavy chain promoter. The age of the animals studied in the atrial contraction experiments was around 150 days. For the contraction experiments (see below), left and right atrial preparations were used as previously described (Gergs et al., 2013). All mice were housed under conditions of optimum light, temperature and humidity with food and water provided ad libitum.

The investigation conformed to the Guide for the Care and Use of Laboratory Animals as published by the National Research Council (2011). The animals were handled and maintained according to the approved protocols of the Animal Welfare Committee of the University of Halle-Wittenberg, Halle, Germany.

2.2. Contractile studies on mouse atrial preparations

In brief, the right or left atrial preparations from the mice were isolated and mounted in organ baths as previously described (Neumann et al., 2003; Gergs et al., 2013). The bathing solution of the organ baths contained 119.8 mM NaCI, 5.4 mM KCI, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 22.6 mM $NaHCO_3$, 0.05 mM Na_2EDTA, 0.28 mM ascorbic acid and 5.05 mM glucose. The solution was continuously gassed with 95 % O_2 and 5 % CO_2 and maintained at 37°C and pH 7.4 (Neumann et al., 1998; Neumann et al., 2003; Kirchhefer et al., 2004). Each atrial preparation was attached to an inductive force transducer in a 10-ml glass tissue chamber containing a bipolar stimulating electrode. Each preparation was pre-stretched to the length of its individual maximum force of contraction in order to record isometric contractions. The left atrial preparations were stimulated electrically by field stimulation at 1 Hz (5 ms rectangular pulses with a stimulating voltage of about 20 % above threshold). The force of contraction was recorded using a PowerLab system containing a bridge amplifier and digitizer (ADInstruments, Oxford, United Kingdom) and all parameters of contraction were calculated by the software LabChart Pro (ADIstruments, Oxford, United Kingdom). Spontaneously beating right atrial preparations (prepared with an intact sinus node) from mice were used to study any chronotropic effects.

The drug application was as follows. After equilibration was reached, 0.1 μM to 10 μM psilocybin or psilocin was added to the left or to the right atrium to establish concentration-response curves. Then, in presence of psilocybin or psilocin, 1 nM to 1 μM 5-HT was cumulatively applied to the preparations.

2.3. Contractile studies on human atrial preparations

The contractile studies on human right atrial preparations were done using the same setup and buffer as in the studies on mouse left atrial preparations, including electrically stimulation at 1 Hz (see Section 2.2) since no stimulus-producing tissue such as the sinoatrial node was present in the human samples. The samples were obtained from 11 male and 2 female patients aged 56–78 years (mean age: 69 ± 7 years) undergoing bypass surgery. The details of patients are listed in Table 1. Our methods used for atrial contraction studies in human samples have been previously published and were not altered in this study (Gergs et al., 2009; Gergs et al., 2017; Gergs et al., 2018; Boknik et al., 2019).

This study complies with the Declaration of Helsinki and has been approved by the local ethics committee (hm-bü 04.08.2005). Informed consent was obtained from all patients included in the study.

2.4. Western blotting

The frozen samples were homogenized in a frozen state in the presence of a sample buffer that inhibited proteolysis, phosphorylation and dephosphorylation. A modified Lowry protocol was used for protein determination. Thereafter, samples were subjected to sodium dodecyl sulphate gel electrophoresis using precast gradient gels (Novex™ 4–20 % "Tris-Glycine Plus Midi Protein Gels", Invitrogen, by Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) followed by transfer to nitrocellulose membranes. Molecular weight markers were identified and used to cut membrane strips at the predicted molecular weight range known from previous published studies. Using our standard lab procedures, the strips were incubated with primary antibodies against the serine-16 phosphorylated form of phospholamban (#A010–12 AP, Badrilla, Leeds, UK; dilution 1:5000) or against the cardiac form of calsequestrin (used as a loading control, #ab3516, abcam, Cambridge, UK; dilution 1:20,000) and then with horseradish peroxidase-labeled secondary antibodies (Sigma-Aldrich, München, Germany). Finally, the bound antibodies were visualized by chemiluminescence (Immobilon™, Millipore, by Merck, Darmstadt, Germany) and an imaging system (Amersham ImageQuant 800, Cytiva, Freiburg im Breisgau, Germany). Quantification was performed following our previously established protocols (Gergs et al., 2009; Boknik et al., 2018; Gergs et al., 2019b; Gergs et al., 2019a).

2.5. Data analysis

Data shown are means ± standard deviation. Statistical significance was estimated using the analysis of variance followed by Bonferroni's t-test. A p-value < 0.05 was considered significant.

2.6. Drugs and materials

Serotonin (5-HT) hydrochloride was purchased from Sigma-Aldrich

Table 1
Patient characteristics.

Patient ID	Gender	Age (years)	NYHA class	CCS Angina Grading Scale	LVEF (%)	Cardiac catheterization findings	Medication
#1	m	74	II	III-IV	60	2 vessel CHD, NSTEMI, post-op AF	Apixaban, atorvastatin, metoprolol, pancreatic enzymes, torasemide
#2	m	78	II	III	65	3 vessel CHD, post-op AF	Apixaban, amlodipine, atorvastatin, bisoprolol, lisinopril, pregabalin, oxycodone/naloxone, pantoprazole, hydrochlorothiazide/triamterene
#3	m	69	II	III	55	3 vessel CHD, NSTEMI	Acetylsalicylic acid 100 mg, clopidogrel, amlodipine, atorvastatin, metoprolol, candesartan, urapidil, hydrochlorothiazide, pantoprazole
#4	m	74	III	IV	40	2 vessel CHD, STEMI	Acetylsalicylic acid 100 mg, atorvastatin, bisoprolol, ramipril, prasugrel, dapagliflozin, sitagliptin, pantoprazole, torasemide
#5	f	56	III	II	60	3 vessel CHD	Acetylsalicylic acid 100 mg, atorvastatin, bisoprolol, ramipril, levothyroxine, pantoprazole, torasemide
#6	m	74	II	III-IV	55	3 vessel CHD, STEMI	Acetylsalicylic acid 100 mg, atorvastatin, bisoprolol, clopidogrel, torasemide, valsartan
#7	m	67	III-IV	III	25	3 vessel CHD, aortic valve stenosis	Acetylsalicylic acid 100 mg, metoprolol, folate, lamotrigine, torasemide
#8	m	57	II	III	50	3 vessel CHD	Acetylsalicylic acid 100 mg, atorvastatin, metoprolol, enalapril, ticagrelor, escitalopram, levetiracetam, pantoprazole, topiramate, torasemide
#9	f	63	III	IV	45	3 vessel CHD, STEMI, post-op.AF	Acetylsalicylic acid 100 mg, metoprolol, apixaban, hydrochlorothiazide, levothyroxine, olmesartan, pantoprazole, simvastatin
#10	m	73	III	II	60	AK-Stenose, 3 vessel CHD	Acetylsalicylic acid 100 mg, candesartan, amlodipine, torasemide, clopidogrel, bisoprolol, insulin
#11	m	68	II	II	60	AK-Stenose, 1 vessel CHD	Acetylsalicylic acid 100 mg, metoprolol, pantoprazole, atorvastatin, torasemide, olmesartan/amlodipine
#12	m	74	III	III	60	3 vessel CHD, STEMI, AF	Apixaban, rosuvastatin, metoprolol, pantoprazole, torasemide, ramipril, clopidogrel
#13	m	63	III	III	35	3 vessel CHD, ICM, AF	Edoxaban, rosuvastatin, metoprolol, pantoprazole, spironolactone, lithium, empagliflozin, carbamazepine, sacubitril/valsartan
Mean ± SD		68.5 ± 6.7			52 ± 11		

NYHA, New York Heart Association; CCS, Canadian Cardiovascular Society; LVEF, left ventricular ejection fraction; CHD, coronary heart disease; AF, atrial fibrillation; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; ICM, ischemic cardiomyopathy

(München, Germany). Psilocin and psilocybin were purchased from LGC (Luckenwalde, Germany). All other chemicals were of the highest purity grade commercially available. Deionized water was used throughout the experiments. Stock solutions were prepared fresh daily.

3. Results

3.1. Studies on isolated atrial preparations from mice

We found that psilocin (original recordings: Fig. 2A, summarized in Fig. 2B) raised force of contraction concentration- and time-dependently in 5-HT_4-TG left atrial preparations but not in WT (Fig. 2A). However, the effect of psilocin was less potent and less effective than the effect of 5-HT (Fig. 2A, B). Accordingly, 5-HT is ineffective in the WT mouse atrium (Fig. 2A and (Gergs et al., 2013). Furthermore, psilocin shortened the time to peak tension and the time of relaxation (Fig. 2D), but these shortenings were not significant. The maximum first derivative of the developed force and the minimum first derivative (Fig. 2E) showed a similar pattern as the force (Fig. 2B). However, the maximum increases due to psilocin were smaller than those to 5-HT (Fig. 2E). Moreover, psilocin raised the beating rate concentration- and time-dependently (Fig. 2C) in right atrial preparations from 5-HT_4-TG but not in WT (data not shown).

Similarly, we found that psilocybin (original recordings: Fig. 3A, summarized in Fig. 3B) to some minor extent raised force of contraction concentration- and time-dependently (Fig. 3A) in left atrial preparation from 5-HT_4-TG (Fig. 3A, B) but not in WT (Fig. 3A). Psilocybin shortened the time of relaxation (not significantly), but apparently not the time to peak tension (Fig. 3D). The maximum first derivative of the developed force and the minimum first derivative (Fig. 3E) showed a similar pattern as the force (Fig. 3B). However, the maximum increases due to psilocybin were smaller than those to 5-HT (Fig. 3E) but also smaller than those to psilocin (compare Figs. 2 and 3). In a similar way, psilocybin raised the beating rate concentration- and time-dependently (Fig. 3C) in right atrial preparations from 5-HT_4-TG but not in WT (data not shown).

3.2. Protein phosphorylation in the mouse

If the underlying mechanisms of psilocybin and psilocin to increase force of contraction involve 5-HT_4 receptor activation, an increase of the phosphorylation state of regulatory proteins should be notable. In the case of 5-HT, we have demonstrated this in previous studies and noted an increase in the phosphorylation state of phospholamban in human and 5-HT_4-TG preparations (Gergs et al., 2009; Gergs et al., 2010). In the present study, we used a comparable approach and noted that the force, relaxation and the phosphorylation state of phospholamban showed a consistent pattern. All parameters increased in left atrial 5-HT_4-TG preparations treated with increasing concentrations of psilocybin or psilocin and then flash frozen. The original Western blot from atrial experiments demonstrates the effect on phospholamban phosphorylation (Fig. 4). Psilocybin and psilocin, each 10 μM, increased the serine 16 phosphorylation of phospholamban in the left atria of 5-HT_4-TG but not WT (Fig. 4).

3.3. Force of contraction in human atrium

In most of the human right atrial preparations used in this study, psilocin and psilocybin led to a positive inotropic effect only if cilostamide, a phosphodiesterase III inhibitor, was added to the organ bath (Figs. 5A and E). The positive inotropic effects of psilocin and psilocybin in the presence of cilostamide could be reversed by 10 μM tropisetron or

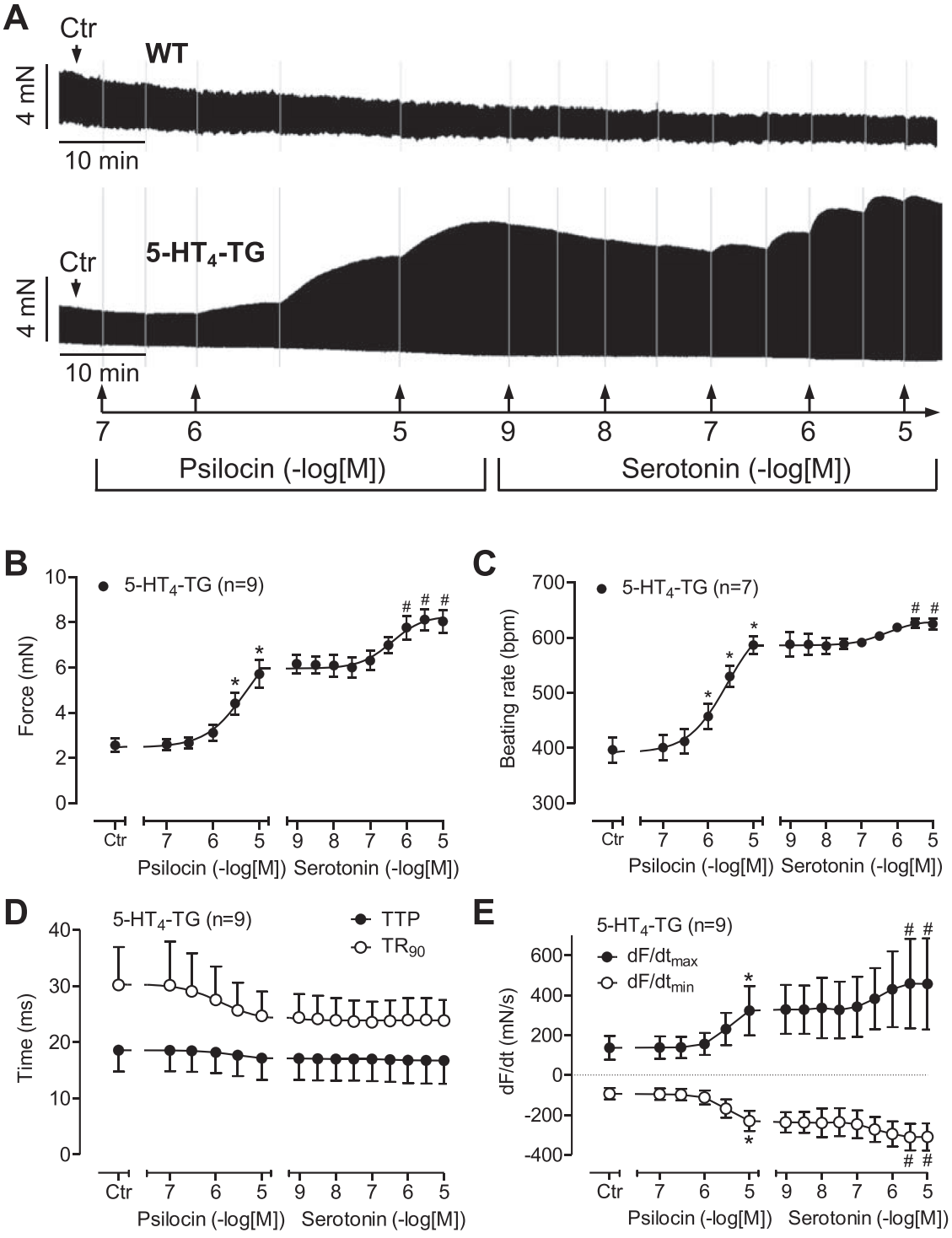


Fig. 2. Psilocin increases contractility in mice overexpressing the 5-HT_4 receptor. (A) The original recordings demonstrate a concentration- and time-dependent positive inotropic effect of psilocin and serotonin in the isolated electrically stimulated (1 Hz) left atrial preparation from 5-HT_4-TG (A, bottom) but not from WT (A, top). To test if psilocin behaves as partial or full agonist, a serotonin concentration response curve was applied in the presence of psilocin. Vertical scale bars depict measured force in milli Newton (mN). Horizontal scale bars indicate time in minutes (min). Because of the missing effects in WT, only data from 5-HT_4-TG preparations (n = 7–9) were summarized: (B) force of contraction of left atria (mN); (C) beating rate of right atria in beats per minute (bpm); (D) time to peak tension (TTP) and time of 90 % relaxation (TR_{90}) of left atria (ms); (E) maximum (dF/dt_{max}) and minimum (dF/dt_{min}) first derivative of the developed force of left atria (mN/s). *$p < 0.05$ versus pre-drug value (Ctr), #$p < 0.05$ versus 10 μM psilocin.

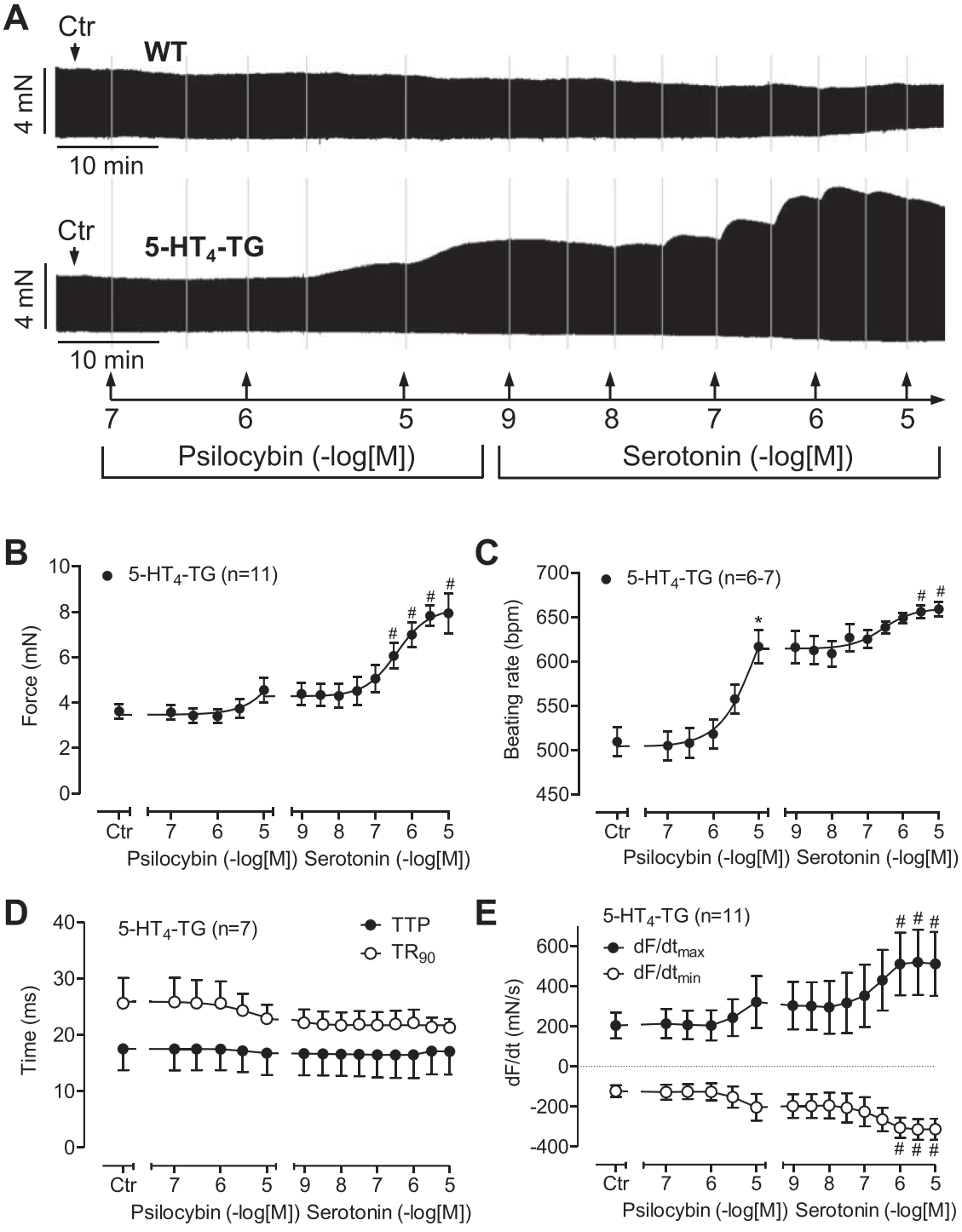
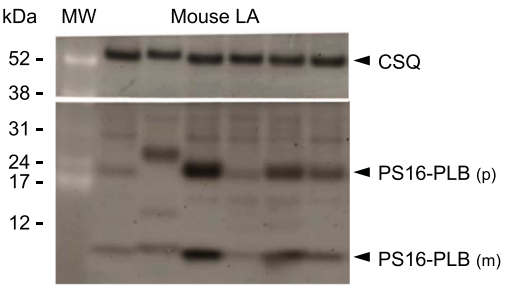


Fig. 3. Psilocybin increases contractility in mice overexpressing the 5-HT_4 receptor. (A) The original recordings demonstrate a concentration- and time-dependent positive inotropic effect of psilocybin and serotonin in the isolated electrically stimulated (1 Hz) left atrial preparation from 5-HT_4-TG (A, bottom) but not from WT (A, top). To test if psilocybin behaves as partial or full agonist, a serotonin concentration response curve was applied in the presence of psilocybin. Vertical scale bars depict measured force in milli Newton (mN). Horizontal scale bars indicate time in minutes (min). Because of the missing effects in WT, only data from 5-HT_4-TG preparations (n = 6–11) were summarized: (B) force of contraction of left atria (mN); (C) beating rate of right atria in beats per minute (bpm); (D) time to peak tension (TTP) and time of 90 % relaxation (TR_{90}) of left atria (ms); (E) maximum (dF/dt_{max}) and minimum (dF/dt_{min}) first derivative of the developed force of left atria (mN/s). *$p < 0.05$ versus pre-drug value (Ctr), #$p < 0.05$ versus 10 μM psilocybin.



LA WT		+	-	-	+	-	-
LA 5-HT$_4$-TG		-	+	+	-	+	+
Psilocin (10 μM)		+	+	+	-	-	-
Psilocybin (10 μM)		-	-	-	+	+	+

Fig. 4. Effect of psilocin and psilocybin on the phosphorylation state of phospholamban (PLB) in 5-HT$_4$-TG mice. By Western blotting, the effect of 10 μM psilocin and psilocybin on PLB serine-16 phosphorylation (PS16-PLB) in isolated paced (1 Hz) left atrium (LA) from wild type (WT) and 5-HT$_4$ transgenic (5-HT$_4$-TG) mice is shown. PLB usually can be detected in its pentameric form (p) as well as in its monomeric form (m) by Western blotting. The protein expression of calsequestrin (CSQ) was utilized as a cardiac myocytes-specific loading control. A pre-stained molecular weight marker (MW) was used to identify the molecular weight range for cutting the membrane. The uncropped Western blots can be seen in the supplementary Figure 1.

by 1 μM GR 125487, a selective 5-HT$_4$ receptor antagonist. This is seen in the original tracings in Fig. 5. Data for force of contraction are summarized and evaluated in Fig. 5B-D (Psilocin) and Fig. 5F-H (Psilocybin). These positive inotropic effects of psilocybin and psilocin were accompanied by an increase in the rate of force development and an increase in the rate of relaxation (Figs. 5C and G). Likewise, the time to peak tension was shortened under our experimental conditions at least for psilocin (Fig. 5D). Moreover, the efficiency of psilocin to increase force of contraction was more pronounced than the efficiency of psilocybin (compare Fig. 5B with F).

In a few of the human right atrial preparations, psilocin and to a much lesser extent psilocybin led to a positive inotropic effect alone, that means without addition of cilostamide (Fig. 6A). At the maximum positive inotropic effect of psilocin or psilocybin, the contracting human atrial preparations were flash frozen and the serine 16 phosphorylation of phospholamban was analyzed by Western blotting (Fig. 6B). Corresponding to the contraction data, only in psilocin-treated preparations, an increased phosphorylation state of phospholamban on serine 16 could be noted (Fig. 6B). For psilocybin-treated preparations, the effect on phospholamban phosphorylation, if any, may be below the detection limit of the method.

4. Discussion

The new finding in the present paper is that psilocin and psilocybin can induce a positive inotropic effect via cardiac human 5-HT$_4$ receptors (Fig. 7). We argue here, that the effects of psilocin and psilocybin in atrial preparations are 5-HT$_4$ mediated because they are present exclusively in 5-HT$_4$-TG but not in their WT littermate, used in parallel experiments under identical conditions as controls. Our data are consistent with the interpretation that psilocin and psilocybin, similar to metoclopramide or cisapride, which we tested in the same transgenic model (Keller et al., 2018; Neumann et al., 2021), are partial agonists on human 5-HT$_4$ receptors. Here, we also demonstrated positive inotropic effects of psilocin and psilocybin in human atrial preparations.

So-called "Magic Mushrooms" contain psilocin (4-hydroxy-N, N-dimethyltryptamine) and its prodrug psilocybin (4-phosphoryloxy-N, N-dimethyltryptamine). Both compounds are heat stable. Therefore, it is not possible to inactivate these drugs by heating or cooking the mushroom extracts. It might be noteworthy that psilocin is an isomer of the psychedelic drug bufotenin (5-hydroxy-N, N-dimethyltryptamine) found in the skin of toads and also in some plants in South America (Nichols, 2020). We have recently reported that bufotenin stimulates cardiac human 5-HT$_4$-receptors (Neumann et al., 2023). Unexpectedly, we found that psilocybin that is commonly regarded as the inactive precursor of psilocin and that has to be metabolized by alkaline phosphatases first (Horita, 1963), is already active in our model. In this context, it is known that orally taken psilocybin undergoes a first pass metabolism in the liver by alkaline phosphatases (Horita, 1963). One hypothesis could be that in the organ bath, inactive psilocybin is converted to active psilocin. However, we did not measure the concentration of psilocin or psilocybin in the atrium at the end of the experiment and thus cannot exclude the possibility that some psilocin was formed and some psilocybin was degraded. Accordingly, if psilocybin was degraded in the atrium one would expect psilocin to be more potent than psilocybin, which was the case for inotropic effects in mouse and human atria but not for chronotropic effects in mouse right atria. This argues against a relevant contribution of psilocin to the effects of psilocybin under our experimental conditions. At least it makes this hypothesis questionable. Hence, it is also possible that not the phenolic ring, but the amino moiety that is the identical part in both compounds (Fig. 1), is important for 5-HT$_4$ receptor binding. However, this is just a speculation that needs to be confirmed through further experiments like, for example, crystal structure analyses.

While it is known that psilocin and psilocybin mediate their neuronal effects via 5-HT$_2$ receptors (Halberstadt and Geyer, 2011; Nichols, 2020), we were able to show, amongst others through antagonist studies, that 5-HT$_4$ receptors are involved in the cardiac effects of these drugs. The fact that 5-HT$_4$ receptor antagonists like tropisetron or GR 125487 antagonized the effects of psilocin and psilocybin is important for two reasons. Firstly, it confirms the conclusion that psilocin and psilocybin act through 5-HT$_4$ receptors. Secondly, it opens the field to treat magic mushroom intoxication, at least cardiac effects, with an approved drug namely tropisetron. Admittedly, tropisetron also blocks 5-HT$_3$ receptors, but that may be an additional benefit, as it would have antiemetic effects via the 5-HT$_3$ receptors. An alternative would be to use selective and potent 5-HT$_4$ antagonists like GR 125487 (Gergs et al., 2013), but they are not approved for use in patients.

Clinical relevance: our data indicate that psilocin and psilocybin could, in principle, cause side effects such as tachycardia in humans by directly stimulating the 5-HT$_4$ receptors in the sinoatrial node. Especially for patients with coronary heart disease, tachycardia could be a problem because the oxygen supply to the heart could deteriorate, leading to angina and myocardial infarction. A possibility to prevent this tachycardia might be the use of the approved drug tropisetron because, as mentioned above, tropisetron inhibits (not only but also) 5-HT$_4$ receptors. Alternatively, for example if depressive patients are treated with psilocybin, addition of a β-adrenoceptor antagonist may be useful to reduce the heart rate, but this depends on the specific patient comorbidities. Currently, 58 studies for the keywords psilocybin and depression can be found on "clinicaltrials.gov" (last access 02/22/2024), for example the recently finished study of Raison et al. (2023). Foremost, one should ask whether in normal dosing, the plasma levels of psilocin or psilocybin are high enough to activate cardiac 5-HT$_4$ receptors. For example, application of 30 mg psilocybin led to maximum plasma levels of psilocin of about 0.1 μM (Madsen et al., 2019). However, this concentration is below any contractile or chronotropic effects noted here. Moreover, in the mouse model, we did not find any pro-arrhythmic effects in spontaneously beating right atria, especially at the relevant low concentrations like 0.1 μM. On the other hand, we have previously reported that the positive inotropic effect of 5-HT in 5-HT$_4$-TG is potentiated by phosphodiesterase inhibition (Neumann et al., 2019). Because phosphodiesterase inhibitors, such as milrinone, levosimendan, roflumilast, theophylline or caffeine, are used clinically or otherwise, we would speculate that taking phosphodiesterase

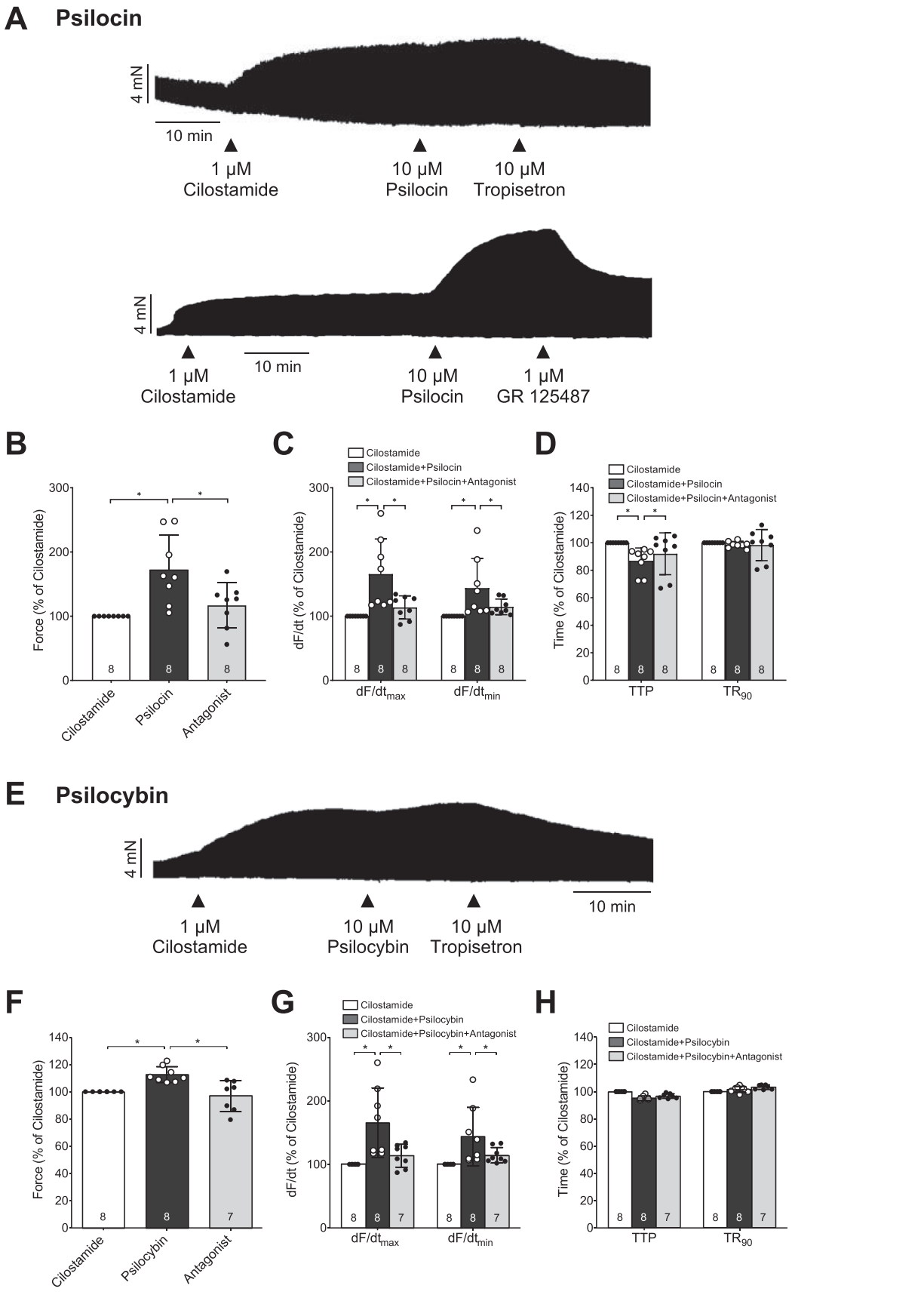


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Fig. 5. Psilocin and psilocybin increase the contractility in human atria. Representative original recordings of positive inotropic effects of (A) psilocin and (E) psilocybin in isolated electrically stimulated (1 Hz) human right atrial preparations. In most patients, a pre-stimulation by the phosphodiesterase III inhibitor cilostamide was necessary to unmask an inotropic effect of psilocin or psilocybin. The positive inotropic effects of psilocin and psilocybin were antagonized by tropisetron as well as by GR 125487 and, therefore, regarded as 5-HT_4-receptor mediated. Because of the high variability of the human preparations, the summarized data (B-D: psilocin; F-H: psilocybin) were normalized to the effect of cilostamide: (B and F) force of contraction, (C and G) maximum (dF/dt_{max}) and minimum (dF/dt_{min}) first derivative of the developed force, (D and H) time to peak tension (TTP) and time of 90 % relaxation (TR_{90}). The effects of tropisetron and GR 125487 were comparable and, therefore, are presented combined as antagonist. *$p < 0.05$ as indicated by brackets. The numbers in the bars of the diagrams indicate the evaluated preparations.

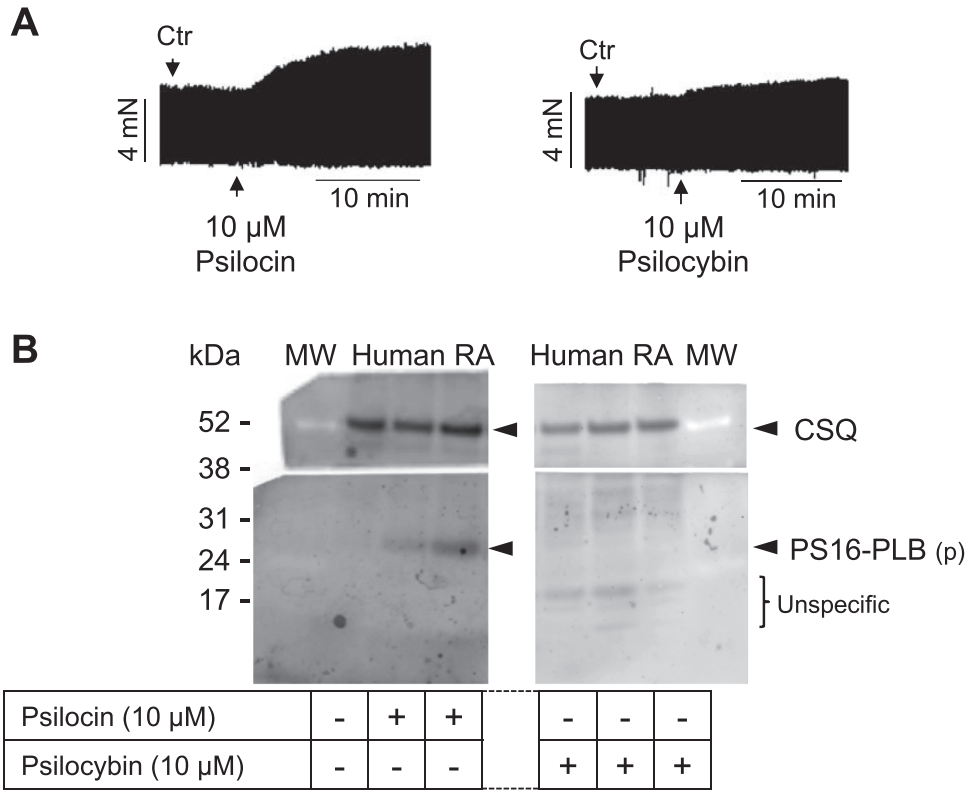

Fig. 6. Effect of psilocin and psilocybin on the phosphorylation state of phospholamban (PLB) in human atria. (A) Representative original recordings of positive inotropic effects of 10 µM psilocin and psilocybin on isolated paced (1 Hz) human right atrial preparations (RA). Please note the different efficiency of psilocin and psilocybin. (B) By Western blotting, the effect of 10 µM psilocin alone, but not the effect of psilocybin alone on PLB serine-16 phosphorylation (PS16-PLB) in human RA could be demonstrated. PLB usually can be detected in its pentameric form (p) as well as in its monomeric form (here not detectable) by Western blotting. As a cardiac myocytes-specific loading control, we used the protein expression of calsequestrin (CSQ). A pre-stained molecular weight marker (MW) was used to identify the molecular weight range for cutting the membrane. The uncropped Western blots can be seen in the supplementary Figure 2.

inhibitors also potentiates the effects of psilocin. Here, we have demonstrated this for cilostamide that is like milrinone a phosphodiesterase III inhibitor. Moreover, because psilocin is degraded by monoamine oxidases (Blei et al., 2020), the risk occurs that in depressive patients taking monoamine oxidase inhibitors, such as moclobemide, selegiline or tranylcypromine, plasma concentrations of psilocin may increase leading to 5-HT_4 receptor-mediated cardiac side effects. A limitation of the study is that we cannot predict the risk of tachycardia in humans because we were unable to obtain human right atrial preparations, for example from explanted hearts, with an intact sinoatrial node. But it is precisely this point that would be of interest, because it appears that the sinoatrial node is about ten times more sensitive to stimulation of the 5-HT_4 receptor compared to the working myocardium (compare Figs. 2B and C or Figs. 3B and C) at least in 5-HT_4-TG mice. Our conclusion regarding cardiac side effects via cardiac 5-HT_4 receptors is that psilocybin or psilocin should be considered safe in therapeutic concentrations, at least with regard to contractile effects. The occurrence of tachycardia, on the other hand, seems entirely possible, but this should be shown by the clinical studies currently underway. Another limitation with regard to the human preparations is that they only came from patients with heart diseases. As shown in Table 1, all patients were treated with medications that affect blood pressure and heart rate. Therefore, the effects of psilocin and psilocybin may be different in heart-healthy patients. Unfortunately, it was not possible to obtain healthy control samples in this study.

In summary, in our 5-HT_4-TG mouse model, we detected cardiac inotropic and chronotropic effects of psilocin and psilocybin. These drugs are taken for recreationally, religiously and clinically purpose, but effects on the heart are not intended. More importantly, we confirmed the results of psilocin and psilocybin in the mouse model with human preparations. Therefore, under certain circumstances there is a risk of cardiac side effects when taking psilocin or psilocybin.

Authorship contribution statement

J.N. and U.G. conceived and designed the research; K.D. and K.A. conducted experiments; K.D., K.A. and U.G. analyzed data; B.H. supplied material and clinical data; J.N. wrote the first draft; J.N. and U.G. wrote and revised the manuscript.

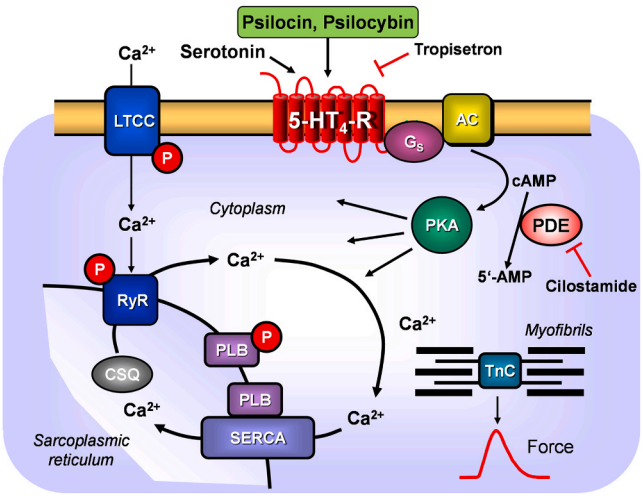

Fig. 7. Suggested mechanism of psilocin and psilocybin signaling via cardiac 5-HT_4 serotonin receptors (5-HT_4-R). The effects of psilocin and psilocybin are transmitted via cAMP-dependent phosphorylation of regulatory proteins and are terminated by phosphodiesterases. Further abbreviations: AC, adenylyl cyclase; cAMP, 3′-5′cyclic adenosine-phosphate; CSQ, cardiac calsequestrin; Gs, stimulatory G-protein; LTCC, L-type Ca^{2+} channel; PDE, phosphodiesterase; PLB, phospholamban; PKA, cAMP-dependent protein kinase; P, phosphorylation; RYR, cardiac ryanodine receptor; SR, sarcoplasmic reticulum; SERCA, SR-Ca^{2+} ATPase; TnC, C subunit of troponin.

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CRediT authorship contribution statement

Joachim Neumann: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Ulrich Gergs:** Writing – review & editing, Visualization, Formal analysis, Conceptualization. **Karyna Azatsian:** Formal analysis, Data curation. **Britt Hofmann:** Resources. **Kiril Dimov:** Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Consent for publication

All authors approved the final manuscript for publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2024.06.006.

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