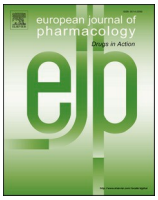
European Journal of Pharmacology 901 (2021) 174074

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Cardiovascular effects of metoclopramide and domperidone on human 5-HT$_4$-serotonin-receptors in transgenic mice and in human atrial preparations

Joachim Neumann [a], Tom Seidler [a], Charlotte Fehse [a], Margaréta Marušáková [a,c], Britt Hofmann [b], Ulrich Gergs [a,*]

[a] Institut für Pharmakologie und Toxikologie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, D-06097, Halle, Germany
[b] Cardiac Surgery, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, D-06097, Halle, Germany
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia

ARTICLE INFO

Keywords:
Serotonin
5-HT$_4$ receptor
Inotropy
Chronotropy
Transgenic mice

ABSTRACT

It is unclear whether metoclopramide and domperidone act on human cardiac serotonin 5-HT$_4$-receptors. Therefore, we studied transgenic mice that only express the human 5-HT$_4$ receptor in cardiomyocytes in the atrium and in the ventricle (5-HT$_4$-TG), their wild type-littermates (WT) and isolated human atrial preparations. We found that only metoclopramide but not domperidone enhanced the force of contraction in left atrial preparations (pEC$_{50}$ = 6.0 ± 0.1; n = 7) from 5-HT$_4$-TG, isolated spontaneously beating right atrial preparations (pEC$_{50}$ = 6.1 ± 0.1; n = 7) from 5-HT$_4$-TG, Langendorff perfused hearts from 5-HT$_4$-TG, living 5-HT$_4$-TG and human right atrial muscle preparations obtained during bypass surgery of patients suffering from coronary heart disease. The maximum inotropic effect of metoclopramide was smaller (81 ± 2%) than that of 5-HT on the left atria from 5-HT$_4$-TG. The maximum increase in the beating rate due to metoclopramide was 93 ± 2% of effect of 5-HT on right atrial preparations from 5-HT$_4$-TG. Metoclopramide and domperidone were inactive in WT.

We found that metoclopramide but not domperidone increased the phosphorylation state of phospholamban in the isolated perfused hearts or muscle strips of 5-HT$_4$-TG, but not in WT. Metoclopramide, but not domperidone, shifted the positive inotropic or chronotropic effects of 5-HT in isolated left atrial and right atrial preparations from 5-HT$_4$-TG dextrally, resp., to higher concentrations: the pEC$_{50}$ of 5-HT for increase in force was in the absence of metoclopramide 8.6 ± 0.1 (n = 5) versus 8.0 ± 0.3 in the presence of 1 μM metoclopramide (n = 5; P < 0.05); and the beating rate was 7.8 ± 0.2 (n = 7) in the absence of metoclopramide versus 7.2 ± 0.1 in the presence of 1 μM metoclopramide (n = 6; P < 0.05). These results suggested that metoclopramide had an antagonistic effect on human cardiac 5-HT$_4$ receptors. In summary, we showed that metoclopramide, but not domperidone, was a partial agonist at human cardiac 5-HT$_4$-receptors.

1. Introduction

Serotonin (5-HT) induces a positive inotropic effect and a relaxant effect in the human heart via human 5-HT$_4$ receptors (Kaumann and Levy 2006; Neumann et al., 2017). Studies on isolated pig heart preparations, anaesthetised pigs and isolated porcine atrial preparations have found that 5-HT can increase force and frequency via porcine 5-HT$_4$ receptors (Kaumann 1990; Villalón et al., 1990). Only in human and pig but not in other mammalian hearts, 5-HT can augment force and beating rate via 5-HT$_4$-receptors. In order to have a small laboratory animal model of the human 5-HT$_4$-receptor, we have used a transgenic mouse containing the human 5-HT$_4$ receptor in cardiomyocytes (5-HT$_4$-TG). The 5-HT could elevate force in atrial and ventricular cardiac preparations of 5-HT$_4$-TG, but not in cardiac preparations of wild-type mice (Gergs et al., 2010, 2013, 2017a,b; Keller et al., 2018; Neumann et al., 2019).

The aim of the present work was to gain further insight into the cardiac effects of two putative 5-HT$_4$ receptor agonists: metoclopramide

* Corresponding author. Institut für Pharmakologie und Toxikologie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, Magdeburger Str. 4, 06112, Halle, Germany.
E-mail address: ulrich.gergs@medizin.uni-halle.de (U. Gergs).

https://doi.org/10.1016/j.ejphar.2021.174074
Received 25 December 2020; Received in revised form 23 March 2021; Accepted 26 March 2021
Available online 31 March 2021
0014-2999/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

(methoxychloroprocainamide) and domperidone. Metoclopramide is probably world-wide used because it is on the WHO list of essential drugs (WHO model list 2019). Hence, a better understanding of its cardiac side effects is of clinical interest. Alone in Germany in the year 2019 about 27.5 million defined daily doses of metoclopramide and 8.4 million defined daily doses of domperidone were prescribed through the national health insurance systems (Schwabe and Ludwig 2020). The indications have been called "less than clearcut" (Schwabe and Ludwig 2020). Hence, one could argue that there is over-prescription of these two drugs which are of doubtful benefit and thus side effects should be very rare, if one really wants to keep them in the market. The structural chemical formula of metoclopramide was based on procainamide and developed as an antiarrhythmic drug (Schulze-Delrieu et al., 1981). However, it was not useful as an antiarrhythmic drug even in animal studies (Cheymol and Mouillé 1975), although it was found to act as a prokinetic and antiemetic drug (Schulze-Delrieu et al., 1981). Metoclopramide passes through the blood–brain barrier and blocks the dopamine D_1, D_2 and D_3 receptors (Grenier und Drolet 2003; Renoux et al., 2016; Lertxundi et al., 2013), although it is ineffective as a neuroleptic drug (Schulze-Delrieu et al., 1981). By blocking D_2-dopamine receptors in the central nervous system, metoclopramide can cause parkinsonism (Grenier und Drolet 2003; Renoux et al., 2016; Lertxundi et al., 2013) and also could increase prolactin and aldosterone levels in peripheral blood (Sala et al., 1988, Schulze-Delrieu et al., 1981). Indeed, intravenous (i.v.) injection of 10 mg metoclopramide elevates aldosterone levels in patients (Jungmann et al., 1985; Matsuoka et al., 1991; Bentsen and Stubhaug. 2002; Tung et al., 2002; Eberhart et al., 2002; Al-Shaer et al., 2015) which might be a harmful side effect.

The antiemetic effect of metoclopramide is usually explained by blockade of the 5-HT_3 receptors in the brainstem (Schulze-Delrieu et al., 1981). Metoclopramide can stimulate 5-HT_4 receptors in the stomach and gut and this agonism is thought to explain its therapeutic usefulness for enteral motility diseases (Tonini et al., 1999).

Domperidone was developed in 1974 as an antiemetic drug that did not pass through the blood–brain barrier and, thus, would not produce unwanted anti-parkinsonoid-like effects on the central nervous system (Champion et al., 1986). Domperidone blocks dopamine D_2 receptors in the brain and stimulates the cardiac beating rate in pigs by acting as an agonist on 5-HT_4 receptors (Villalón et al., 1990). Likewise, metoclopramide can stimulate cardiac 5-HT_4 receptors in pigs (Medhurst and Kaumann 1993).

In this study, we tested the hypothesis that metoclopramide and domperidone act as agonists and/or antagonists on human cardiac 5-HT_4 receptors. A progress report of this work has been previously published in abstract form (Seidler et al., 2020).

2. Materials and methods

2.1. Transgenic mice

A mouse with cardiomyocyte-specific expression of the human 5-$HT_{4(a)}$ receptor has been generated in our laboratories (Gergs et al., 2010). The cardiac myocyte-specific expression was achieved by the use of the α-myosin heavy chain promoter. The age of the animals studied in the atrial contraction experiments was around 154 days. All mice were housed under conditions of optimum light, temperature and humidity with food and water provided ad libitum. The animals were handled and maintained according to the approved protocols of the Animal Welfare Committee of the University of Halle-Wittenberg, Halle, Germany (Approval number 42502-2-1600-MLU).

2.2. Contractile studies in mice

In brief, the right or left atrial preparations from the mice were isolated and mounted in organ baths as previously described (Gergs et al., 2013; Neumann et al., 2003). The bathing solution of the organ baths contained 119.8 mM NaCl, 5.4 mM KCl, 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, 2003; Kirchhefer et al., 2004). Spontaneously beating right atrial preparations from mice were used to study any chronotropic effects.

The drug application (Fig. 1) was as follows and is depicted in Fig. 1A: we performed a cumulative concentration-response curve with 1 nM–10 μM metoclopramide and 10 μM metoclopramide (MCP) was kept in the organ bath until complete stabilization which took 10 min. Thereafter, and without any washout, 1 nM to 10 μM 5-HT was cumulatively applied to the organ baths containing the atrial preparations. Finally, after the response to 10 μM 5-HT stabilized and without any washout, 1 μM isoprenaline was added to test whether the efficacy of 10 μM 5-HT was different from the efficacy of 1 μM isoprenaline. The same procedure was followed using domperidone instead of metoclopramide (Fig. 1B). In a further experimental design (Fig. 1C), either no MCP (control) or 0.3 μM MCP or 1 μM MCP were applied to three different atrial preparations to the organ bath. After stabilization, 5-HT was cumulatively additionally applied (1 nM- 1 μM). After a complete washout of 5-HT, 1 nM to 10 μM 5-HT was again cumulatively applied (Fig. 1C). The second application of 5-HT was a control for desensitisation: we had reported that after an initial concentration response curve to 5-HT and complete wash out of 5-HT (or generally a 5-HT_4-receptor-agonist), a second concentration response curve to 5-HT was dextrally shifted, indicative of homologous desensitisation (Gergs et al., 2017a). This desensitisation was expected to occur if the first concentration response curve was done with no addition of metoclopramide. But it was conceivable that initial treatment with a high single concentration of MCP (0.3 or 1 μM) might lead to desensitisation (Fig. 1C).

2.3. Contractile studies on human preparations

The contractile studies on human preparations were done using the same setup and buffer as used in the mouse studies (see section 2.2). The samples were obtained from male patients aged 63–75 years. Drug therapy included metoprolol, furosemide, apixaban and acetyl salicylic acid. 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, 2017b, 2018; Boknik et al., 2019).

2.4. Western blotting

The homogenization of the samples, protein measurements, electrophoresis, primary and secondary antibody incubation and quantification were performed following our previously established protocols (Gergs et al., 2009, 2019a,b; Boknik et al., 2018).

2.5. Echocardiography

Echocardiography was performed by following our previously published methods (Boknik et al., 2019; Gergs et al., 2018). Anaesthesia of the mice was induced using an inhalation of isoflurane (Forene®, AbbVie, USA). Then 1 mM metoclopramide (metoclopramide hydrochloride, Sigma-Aldrich Chemie GmbH, Germany), 1 mM 5-HT (5-hydroxytryptamine hydrochloride, Sigma-Aldrich Chemie GmbH, Germany) as a control and 1 mM isoprenaline (Isoproterenol bitartrate salt, Sigma-Aldrich Chemie GmbH, Germany) were intravenously injected. The heart was visualized using the Vevo 2100 Linear Imaging System (VisualSonics Inc., Canada).

2.6. Langendorff hearts

We followed previously reported methods for the treatment of the animals. This included the removal of the heart and lungs from the

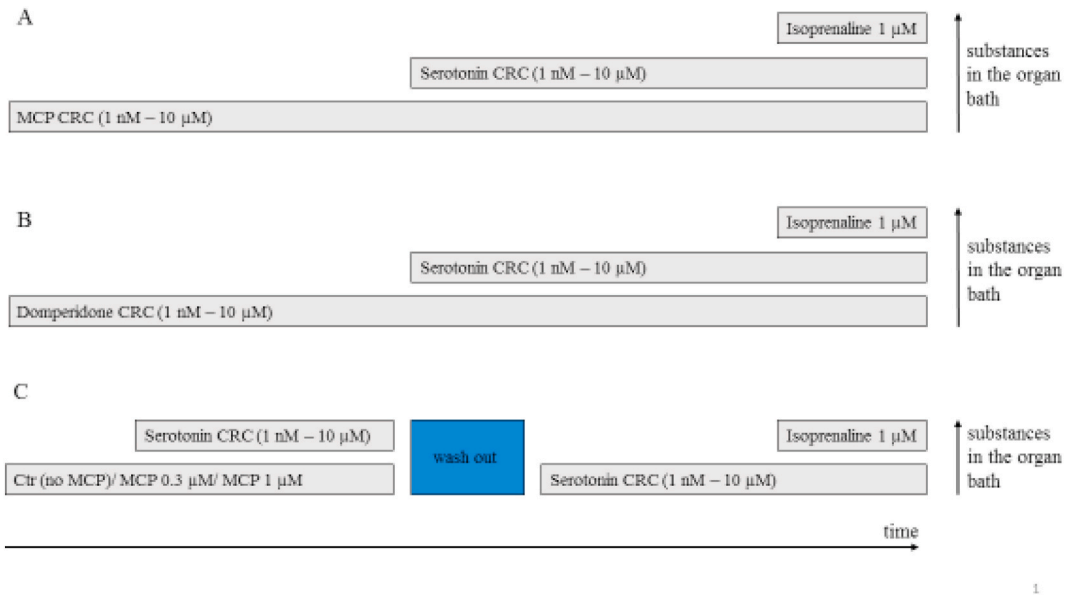


Fig. 1. Schematic representation of the sequential drug additions in this study. Protocol A: first a cumulative concentration response (CRC) curve to metoclopramide (MCP) was constructed in right and left atrial preparation from mouse hearts. In the presence of 10 µM metoclopramide, serotonin was cumulative applied. Finally, in addition 1 µM isoprenaline was added. This was done to assess the efficacy of metoclopramide compared to isoprenaline.
Protocol B: first a cumulative concentration response curve to domperidone was constructed. In the presence of 10 µM domperidone, serotonin was cumulative applied. Finally, in addition 1 µM isoprenaline was added. This was done to assess the efficacy of domperidone compared to isoprenaline.
Protocol C: here, three different atria were studied. One without drug addition (Ctr), one with 0.3 µM metoclopramide, the third group with 1 µM metoclopramide. Then serotonin was in addition added in cumulative manner. Thereafter, atria were washed out of drugs. Then serotonin alone was given in cumulative manner and finally isoprenaline was applied.

thoracic cavity, placing the dissected heart onto a cannula, the use of custom-made equipment, the buffer composition, quantification of the force from the left ventricular apex, digitization of the recordings and freeze clamping the hearts (Boknik et al., 2019; Gergs et al., 2019c).

2.7. Data analysis

Data shown are means ± standard error of the mean. Statistical significance was estimated using the analysis of variance followed by Bonferroni's *t*-test or the χ^2 test as appropriate. A P-value < 0.05 was considered to be significant.

2.8. Drugs and materials

The drugs isoprenaline (bitartrate salt), serotonin (5-HT) hydrochloride, domperidone and metoclopramide hydrochloride were purchased from Sigma-Aldrich (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 in isolated left atria from mice

We showed before, that serotonin (5-HT) increases the force of contraction in the atria from 5-HT_4-TG, but not in the atria from wild type litter mate control mice (WT: Gergs et al., 2013). As a next step, we wanted to extend those data and to determine whether metoclopramide and domperidone also exerted positive inotropic effects in 5-HT_4-TG. We found that metoclopramide (Fig. 2A and C) raised force in a concentration- and time-dependent manner (for experimental design see Fig. 1A); these results are summarized in Fig. 3A. However, 1 nM to 10 µM domperidone, cumulatively applied (for experimental design see Fig. 1B) in the organ bath (Fig. 2B), left force of contraction unchanged in the electrically driven left atrial preparations from TG mice (see Fig. 2B for an original recording and summarized data are shown in Fig. 3A). The contractile effects were also quantified in single atrial muscle contractions plotted at a high time resolution (Fig. 2C). Moreover, in isolated electrically driven left atrial preparations from WT, metoclopramide and domperidone could not alter contractility (Fig. 2A and B original recordings, plotted data in Fig. 3A). In addition, in left atrium from 5-HT_4-TG, metoclopramide shortened the time to peak tension (Fig. 3B), which was further reduced by the addition of serotonin (Fig. 3B). In contrast, metoclopramide did not alter the time of tension development in WT (Fig. 3B). Domperidone did not shorten the time to peak tension in 5-HT_4-TG or in WT (Fig. 3B). In left atrium from 5-HT_4-TG, metoclopramide also shortened the time of tension relaxation (Fig. 3C), which was further substantially shortened by the addition of serotonin (Fig. 3C). Metoclopramide failed to shorten the time of relaxation in WT (Fig. 3C). Domperidone did not shorten the time of relaxation in 5-HT_4-TG or in WT (Fig. 3C).

The first derivate of the developed force, namely the maximum rate of tension development (Fig. 3D), and the minimum rate of tension development (Fig. 3E) showed qualitatively a similar pattern as the force of contraction in milli Newton (Fig. 3A). However, the increase in rate of tension development due to the metoclopramide was much more pronounced (as percent of pre-drug value) than the increase in force due to metoclopramide. Domperidone (Dom) did not affect the rate of tension development (Fig. 3D) or the rate of tension relaxation (Fig. 3E).

We also wanted to determine, whether metoclopramide (MCP) or domperidone could attenuate the positive inotropic effect of 5-HT. In other words, whether metoclopramide or domperidone acted also as antagonist at 5-HT_4–receptors, which would be typical for partial agonists manner (for experimental design see Fig. 1C). In separate experiments, either only buffer or 300 nM metoclopramide or 1 µM were administered (see also Fig. 1C for experimental design). Both 300 nM metoclopramide (Fig. 4A) or 1 µM metoclopramide (Fig. 4B), in this protocol non cumulatively applied, exerted a positive inotropic effect. When additionally, serotonin was cumulatively applied (for experimental design see Fig. 1C), the effects of 5-HT were dextrally shifted,

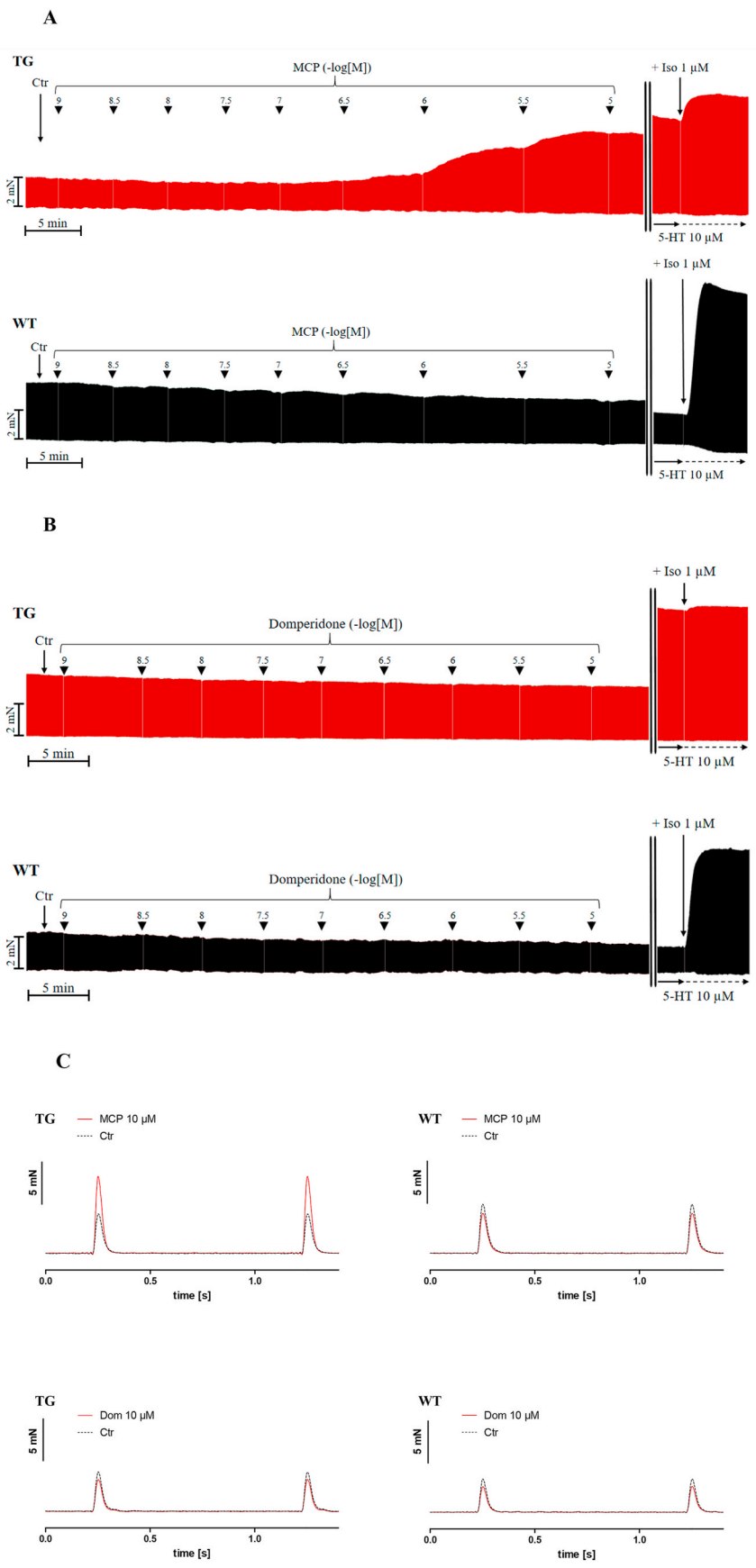


Fig. 2. Original mechanical recordings in left atrium
(**A**) Effect of cumulatively applied metoclopramide (MCP) on force of contraction in isolated electrically driven (1 Hz) left atrium of 5-HT_4 receptor overexpressing mice (TG, top) or wild type mice (WT, bottom).
(**B**) Effect of cumulatively applied domperidone on force of contraction in isolated electrically driven (1 Hz) left atrium of 5-HT_4receptor overexpressing mice (top) or wild type mice (WT, bottom). (**C**) Single contractions in the presence of metoclopramide (MCP, top) or domperidone (Dom, bottom) in isolated electrically driven (1 Hz) left atrium of 5-HT_4-receptor overexpressing mice (TG, left) or wild type mice (WT, right hand side).
Vertical bars indicate force of contraction in milli Newton (mN) and horizontal bars represent time in minutes (min) or seconds (s). At the end of each experiment isoprenaline was applied (compare Fig. 1 for details).

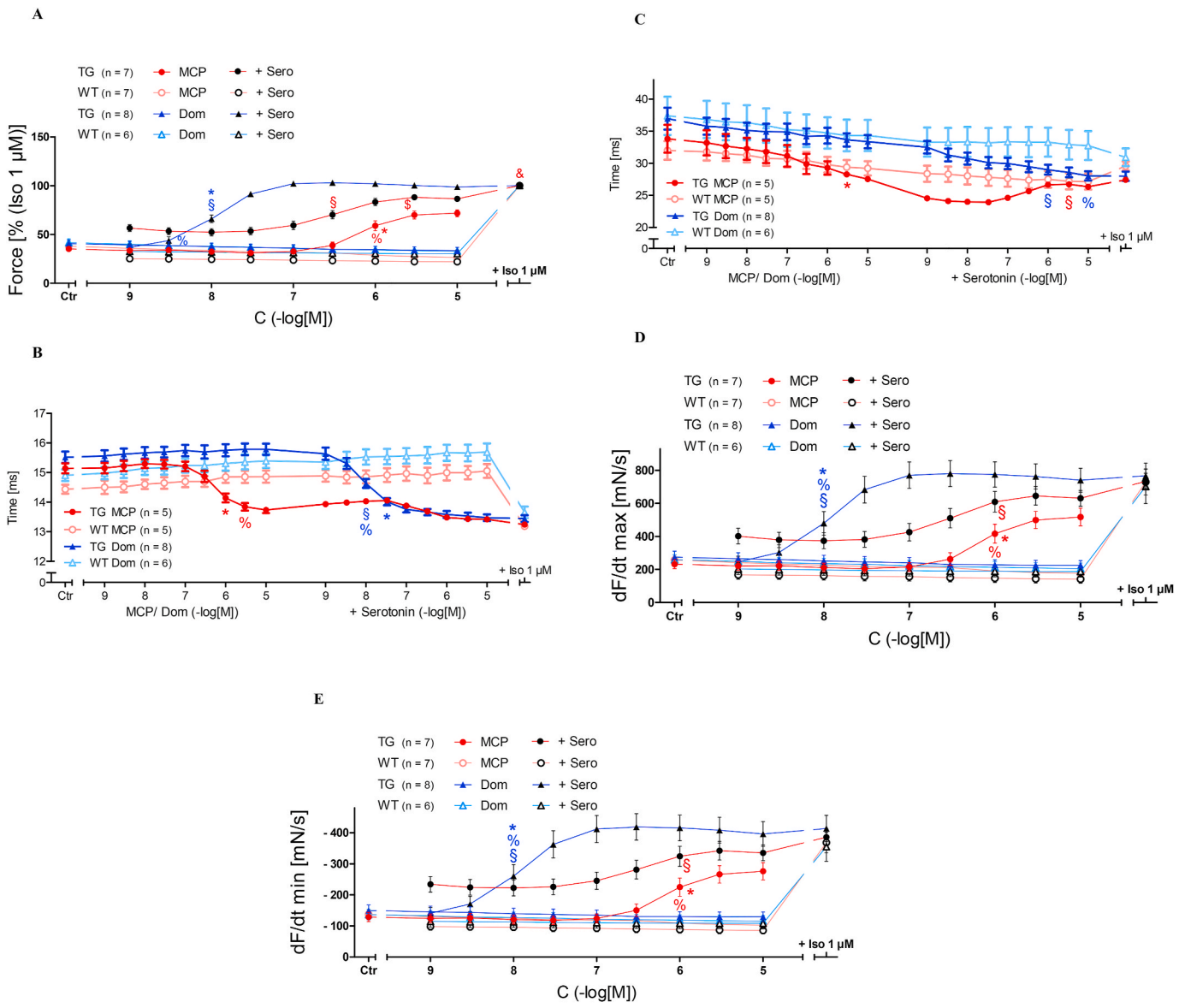


Fig. 3. Summarized left atrial contraction results

Effects of metoclopramide (MCP) or domperidone (Dom) on force of contraction (**A**) in percent of the force induced by 1 µM isoprenaline, time to peak tension (**B**), time of relaxation (**C**), maximum rate of tension development (**D**) and minimum rate of tension relaxation (**E**) in isolated electrically driven (1 Hz) left atrium of 5-HT_4-receptor overexpressing mice or wild type mice. First metoclopramide or domperidone were cumulatively applied (see Fig. 1A and B), then serotonin (Sero) concentrations were cumulatively applied; numbers in brackets indicate number of experiments. Basal force of contraction amounted to 4.2 ± 0.2 mN. Pre-drug time to peak tension amounted to 15.06 ± 0.12 ms. Pre-drug time of relaxation amounted to 35.4 ± 1.1 ms. Pre-drug maximum rate of tension development amounted to 256 ± 15 mN/s. Pre-drug minimum rate of tension development amounted to −138 ± 8 mN/s. Ctr, pre-drug contraction; Iso, isoprenaline (1 µM, maximum β-adrenergic stimulation). *indicates first significant difference ($P < 0.05$) vs. Ctr; $^{\%}$first significant difference ($P < 0.05$) vs. WT; §indicates first significant difference ($P < 0.05$) vs. 5-HT 1 nM; $^{\$}$indicates significant difference ($P < 0.05$) vs. MCP 10 µM; $^{\&}$indicates significant difference ($P < 0.05$) vs. 5-HT 3 µM. Abscissae indicate negative decadic logarithm of drug concentration.

that is to higher concentrations of serotonin (Fig. 4A and B, Table 1). For comparison a second concentration response curve to 5-HT after washout (open circles, Fig. 4A and B) was added (Fig. 1C for experimental design. This was done to assess the shift in the curves to 5-HT due to putative receptor desensitisation. Indeed, under control conditions the second concentration response curve to 5-HT was dextrally shifted in comparison to the first concentration response curve to 5-HT (open squares in Fig. 4A and B, Table 1 for statistical evaluation of potencies) which is in line with our previous reports under the very same experimental conditions (Gergs et al., 2017a).

Both metoclopramide and 5-HT were less effective than isoprenaline to raise force of contraction (Fig. 3A): The efficacies of metoclopramide and 5-HT were 59% ± 5% and 86% ± 1%, respectively, of that of isoprenaline (Fig. 3A). Metoclopramide was also less effective and less potent than 5-HT for the time to peak tension (Fig. 3B) and the effects on the rate of tension development (Fig. 3D) and the rate of relaxation (Fig. 3E, Table 1). Isoprenaline was also more effective than metoclopramide and 5-HT in reducing the time to peak tension (Fig. 3B, Table 1).

3.2. *Langendorff hearts*

Isolated hearts were prepared according to the Langendorff method. In this study, we found that 10 µM metoclopramide increased the force of contraction in isolated Langendorff hearts in 5-HT_4-TG from 12.5 ± 0.72 mN to 19.3 ± 1.22 mN, the rate of tension development from 411 ±

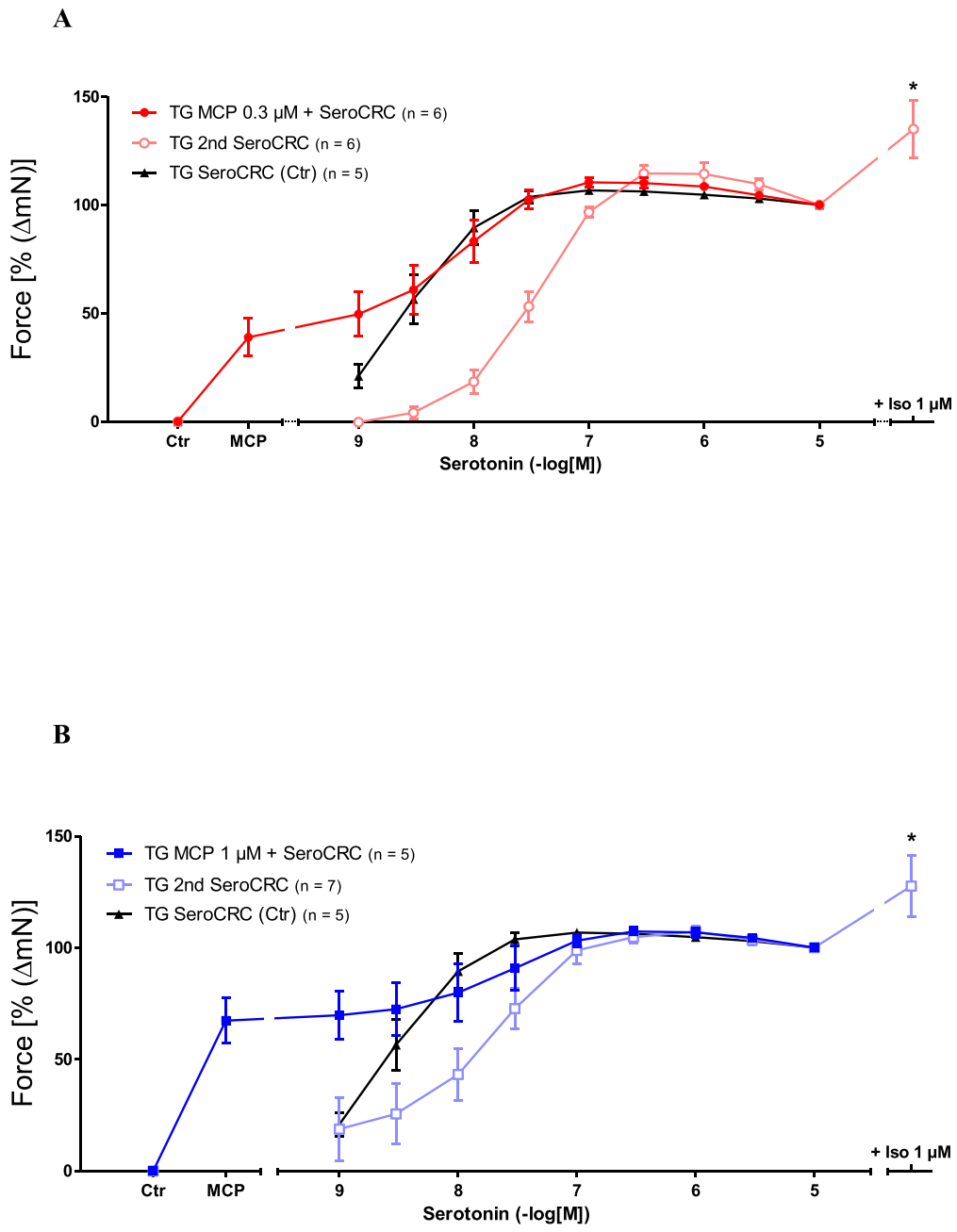


Fig. 4. Antagonistic effects in left atrium. Effect of metoclopramide (MCP) or domperidone (Dom) on force of contraction in isolated electrically driven (1 Hz) left atrium from 5-HT_4 receptor overexpressing mice (TG). Buffer alone or 0.3 µM metoclopramide (**A**) or 1 µM metoclopramide (**B**) were given and then additionally serotonin was cumulatively applied (see Fig. 1C: left hand side); numbers in brackets indicate number of experiments. Basal force of contraction amounted to 5.5 ± 0.5 mN (**A**)/5.6 ± 0.4 mN (**B**). Ctr, basal contraction; Iso, isoprenaline (1 µM, maximum β-adrenergic stimulation). *indicates significant difference ($P < 0.05$) vs. Sero (10 µM). Ordinates indicate change in force of contraction in percent of the effect of 10 µM serotonin. Abscissae in indicate pre-drug values (Ctr), effect of 300 nM MCP (**A**) or 1 µM MCP (**B**) and the decadiac logarithm of the applied final concentration of serotonin in the organ bath. CRC, concentration response curve.

33 mN/s to 700 ± 61 mN/s and the rate of tension relaxation from 341 ± 22 mN/s to 682 ± 90 mN/s (n = 5; P < 0.05). However, metoclopramide was inactive in Langendorff hearts from WT mice (data not shown, n = 3).

3.3. Echocardiography

An injection of 100 µl solution of 1 mM metoclopramide into the peritoneum of mice (to ensure complete absorption and rapid distribution of drugs) increased the left ventricular ejection fraction in anaesthetised 5-HT_4-TG but not in WT (Fig. 5). Domperidone failed to alter ejection fraction (data not shown). The left ventricular ejection fraction in echocardiography is an established non-invasive parameter which is widely used to measure of left ventricular contractility in mice and humans.

3.4. Studies in isolated right atria from mice

Next, we wanted to know whether metoclopramide or domperidone had an effect on the beating rate in 5-HT_4-TG or WT. Similar to serotonin, metoclopramide (for experimental design see Fig. 1A) did not alter the beating rate in the atrium of WT (Fig. 6A and B). However, 1

Table 1
Negative logarithmic EC_{50} -values of receptor agonists in isolated atrial preparations from transgenic (TG) mice.

	Force		Beating rate		TPT	
	-logEC_{50}	n	-logEC_{50}	n	-logEC_{50}	n
Isoprenaline	7.6 ± 0.1	6	8.5 ± 0.1[e]	7	–	–
5-HT	8.6 ± 0.1[a]	5	7.8 ± 0.2[ae]	7	–	–
Metoclopramide (MCP)	6.0 ± 0.1[ab]	7	6.1 ± 0.1[ab]	7	6.1 ± 0.1	7
5-HT (after MCP CRC)	6.5 ± 0.1[ab]	7	6.2 ± 0.2[ab]	6	6.5 ± 0.1[c]	7
5-HT (after Dom CRC)	8.0 ± 0.1[bcd]	8	7.7 ± 0.1[acd]	7	8.0 ± 0.1[cd]	8
5-HT (after MCP 3 × 10^{-7}M)	8.4 ± 0.2[acd]	6	7.5 ± 0.1[acde]	4	–	–
5-HT (after MCP 10^{-6}M)	8.0 ± 0.3[cd]	5	7.2 ± 0.1[abcd]	6	–	–
2nd 5-HT	8.0 ± 0.1[cd]	5	7.5 ± 0.1[acde]	7	–	–
2nd 5-HT (MCP 3 × 10^{-7}M)	7.6 ± 0.1[bcd]	6	7.0 ± 0.2[abcd]	5	–	–
2nd 5-HT (MCP 10^{-6}M)	7.7 ± 0.1[bcd]	7	7.0 ± 0.1[abcde]	5	–	–

One way ANOVA: [a]P < 0.05 vs. isoprenaline, [b]P < 0.05 vs. 5-HT, [c]P < 0.05 vs. metoclopramide, [d]P < 0.05 vs. 5-HT (after MCP concentration response curves = CRC), [e]P < 0.05 vs. Force was measured in left atrium, beating rate was determined in right atrium,; n = number of animals. Dom = domperidone; TPT = time to peak tension.

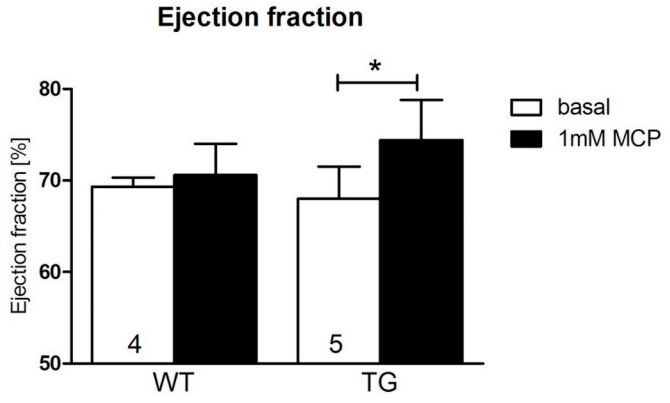


Fig. 5. Echocardiography. Effect of metoclopramide (MCP, 100 µl injected of a 1 mM MCP solution) on left ventricular ejection fraction in wild type mice (WT) and 5-HT_4 receptor overexpressing mice (TG) compared to pre-drug values. Results are shown as average ± S.E.M (n_{WT} = 4, n_{TG} = 5), ⋆ P < 0.05 vs. pre-drug.

nM to 10 µM domperidone cumulatively applied decreased the beating rate in the atrium of WT manner (for experimental design see Fig. 1B). Original recordings are shown in Fig. 6A, and several experiments are summarized in Fig. 6B. Furthermore, domperidone had, similar to WT, a negative chronotropic effect also on the atrial preparations of 5-HT_4-TG (Fig. 6A and B). In contrast to domperidone, metoclopramide exerted concentration- and time-dependent positive chronotropic effects on spontaneously beating right atrial preparations from 5-HT_4-TG (the original recording is shown in Fig. 6A and several experiments have been collected in Fig. 6B). Serotonin, as was to be expected, increased the beating rate in a concentration-dependent manner with a plateau reached at about 1 µM 5-HT (Fig. 6B). Fig. 6A and (upper traces) show the typical recordings from the right atrial preparations, the positive chronotropic effects of metoclopramide only in 5-HT_4-TG and not in WT and the negative chronotropic effects of domperidone in 5-HT_4-TG and WT. In order to assess any potential antagonistic effects of metoclopramide or domperidone at 5-HT_4-receptors in right atrial preparations, experiments were performed in the presence of buffer (as a control) or 300 nM or 1 µM metoclopramide and domperidone (Protocol C in Fig. 1). As above, we found that 300 nM or 1 µM metoclopramide induced only a positive chronotropic effect in 5-HT_4-TG (Fig. 6C and D). In the continued presence of metoclopramide (300 nM or 1 µM), we noted (for experimental design see Fig. 1C) a dextral displacement of the subsequently performed concentration–response curve for serotonin in 5-HT_4-TG (300 nM = Figs. 6C and 1 µM = Fig. 6D). For comparison, a second concentration response curve to 5-HT after washout (open circles, Fig. 6C and D) was added (Fig. 1C for experimental design). This was done to assess the shift in the curves to 5-HT due to putative receptor desensitisation. Indeed, under control conditions, the second concentration response curve to 5-HT was dextrally shifted in comparison to the first concentration response curve to 5-HT (open squares in Fig. 6C and D, Table 1 for statistical evaluation of potencies) which is in line with our previous reports under the very same experimental conditions (Gergs et al., 2017a).

3.5. Arrhythmias in isolated atria from TG and WT mice

In the series of experiments presented in this report, we studied completely different animals from those in previous reports (Gergs et al. 2010, 2013). Hence, we wanted to know if there were increased arrhythmias under basal conditions (i.e., before the addition of the drugs) or spontaneous arrhythmias as we saw in previous studies (Gergs et al. 2010, 2013; Keller et al., 2018). Three of the 44 atria from WT mice exhibited arrhythmias, while 10 of the 74 atria from TG mice showed arrhythmias (P < 0.05). In this study, we also wanted to know whether metoclopramide or domperidone could induce concentration-dependent arrhythmias in the left or right atrial preparations from TG and WT mice. We found that arrhythmias, in the presence of metoclopramide (experimental design as in Fig. 1A), occurred in 3 of the 11 atria from WT and in 4 of the 11 atria from 5-HT_4-TG (P > 0.05). However, no arrhythmias were being noticed in the presence of domperidone in the 11 atria from WT and in the 12 atria from 5-HT_4-TG (experimental design as in Fig. 1B).

3.6. Protein phosphorylation

Next, we wanted to understand better the underlying mechanisms of the contractile effects of metoclopramide. In previous studies, we have shown that 5-HT could increase the phosphorylation state of phospholamban in human and 5-HT_4-TG preparations (Gergs et al. 2009, 2010). In this study, we used a comparable approach and noted that the force, relaxation and the phosphorylation state of phospholamban showed a similar pattern. We studied isoprenaline as a positive control and detected a robust increase in the phosphorylation state of phospholamban in quiescent ventricular strips (Fig. 7A right hand side for original data and left hand side for summarized data) and perfused hearts (Fig. 7B). An increase in the phosphorylation state of phospholamban on amino acid serine 16 was noted in 5-HT_4-TG preparations that were treated with metoclopramide (Fig. 7A). In spontaneously beating isolated, perfused mouse hearts (Langendorff preparation, Fig. 7B and C), metoclopramide increased the phosphorylation state of both phospholamban and of C-protein (myosin binding protein C) (Fig. 7B and C). For comparison, the increase in phosphorylation of phospholamban due to 1 µM isoprenaline is shown in Fig. 7A. At right hand side of Fig. 7A, at the top of Fig. 7B and C, original Western blots for phosphorylation of phospholamban or C-protein are depicted. The uppermost strip in Fig. 7A, B and Fig. 7C depict calsequestrin which we always use as a loading control between lanes. The ratio of phospholamban Serine 16 phosphorylation to calsequestrin is plotted in Fig. 7A and Fig. B as normalized increase in phosphorylation in the ordinates. A similar approach was used in Fig. 7C for assessing the phosphorylation state of C-protein: metoclopramide increased the phosphorylation state of C-protein while domperidone was without effect (data not shown).

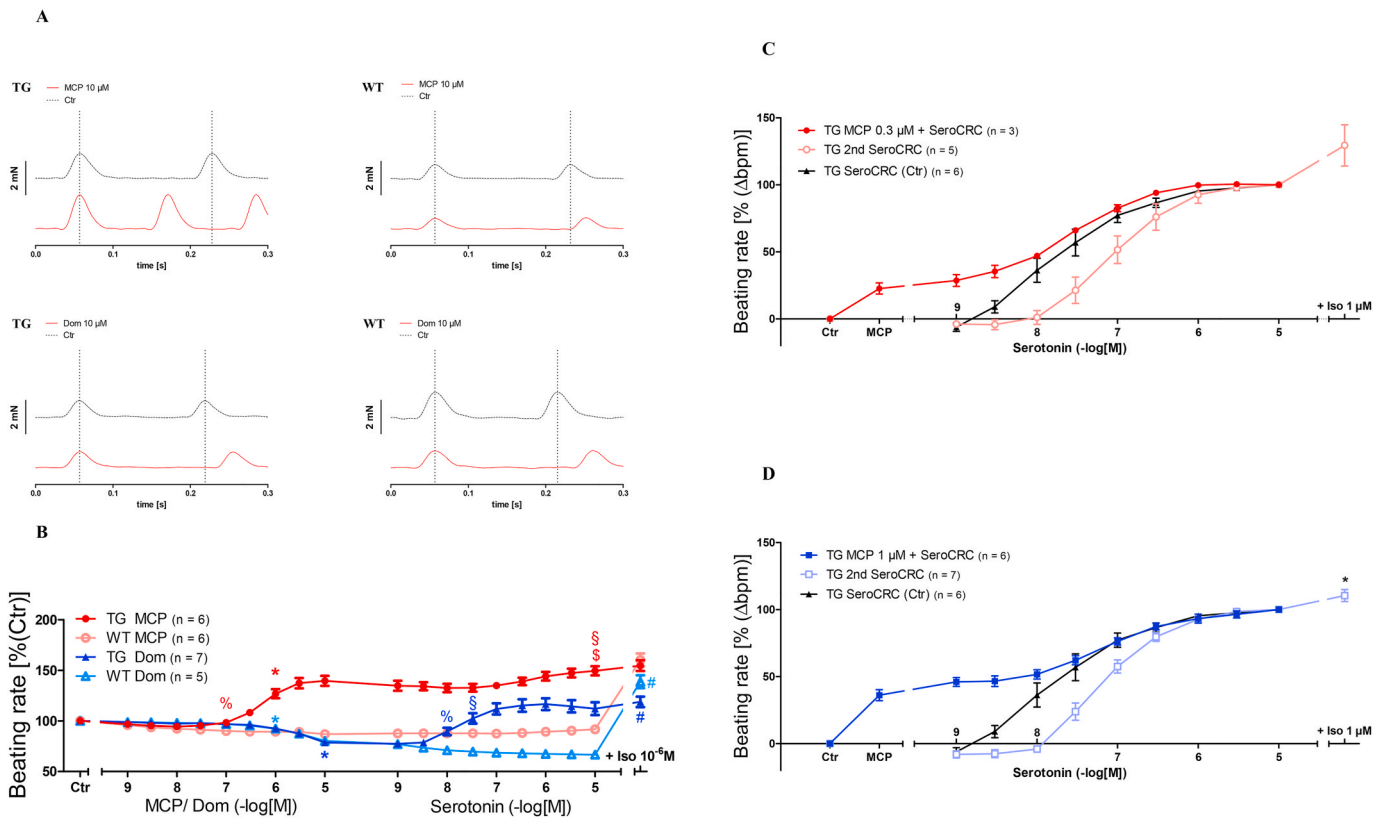


Fig. 6. Original recordings and summarized data of agonistic and antagonistic effects in right atrium

(**A**) Original recordings. Metoclopramide (MCP, 10 µM) exerts a positive chronotropic effect in isolated spontaneously beating right atrial preparations of 5-HT_4 receptor overexpressing mice (TG) but not in wild type mice (WT). Domperidone (Dom, 10 µM) reduced beating rate in TG and WT. The upper tracing in each of the plots was measured before and the lower after drug addition.

(**B**) Effects of metoclopramide or domperidone on beating rate in isolated right atrial preparation of 5-HT_4 receptor overexpressing mice (TG) or WT. First metoclopramide or domperidone were cumulatively applied (see Fig. 1A and B), then serotonin concentrations were cumulatively applied to the organ bath. Numbers in brackets indicate number of experiments. Pre-drug beating rate amounted to 391 ± 8 bpm. Ctr, basal contraction; Iso, isoprenaline (1 µM, maximum β-adrenergic stimulation). ★indicates first significant difference ($P < 0.05$) vs. Ctr; %first significant difference ($P < 0.05$) vs. WT; §indicates first significant difference ($P < 0.05$) vs. 5-HT 1 nM; $indicates significant difference ($P < 0.05$) vs. MCP 10 µM; #indicates significant difference ($P < 0.05$) vs. TG/WT MCP. (**C/D**) Effect of metoclopramide (MCP) on beating rate in isolated right atrial preparation of 5-HT_4 receptor overexpressing mice (TG). Buffer alone or 0.3 µM metoclopramide (**C**) or 1 µM metoclopramide (**D**) and then additionally serotonin was cumulatively applied (see Fig. 1C, left hand side). Numbers in brackets indicate number of experiments. Pre-drug beating rate amounted to 431 ± 12 bpm (**C**)/410 ± 9 bpm (**D**). Ctr, pre-drug beating rate; Iso, isoprenaline (1 µM, maximum β-adrenergic stimulation). ★indicates significant difference ($P < 0.05$) vs. 5-HT (10 µM).

3.7. Human atrium

In the final experiments, metoclopramide was cumulatively applied to isolated paced human atrial trabeculae and like in TG led to a positive inotropic effect (Fig. 8A and B). Domperidone, which was also cumulatively applied, was inactive up to 10 µM, the highest concentration studied (data not shown). The positive inotropic effects of metoclopramide were accompanied by an elevation in the rate of tension development and an increase in the rate of tension relaxation (Fig. 8D), while the time to peak tension and the time of relaxation remained unaltered under these experimental conditions (Fig. 8C). We would, finally, like to summarize the findings in Fig. 9.

4. Discussion

4.1. Inotropic effects

The present study showed that metoclopramide exerted a concentration-dependent positive inotropic effect, was less potent than 5-HT and antagonized the positive inotropic effect of 5-HT on human 5-HT_4 receptors (see Fig. 9). In a previous study, we noted that the efficacies of cisapride and prucalopride to increase force of contraction in the left atrium of 5-HT_4-TG were about 81% and 100%, respectively, of that of 5-HT (Keller et al., 2018). Thus, we present data that metoclopramide, similar to cisapride, was a partial agonist on 5-HT_4 receptors, in keeping with this, it took more time to reach a plateau than 5-HT in 5-HT_4-TG. In this study, we found that metoclopramide exerted a positive inotropic effect on human atrial preparations. Moreover, we could show that metoclopramide can raise the phosphorylation state of phospholamban and C-protein. Increased phosphorylation of phospholamban (Tada et al., 1976) and C-protein (Hartzell. 1984; Heling et al., 2020), both can explain, at least in part, why metoclopramide reduced time to relaxation and increased rate of tension relaxation in atrial and ventricular preparations from 5-HT_4-TG mice: phosphorylated phospholamban increases the rate at which calcium cations are pumped from the cytosol into the sarcoplasmic reticulum, less calcium cations bind to the myofilaments and myofilaments relax faster (Tada et al., 1976; Hamstra et al., 2020, Fig. 9). Phosphorylated C-protein might reduce the affinity of cytosolic calcium cations for myofilaments and thus facilitate cardiac relaxation (Heling et al., 2020). We had previously reported that 5-HT increased the phosphorylation state of phospholamban in isolated perfused heart of 5-HT_4-TG (Gergs et al., 2010) but also in isolated human atrial preparations (Gergs et al., 2009). Moreover, we found that the inotropic effects of metoclopramide were not confined to the atrium of 5-HT_4-TG, but were also present in the ventricle when measuring the ejection fraction of the left ventricle in living 5-HT_4-TG or directly in

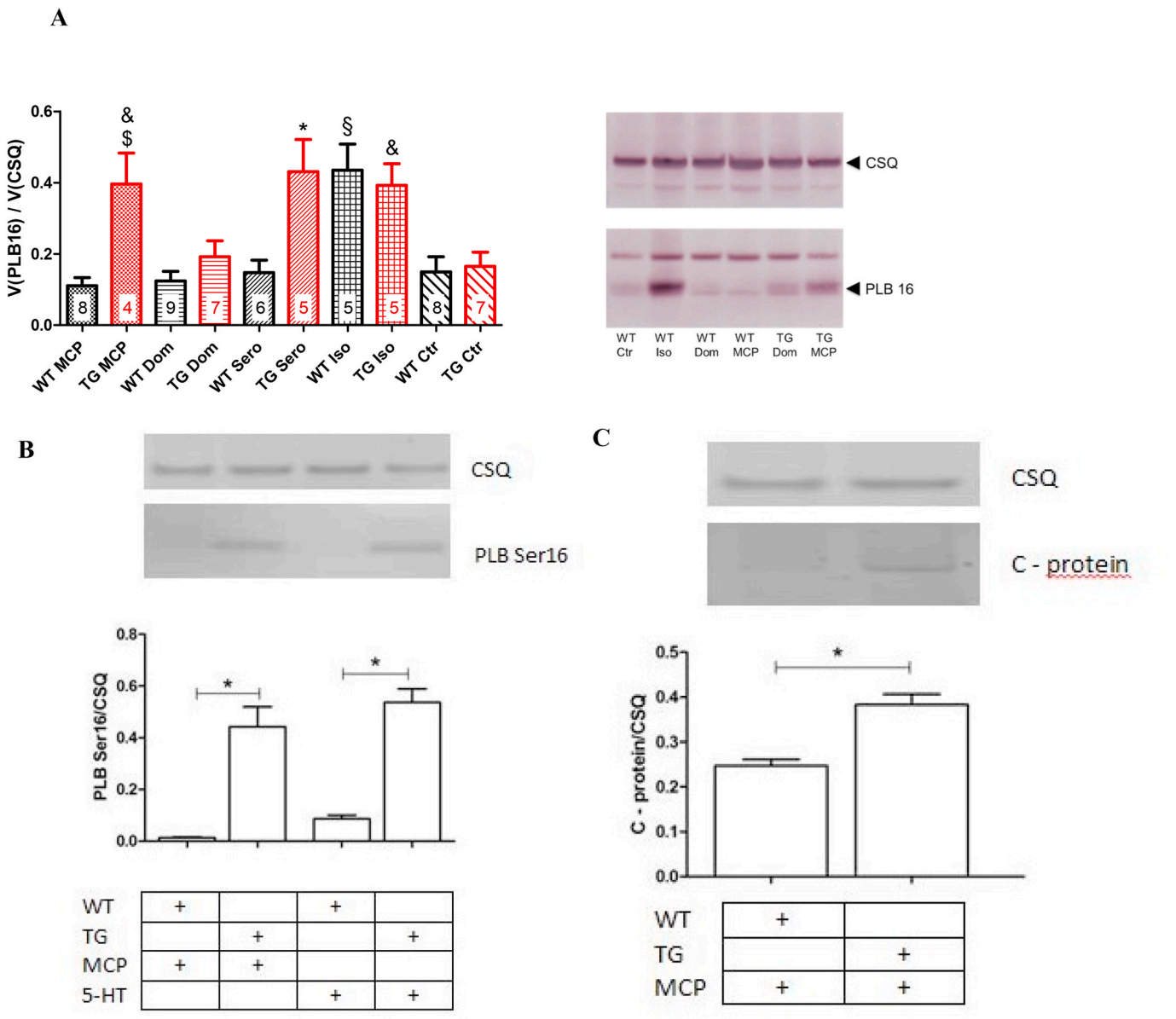


Fig. 7. Effects on protein phosphorylation

(**A**) Effect of 10 μM metoclopramide (MCP) or 10 μM domperidone (Dom) or 1 μM serotonin (Sero) or 1 μM isoprenaline (Iso) on the phosphorylation state of phospholamban at amino acid serine 16 (PLB 16) in relation to the expression of calsequestrin 2 (CSQ), as gel loading control, in quiescent left ventricular strips from 5-HT_4 receptor overexpressing mice and wild type mice (WT). On the left hand side (**A**) the summarized data and on the right a picture of an original Western Blot are displayed. Numbers in bars indicate number of experiments. Ctr, pre-drug PLB 16 phosphorylation. ★indicates significant difference ($P < 0.05$) vs. TG, Ctr; §indicates significant difference ($P < 0.05$) vs. WT, Ctr; $indicates significant difference ($P < 0.05$) vs. WT MCP; &indicates significant difference ($P < 0.05$) vs. TG Ctr.
(**B**) Effect of 10 μM metoclopramide (MCP) or 1 μM serotonin (5-HT) on PLB Ser 16 phosphorylation in isolated perfused hearts from TG or WT as depicted in the table below the bar diagram (n = 4 each). Ctr, basal PLB 16 phosphorylation. ★indicates significant difference ($P < 0.05$) vs. WT. Hearts were frozen in liquid nitrogen at the top of the inotropic effect (after 5 min of drug perfusion). (**C**) Effect of 10 μM metoclopramide (MCP) on C-protein phosphorylation in the same mouse heart ventricles as in (B). ★indicates significant difference ($P < 0.05$) vs. WT.

vitro with the Langendorff hearts of 5-HT_4-TG.

4.2. Domperidone

Interestingly, domperidone failed to increase the force in the atrial preparations from 5-HT_4-TG or WT or humans up to concentration of 10 μM. Thus, either the intrinsic activity of domperidone on human 5-HT_4 receptors was below our level of functional detection or the action on human 5-HT_4 receptors did not exist, which we find more plausible. In any case, the action of domperidone on cardiac 5-HT_4 receptors cannot explain the arrhythmias that were seen with the drug in patients, while the agonism of metoclopramide on the cardiac 5-HT_4 receptors might in part explain why metoclopramide can induce potentially fatal arrhythmias in patients (Fig. 9). It can be argued that we failed to detect relevant contractile effects of domperidone because we used subtherapeutic concentrations of the drug. The metabolism of domperidone can be blocked by other drugs, which can lead to high putative toxic plasma levels of domperidone. The peroral bioavailability of domperidone is about 15% (Heykants et al., 1981). Domperidone is mainly metabolized by CYP3A4; the half-life is 8–20 h (Lertxundi et al., 2013). One study on humans found that the peak plasma values of domperidone increased to 68 ng/ml, or 169 nM (Boyce et al., 2012). In the gut, as well as elsewhere

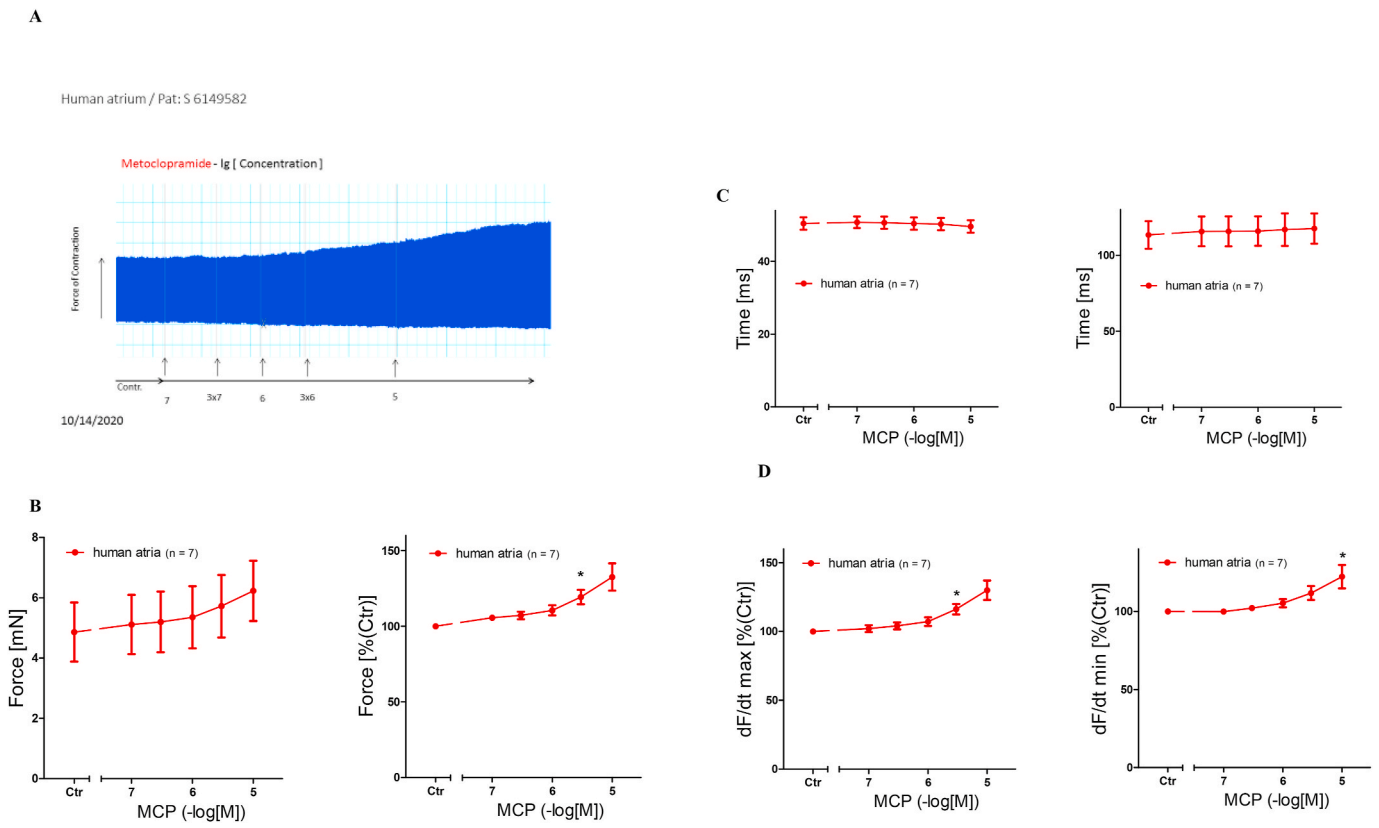


Fig. 8. Effects in human atrial preparations
(**A**) Original recording: concentration dependent positive inotropic effects of metoclopramide in one isolated electrically driven right atrial strip from one human heart in mN. Patient number and date of the contraction experiment are also displayed.
(**B**) Concentration dependent positive inotropic effects of metoclopramide (MCP) on isolated electrically driven right atrial strips from human hearts in mN (left) or percent of pre-drug value (right). First significant difference (★ $P < 0.05$) vs. pre-drug values (Ctr).
(**C**) Concentration dependent effects of metoclopramide (MCP) on time to peak tension (left) and time of relaxation (right) in isolated electrically driven right atrial strips from human hearts in milliseconds (in ms). Numbers in brackets indicate number of experiments. Ctr, pre-drug value. First significant difference (★ $P < 0.05$) vs. pre-drug values (Ctr). (**D**) Concentration dependent effects of metoclopramide (MCP) on rate of tension development (left) or rate of tension relaxation (right) in isolated electrically driven right atrial strips from human hearts in percent of pre-drug value (Ctr); numbers in brackets indicate number of experiments. Pre-drug values for rate of tension development amounted to 95 ± 19 mN/s. Pre-drug for rate of tension relaxation amounted to −56 ± 9 mN/s. First significant difference (★ $P < 0.05$) vs. pre-drug values.

in the body, domperidone is a substrate for P-glycoprotein (Tsujikawa et al., 2003; Yasuda et al., 2002). Thus, inhibitors of P-glycoprotein will increase the bioavailability of domperidone and could increase its plasma levels seven-fold. Inhibitors of P-glycoprotein include cyclosporin, verapamil and ketoconazole (Boyce et al., 2012; Yasuda et al., 2002; Lertxundi et al., 2013). The 5-HT_4 receptors in pigs have a different amino acid sequence than the human receptors, which might explain why we found that domperidone was inactive in the right atria of TG mice and in human atrium. Alternatively, we could speculate that the domperidone led to vasodilatation in pigs, which indirectly caused compensatory tachycardia. For instance, a study found that domperidone increased the cAMP content in the mesenteric arteries in rabbits, which could lead to vasodilatation (Zhu et al., 2000).

4.3. Chronotropy

Medhurst and Kaumann (1993) showed that metoclopramide elevated the beating rate in the isolated right atrial preparations of pigs via the 5-HT_4 receptors. In anaesthetised living pigs, both metoclopramide and domperidone had positive chronotropic effects through the 5-HT_4 receptors (Villalón et al., 1991). However, these drugs were less potent than serotonin and could antagonize its chronotropic effects; therefore, Villalón et al. (1991) regarded metoclopramide and domperidone as weak agonists with antagonistic properties at 5-HT_4-receptors. In the present study, we found that the maximum positive chronotropic effect of metoclopramide was 93% ± 2% of that of 5-HT in the right atria of TG mice.

4.4. Clinical implications

Peroral administration of metoclopramide can cause cardiac arrhythmias in humans (Pollera et al., 1984; Malkoff et al., 1995, Midttun and Oberg 1994; Chou and Wu 2001; Bentsen and Stubhaug 2002; Ellidokuz and Kaya 2003; Grenier und Drolet 2003; Siddique et al., 2009; Schwartz 2010; Rumore et al., 2011; Al-Shaer et al., 2015). Likewise, peroral administration of domperidone can elicit cardiac arrhythmias in patients (Roussak et al., 1984; Giaccone et al., 1984; Osborne et al., 1985; Bruera et al., 1986; Drolet et al., 2000; Renoux et al., 2016; Boyce et al., 2012; Michaud and Turgeon 2013; Buffery and Strother 2015). In the present study, we detected contractile responses to metoclopramide only in concentrations hardly reached in human plasma after peroral application. One study reported that peroral 10 mg metoclopramide led to peak plasma levels of 40–80 ng/ml, or 0.132–0.264 μM (Schulze-Delrieu et al., 1981). When metoclopramide was given i. v., the peak plasma levels were 200–300 nM (Ross-Lee et al., 1981). Another study reported that an overdose of metoclopramide led to plasma levels of 4.4 mg/l, or 1.42 μM (Beno and Nemeth 1991). The study found that 20% of metoclopramide is metabolized and that the

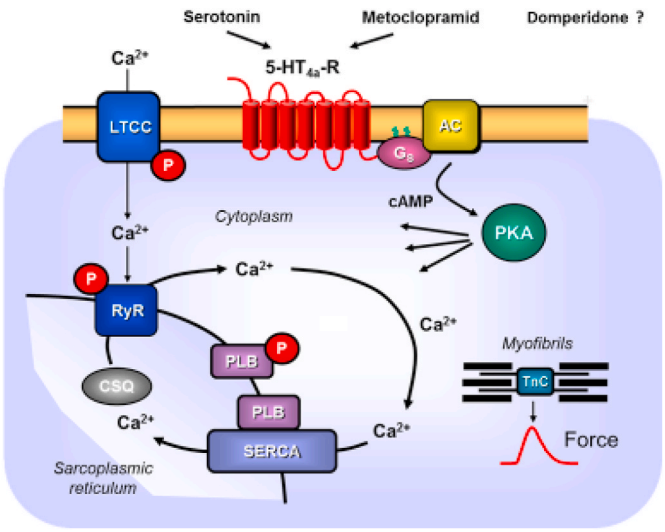


Fig. 9. Schematic presentation of a cardiomyocyte: serotonin acts via a cascade starting with the 5-HT_4-receptor in the sarcolemma, the occupation of which by serotonin elevates the activity of adenylyl cyclase (AC) in the sarcolemma via stimulatory G-proteins (Gs), augments the subsequent production of cAMP and, thereby, activates cAMP-dependent protein kinase (PKA) in the cytosol. PKA increases cardiac force generation and relaxation by increasing the phosphorylation state (P) of the L-type calcium channel (LTCC), of phospholamban (PLB) in the free sarcoplasmic reticulum, and other regulatory proteins like the ryanodine receptor 2 (RYR). Trigger Ca^{2+} initiates release of Ca^{2+} from the sarcoplasmic reticulum via ryanodine receptors into the cytosol, where Ca^{2+} activates myofilaments and leads to increased inotropy. In diastole, Ca^{2+} is taken up into the sarcoplasmic reticulum via a sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), whose activity is higher when the phosphorylation state of PLB is elevated by PKA and this hastens mechanical relaxation of the heart. Phosphorylation of C-protein in the myofibrils (not depicted) probably contributes to cardiac relaxation. Instead of serotonin, also metoclopramide but not domperidone can stimulate the HT_4-receptor and leads to phosphorylation of phospholamban and C-protein. Moreover, metoclopramide can also antagonize the function of serotonin via the HT_4-receptor.

half-life is 4 h (Schulze-Delrieu et al., 1981). Any metabolism of the metoclopramide is due to CYP2D6, CYP3A4, CYP2C19 and CYP1A2 enzymes (Desta et al., 2002). Patients with low activity mutants of CYP2D6 exhibit high plasma concentrations of metoclopramide and develop signs of drug-induced parkinsonism (van der Padt et al., 2006). High levels of metoclopramide have also been measured in patients with renal impairment (van der Meer et al., 2014) and liver cirrhosis (Magueur et al., 1991).

Hence, we suggest high concentrations of metoclopramide occur after intravenous application of metoclopramide, with intoxication by metoclopramide or because an interaction with drugs that inhibit the metabolism of metoclopramide. These higher concentrations can then exert contractile effects in the atrial preparations of 5-HT_4-TG, because inotropic effects started at 300 nM metoclopramide (Fig. 4A). We report here also force increasing effects of metoclopramide in human atria, which might be relevant under clinical conditions. However, these effects started at tenfold higher concentrations of metoclopramide than in 5-HT_4-TG (3 μM, Fig. 8B). However, contractile effects of 5-HT in human cardiac preparations can be shifted to lower concentrations by phosphodiesterase inhibitors in isolated human ventricular preparations (Afzal et al., 2008) and isolated 5-HT_4-TG atrial preparations (Neumann et al., 2019). In clinical practice, phosphodiesterase inhibitors include milrinone, levosimendan and theophylline. In the presence of these inhibitors, metoclopramide might, therefore, nevertheless induce arrhythmias via the cardiac 5-HT_4 receptor in patients.

In summary, using the 5-HT_4-TG model, we were able to detect cardiac inotropic and chronotropic effects for a clinically prescribed drug that is not intended to act on the heart, namely metoclopramide. In addition, our model was also able to rule out cardiac effects of domperidone via the 5-HT_4 receptors. More importantly, we successfully predicted the qualitative effects of domperidone and metoclopramide on the contractility in the human heart.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

The work contains parts of the medical theses of Tom Seidler and Charlotte Fehse. The technical assistance of S. Reber and P. Willmy is gratefully acknowledged.

References

Afzal, F., Andressen, K.W., Mørk, H.K., Aronsen, J.M., Sjaastad, I., Dahl, C.P., Skomedal, T., Levy, F.O., Osnes, J.B., Qvigstad, E., 2008 Dec. 5-HT4-elicited positive inotropic response is mediated by cAMP and regulated by PDE3 in failing rat and human cardiac ventricles. Br. J. Pharmacol. 155 (7), 1005–1014.

Al-Shaer, M.H., Mustafa, M.S., Scalese, M.J., 2015 May. Metoclopramide-induced asystolic cardiac arrest. Ann. Pharmacother. 49 (5), 610–611. https://doi.org/10.1177/1060028015577446.

Beno, J.M., Nemeth, D.R., 1991 Sep-Oct. Diltiazem and metoclopramide overdose. J. Anal. Toxicol. 15 (5), 285–287. https://doi.org/10.1093/jat/15.5.285.

Bentsen, G., Stubhaug, A., 2002 Aug. Cardiac arrest after intravenous metoclopramide - a case of five repeated injections of metoclopramide causing five episodes of cardiac arrest. Acta Anaesthesiol. Scand. 46 (7), 908–910. https://doi.org/10.1034/j.1399-6576.2002.460725.x.

Boknik, P., Drzewiecki, K., Eskandar, J., Gergs, U., Grote-Wessels, S., Fabritz, L., Kirchhof, P., Müller, F.U., Stümpel, F., Schmitz, W., Zimmermann, N., Kirchhefer, U., Neumann, J., 2018 Jan 22. Phenotyping of mice with heart specific overexpression of A2A-adenosine receptors: evidence for cardioprotective effects of A2A-adenosine receptors. Front. Pharmacol. 9, 13. https://doi.org/10.3389/fphar.2018.00013. eCollection.2018.

Boknik, P., Drzewiecki, K., Eskandar, J., Gergs, U., Hofmann, B., Treede, H., Grote-Wessels, S., Fabritz, L., Kirchhof, P., Fortmüller, L., Müller, F.U., Schmitz, W., Zimmermann, N., Kirchhefer, U., Neumann, J., 2019 Sep 18. Evidence for arrhythmogenic effects of A2A-adenosine receptors. Front. Pharmacol. 10, 1051. https://doi.org/10.3389/fphar.2019.01051.

Boyce, M.J., Baisley, K.J., Warrington, S.J., 2012 Mar. Pharmacokinetic interaction between domperidone and ketoconazole leads to QT prolongation in healthy volunteers: a randomized, placebo-controlled, double-blind, crossover study. Br. J. Clin. Pharmacol. 73 (3), 411–421. https://doi.org/10.1111/j.1365-2125.2011.04093.x.

Bruera, E., Villamayor, R., Roca, E., Barugel, M., Tronge, J., Chacon, R., 1986 Apr. Q-T interval prolongation and ventricular fibrillation with i.v. domperidone. Canc. Treat Rep. 70 (4), 545–546.

Buffery, P.J., Strother, R.M., 2015 Jun 12. Domperidone safety: a mini-review of the science of QT prolongation and clinical implications of recent global regulatory recommendations. N. Z.Med. J. 128 (1416), 66–74.

Champion, M.C., Hartnett, M., Yen, M., 1986 Sep 1. Domperidone, a new dopamine antagonist. CMAJ (Can. Med. Assoc. J.) 135 (5), 457–461.

Cheymol, G., Mouillé, P., 1975 May. [Anti-arrhythmic effects of derivatives of metoclopramide]. Arch. Int. Pharmacodyn. Ther. 215 (1), 150–159.

Chou, C.C., Wu, D., 2001 Dec. Torsade de pointes induced by metoclopramide in an elderly woman with preexisting complete left bundle branch block. Chang Gung Med. J. 24 (12), 805–809.

Desta, Z., Wu, G.M., Morocho, A.M., Flockhart, D.A., 2002 Mar. The gastroprokinetic and antiemetic drug metoclopramide is a substrate and inhibitor of cytochrome P450 2D6. Drug Metab. Dispos. 30 (3), 336–343. https://doi.org/10.1124/dmd.30.3.336.

Drolet, B., Rousseau, G., Daleau, P., Cardinal, R., Turgeon, J., 2000 Oct 17. Domperidone should not be considered a no-risk alternative to cisapride in the treatment of gastrointestinal motility disorders. Circulation 102 (16), 1883–1885. https://doi.org/10.1161/01.cir.102.16.1883.

Eberhart, L.H.J., Morin, A.M., Geldner, G., 2002. Short-term arterial hypotension induced by intravenous administration of metoclopramide. Anasthesiol. Intensivmed. 43, 341–345.

Ellidokuz, E., Kaya, D., 2003 Jul 1. The effect of metoclopramide on QT dynamicity: double-blind, placebo-controlled, cross-over study in healthy male volunteers. Aliment. Pharmacol. Ther. 18 (1), 151–155. https://doi.org/10.1046/j.1365-2036.2003.01641.x.

Gergs, U., Neumann, J., Simm, A., Silber, R.E., Remmers, F.O., Läer, S., 2009 Apr. Phosphorylation of phospholamban and troponin I through 5-HT4 receptors in the isolated human atrium. Naunyn-Schmiedeberg's Arch. Pharmacol. 379 (4), 349–359. https://doi.org/10.1007/s00210-008-0371-y.

Gergs, U., Baumann, M., Böckler, A., Buchwalow, I.B., Ebelt, H., Fabritz, L., Günther, S., Hauptmann, S., Kirchhof, P., Klöckner, U., Pönicke, K., Rückschloß, U., Schmitz, W., Schulz, N., Werner, F., Neumann, J., 2010 Sep. Cardiac overexpression of the human

5-HT$_4$-receptor in mice. Am. J. Physiol. Heart Circ. Physiol. 299 (3), H788–H798. https://doi.org/10.1152/ajpheart.00691.2009.
Gergs, U., Böckler, A., Ebelt, H., Hauptmann, S., Keller, N., Otto, V., Pönicke, K., Schmitz, W., Neumann, J., 2013 May. Human 5-HT$_4$ receptor stimulation in atria of transgenic mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 386 (5), 357–367. https://doi.org/10.1007/s00210-013-0831-x.
Gergs, U., Fritsche, J., Fabian, S., Christ, J., Neumann, J., 2017a. Desensitization of the human 5-HT4 receptor in isolated atria of transgenic mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 390 (10), 987–996. https://doi.org/10.1007/s00210-017-1403-2.
Gergs, U., Jung, F., Buchwalow, I.B., Hofmann, B., Simm, A., Treede, H., Neumann, J., 2017b. Pharmacological and physiological assessment of serotonin formation and degradation in isolated preparations from mouse and human heart. Am. J. Physiol. Heart Circ. Physiol. 313 (6), H1087–H1097. https://doi.org/10.1152/ajpheart.00350.2017.
Gergs, U., Rothkirch, D., Hofmann, B., Treede, H., Robaye, B., Simm, A., Müller, C.E., Neumann, J., 2018 Jul 5. Mechanism underlying the contractile activity of UTP in the mammalian heart. Eur. J. Pharmacol. 830, 47–58. https://doi.org/10.1016/j.ejphar.2018.04.013.
Gergs, U., Mangold, W., Langguth, F., Hatzfeld, M., Hauptmann, S., Bushnaq, H., Simm, A., Silber, R.E., Neumann, J., 2019a. Alterations of protein expression of phospholamban, ZASP and plakoglobin in human atria in subgroups of seniors. Sci. Rep. 9 (1), 5610. https://doi.org/10.1038/s41598-019-42141-w.
Gergs, U., Jahn, T., Werner, F., Köhler, C., Köpp, F., Großmann, C., Neumann, J., 2019b. Overexpression of protein phosphatase 5 in the mouse heart: reduced contractility but increased stress tolerance - two sides of the same coin? PloS One 14 (8), e0221289. https://doi.org/10.1371/journal.pone.0221289.eCollection.2019.
Gergs, U., Bernhardt, G., Buchwalow, I.B., Edler, H., Fröba, J., Keller, M., Kirchhefer, U., Köhler, F., Mißlinger, N., Wache, H., Neumann, J., 2019c. Initial characterization of transgenic mice overexpressing human histamine H2 receptors. J. Pharmacol. Exp. Therapeut. 369 (1), 129–141. https://doi.org/10.1124/jpet.118.255711.
Giaccone, G., Bertetto, O., Calciati, A., 1984 Dec 8. Two sudden deaths during prophylactic antiemetic treatment with high doses of domperidone and methylprednisolone. Two sudden deaths during prophylactic antiemetic treatment with high doses of domperidone and methylprednisolone. Lancet 2 (8415), 1336–1337. https://doi.org/10.1016/s0140-6736(84)90841-9.
Grenier, Y., Drolet, P., 2003 Apr. Asystolic cardiac arrest: an unusual reaction following iv metoclopramide. Can. J. Anaesth. 50 (4), 333–335. https://doi.org/10.1007/BF03021028.
Hamstra, S.I., Whitley, K.C., Baranowski, R.W., Kurgan, N., Braun, J.L., Messner, H.N., Fajardo, V.A., 2020 Oct 1. The role of phospholamban and GSK3 in regulating rodent cardiac SERCA function. Am. J. Physiol. Cell Physiol. 319 (4), C694–C699. https://doi.org/10.1152/ajpcell.00318.2020.
Hartzell, H.C., 1984 Apr. Phosphorylation of C-protein in intact amphibian cardiac muscle. Correlation between 32P incorporation and twitch relaxation. J. Gen. Physiol. 83 (4), 563–588. https://doi.org/10.1085/jgp.83.4.563.
Heling, L.W.H.J., Geeves, M.A., Kad, N.M., 2020 Mar. MyBP-C: one protein to govern them all. J. Muscle Res. Cell Motil. 41 (1), 91–101. https://doi.org/10.1007/s10974-019-09567-1.
Heykants, J., Hendriks, R., Meuldermans, W., Michiels, M., Scheygrond, H., Reyntjens, H., 1981. On the pharmacokinetics of domperidone in animals and man. IV. The pharmacokinetics of intravenous domperidone and its bioavailability in man following intramuscular, oral and rectal administration. Eur. J. Drug Metab. Pharmacokinet. 6 (1), 61–70. https://doi.org/10.1007/BF03189516.
Jungmann, E., Störger, H., Althoff, P.H., Hadler, D., Fassbinder, W., Bussmann, W.D., Kaltenbach, M., Schöffling, K., 1985. Aldosterone and prolactin responsiveness after prolonged treatment of congestive heart failure with captopril. Eur. J. Clin. Pharmacol. 28 (1), 1–4. https://doi.org/10.1007/BF00635699.
Kaumann, A.J., 1990 Nov. Piglet sinoatrial 5-HT receptors resemble human atrial 5-HT4-like receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 342 (5), 619–622. https://doi.org/10.1007/BF00169055.
Kaumann, A.J., Levy, F.O., 2006 Sep. 5-hydroxytryptamine receptors in the human cardiovascular system. Pharmacol. Ther. 111 (3), 674–706. https://doi.org/10.1016/j.pharmthera.2005.12.004.
Keller, N., Dhein, S., Neumann, J., Gergs, U., 2018 Sep. Cardiovascular effects of cisapride and prucalopride on human 5-HT4 receptors in transgenic mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 391 (9), 975–985. https://doi.org/10.1007/s00210-018-1519-z.
Kirchhefer, U., Baba, H.A., Hanske, G., Jones, L.R., Kirchhof, P., Schmitz, W., Neumann, J., 2004 Nov. Age-dependent biochemical and contractile properties in atrium of transgenic mice overexpressing junctin. Am. J. Physiol. Heart Circ. Physiol. 287 (5), H2216–H2225. https://doi.org/10.1152/ajpheart.00137.2004.
Lertxundi, U., Domingo-Echaburu, S., Soraluce, A., García, M., Ruiz-Osante, B., Aguirre, C., 2013 Feb. Domperidone in Parkinson's disease: a perilous arrhythmogenic or the gold standard? Curr. Drug Saf. 8 (1), 63–68. https://doi.org/10.2174/1574886311308010009.
Magueur, E., Hagege, H., Attali, P., Singlas, E., Etienne, J.P., Taburet, A.M., 1991 Feb. Pharmacokinetics of metoclopramide in patients with liver cirrhosis. Br. J. Clin. Pharmacol. 31 (2), 185–187. https://doi.org/10.1111/j.1365-2125.1991.tb05511.x.
Malkoff, M.D., Ponzillo, J.J., Myles, G.L., Gomez, C.R., Cruz-Flores, S., 1995 Apr. Sinus arrest after administration of intravenous metoclopramide. Ann. Pharmacother. 29 (4), 381–383. https://doi.org/10.1177/106002809502900408.
Matsuoka, H., Fukui, K., Dan, Y., Ishimitsu, T., Hirata, Y., Kimura, K., Sugimoto, T., Ishii, M., Kangawa, K., Matsuo, H., 1991 Aug. Atrial natriuretic peptide inhibits the aldosterone response to metoclopramide in patients with glomerular disease and essential hypertension. Clin. Exp. Pharmacol. Physiol. 18 (8), 557–562. https://doi.org/10.1111/j.1440-1681.1991.tb01491.x.
Medhurst, A.D., Kaumann, A.J., 1993 Nov. Characterization of the 5-HT4 receptor mediating tachycardia in piglet isolated right atrium. Br. J. Pharmacol. 110 (3), 1023–1030. https://doi.org/10.1111/j.1476-5381.1993.tb13916.x.
Schulze-Delrieu, K., 1981 Jul 2. Metoclopramide. In: Koch-Weser, J. (Ed.), Drug Therapy. N Engl J Med, vol. 305, pp. 28–33.
Michaud, V., Turgeon, J., 2013 Mar. Domperidone and sudden cardiac death: how much longer should we wait? J. Cardiovasc. Pharmacol. 61 (3), 215–217. https://doi.org/10.1097/FJC.0b013e31827e2573.
Midttun, M., Oberg, B., 1994 Jan 15. Total heart block after intravenous metoclopramide. Lancet 343 (8890), 182–183. https://doi.org/10.1016/s0140-6736(94)90978-4.
Neumann, J., Boknik, P., DePaoli-Roach, A.A., Field, L.J., Rockman, H.A., Kobayashi, Y. M., Kelley, J.S., Jones, L.R., 1998 Oct. Targeted overexpression of phospholamban to mouse atrium depresses Ca2+ transport and contractility. J. Mol. Cell. Cardiol. 30 (10), 1991–2002. https://doi.org/10.1006/jmcc.1998.0760.
Neumann, J., Boknik, P., Matherne, G.P., Lankford, A., Schmitz, W., 2003 Jan. Pertussis toxin sensitive and insensitive effects of adenosine and carbachol in murine atria overexpressing A_1–adenosine receptors. Br. J. Pharmacol. 138 (1), 209–217. https://doi.org/10.1038/sj.bjp.0705012.
Neumann, J., Hofmann, B., Gergs, U., 2017. Production and function of serotonin in cardiac cells. In: Fatima Shad, Kaneez (Ed.), Serotonin - A Chemical Messenger between All Types of Living Cells, pp. 271–305 (Chapter 13).
Neumann, J., Käufler, B., Gergs, U., 2019 Aug. Which phosphodiesterase can decrease cardiac effects of 5-HT4 receptor activation in transgenic mice? Naunyn-Schmiedeberg's Arch. Pharmacol. 392 (8), 991–1004. https://doi.org/10.1007/s00210-019-01653-y.
Osborne, R.J., Slevin, M.L., Hunter, R.W., Hamer, J., 1985. Cardiotoxicity of intravenous domperidone. Lancet 2 (8451), 385. https://doi.org/10.1016/s0140-6736(85)92515-2.
Pollera, C.F., Cognetli, F., Nardi, M., Mozza, D., 1984 Aug 25. Sudden death after acute dystonic reaction to high-dose metoclopramide. Lancet 2 (8400), 460–461. https://doi.org/10.1016/s0140-6736(84)92931-3.
Renoux, C., Dell'Aniello, S., Khairy, P., Marras, C., Bugden, S., Turin, T.C., Blais, L., Tamim, H., Evans, C., Steele, R., Dormuth, C., Ernst, P., 2016 Aug. Canadian Network for Observational Drug Effect Studies (CNODES) investigators. Ventricular tachyarrhythmia and sudden cardiac death with domperidone use in Parkinson's disease. Br. J. Clin. Pharmacol. 82 (2), 461–472. https://doi.org/10.1111/bcp.12964.
Ross-Lee, L.M., Eadie, M.J., Hooper, W.D., Bochner, F., 1981. Single-dose pharmacokinetics of metoclopramide. Eur. J. Clin. Pharmacol. 20 (6), 465–471. https://doi.org/10.1007/BF00542101.
Roussak, J.B., Carey, P., Parry, H., 1984 Dec 8. Cardiac arrest after treatment with intravenous domperidone. Br. Med. J. 289 (6458), 1579. https://doi.org/10.1136/bmj.289.6458.1579.
Rumore, M.M., Lee, S.E., Wang, S., Farmer, B., 2011 Nov 2. Metoclopramide-induced cardiac arrest. Clin. Pract. 1 (4), e83. https://doi.org/10.4081/cp.2011.e83.
Sala, C., Morganti, A., Ferraris, P., Ardeleani, G., Venegoni, P., Quorso, P., Ambrosi, B., Zanchetti, A., 1988 Dec. Dopaminergic control of aldosterone secretion is not mediated by atrial natriuretic factor in patients with essential hypertension. J. Hypertens. Suppl. 6 (4), S336–S338. https://doi.org/10.1097/00004872-198812040-00104.
Schwabe, U., Ludwig, W.D., 2020. Arzneiverordnungsreport. Springer-Verlag, Heidelberg, Germany, p. 628.
Schwartz, B.G., 2010 Jun. Metoclopramide and digoxin cause 22 episodes of bradyarrhythmias. Am. J. Med. 123 (6), e5–6. https://doi.org/10.1016/j.amjmed.2009.10.016.
Seidler, T., Gergs, U., Neumann, J., 2020. Metoclopramide but not domperidone affect cardiac contractility via 5-HT4 receptors in transgenic mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 393 (Suppl. 1), S31–S32.
Siddique, S.M., Shariff, N., Vesuwala, N., Hafiz, T., 2009 Apr 7. Metoclopramide as a possible cause of prolonged QT syndrome and torsade de pointes in a patient with heart failure and renal insufficiency. Ann. Intern. Med. 150 (7), 502–504. https://doi.org/10.7326/0003-4819-150-7-200904070-00016.
Tada, M., Kirchberger, M.A., Katz, A.M., 1976. Regulation of calcium transport in cardiac sarcoplasmic reticulum by cyclic AMP-dependent protein kinase. Recent Adv. Stud. Card. Struct. Metabol. 9, 225–239.
Tonini, M., De Ponti, F., Di Nucci, A., Crema, F., 1999 Dec. Review article: cardiac adverse effects of gastrointestinal prokinetics. Aliment. Pharmacol. Ther. 13 (12), 1585–1591. https://doi.org/10.1046/j.1365-2036.1999.00655.x.
Tsujikawa, K., Dan, Y., Nogawa, K., Sato, H., Yamada, Y., Murakami, H., Ohtani, H., Sawada, Y., Iga, T., 2003 Apr. Potentiation of domperidone-induced catalepsy by a P-glycoprotein inhibitor, cyclosporin A. Biopharm Drug Dispos. 24 (3), 105–114. https://doi.org/10.1002/bdd.343.
Tung, A., Sweitzer, B., Cutter, T., 2002 Dec. Cardiac arrest after labetalol and metoclopramide administration in a patient with scleroderma. Anesth. Analg. 95 (6), 1667–1668. https://doi.org/10.1097/00000539-200212000-00035 table of contents.
van der Meer, Y.G., Venhuizen, W.A., Heyland, D.K., van Zanten, A.R., 2014 Sep 23. Should we stop prescribing metoclopramide as a prokinetic drug in critically ill patients? Crit. Care 18 (5), 502. https://doi.org/10.1186/s13054-014-0502-4.
van der Padt, A., Van Schaik, R.H., Sonneveld, P., 2006. Acute dystonic reaction to metoclopramide in patients carrying homozygous cytochrome P450 2D6 genetic polymorphisms. Neth. J. Med. 64, 160–162.
Villalón, C.M., den Boer, M.O., Heiligers, J.P., Saxena, P.R., 1990 Aug. Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT$_4$ receptor.

Br. J. Pharmacol. 100 (4), 665–667. https://doi.org/10.1111/j.1476-5381.1990.tb14073.x.

Villalón, C.M., den Boer, M.O., Heiligers, J.P., Saxena, P.R., 1991 Jan. Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT4 receptor mediating tachycardia in the pig. Br. J. Pharmacol. 102 (1), 107–112. https://doi.org/10.1111/j.1476-5381.1991.tb12140.x.

WHO 2019 21 st WHO model list of essential medicines, 2019. https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1.

Yasuda, K., Lan, L.B., Sanglard, D., Furuya, K., Schuetz, J.D., Schuetz, E.G., 2002 Oct. Interaction of cytochrome P450 3A inhibitors with P-glycoprotein. J. Pharmacol. Exp. Therapeut. 303 (1), 323–332. https://doi.org/10.1124/jpet.102.037549.

Zhu, L., Zhao, R.R., Zhang, W.F., 2000 Jun. Effects of dopamine receptor agonists on the cAMP content in arteries of the rabbit. Sheng Li Xue Bao 52 (3), 247–251.