β-arrestin biased signaling is not involved in the hypotensive actions of 5-HT$_7$ receptor stimulation: use of Serodolin

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Abstract

The 5-hydroxytryptamine 7 receptor (5-HT$_7$) is necessary for 5-HT to cause a concentration-dependent vascular relaxation and hypotension. 5-HT$_7$ is recognized as having biased signaling, transduced through either Gs or β -arrestin. It is unknown whether 5-HT$_7$ signals in a biased manner to cause vasorelaxation/hypotension. We used the recently described β-arrestin selective 5-HT$_7$ receptor agonist serodolin to test the hypothesis that 5-HT$_7$ activation does not cause vascular relaxation or hypotension *via* the β -arrestin pathway. Isolated abdominal aorta (no functional 5-HT$_7$) and vena cava (functional 5-HT$_7$) from male Sprague Dawley rats were used in isometric contractility studies. Serodolin (1 nM – 10 μM) did not change baseline tone of isolated tissues and did not relax the endothelin-1 (ET-1)-contracted vena cava or aorta. In the aorta, serodolin acted as a 5-HT$_{2A}$ receptor antagonist, evidenced by a rightward shift in 5-HT-induced concentration response curve [pEC$_{50}$ 5-HT [M]: Veh = 5.2α0.15; Ser (100 nM) = 4.49α0.08; p<0.05]. In the vena cava, serodolin acted as a 5-HT$_7$ receptor antagonist, shifting the concentration response curve to 5-HT left and upward (%10 μM NE contraction; Veh = 3.2α1.7;

Ser (10 nM) = 58α11; p< 0.05) and blocking relaxation of pre-contracted tissue to the 5-HT$_{1A/7}$ agonist 5-carboxamidotryptamine. In anesthetized rats, 5-HT or serodolin was infused at 5, 25 and 75 μg/kg/min, iv. Though 5-HT caused concentration-dependent depressor responses, serodolin caused an insignificant small depressor responses at all three infusion rates. With the final dose of serodolin on board, 5-HT was unable to reduce blood pressure. Collectively the data indicate that serodolin functions as a 5-HT$_7$ antagonist with additional 5-HT$_{2A}$ blocking properties. 5-HT$_7$ activation does not cause vascular relaxation or hypotension *via* the β -arrestin pathway.

Graphical Abstract

Gs Beta-arrestin

Potential Therapeutic Agents
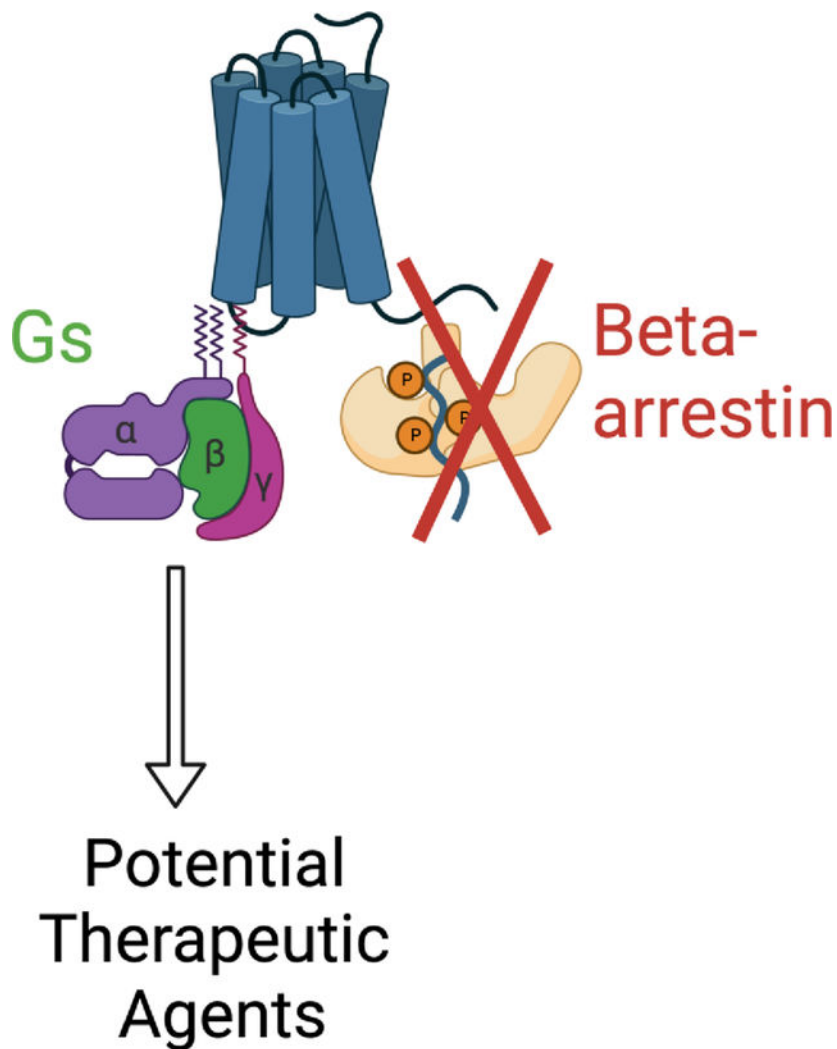
Keywords

5-HT; 5-HT$_7$ receptor; hypotension; biased agonist; cardiovascular disease

1.0   INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT) was discovered as a vasoconstrictor, a substance that could change blood pressure in animal and human (Page and McCubbin 1953). It is without question that 5-HT is a vasoconstrictor primarily through activation of 5-HT$_{2A}$ receptor. However, with the discovery of other classes of 5-HT receptors, the (cardio)vascular actions of 5-HT expanded (Watts et al, 2012). Of specific interest in this study is the 5-HT$_7$ receptor, cloned in 1993 (Ruat et al, 1993; Shen et al, 1993).

The 5-HT$_7$ receptor is best known to play a role in circadian rhythm and photic response (Gardani and Biello, 2008; Sprouse et al, 2005); sleep and wakefulness (Monti and Jantos, 2014); temperature regulation (Hedlund et al, 2003); learning and memory (Roberts and Hedlund, 2012) and inflammation (Guseva et al, 2014). Relative to its function in the cardiovascular system, the 5-HT$_7$ receptor mRNA was first localized to the blood vessels, both arteries and veins, by Ullmer et al (1995).

The functions of this receptor in control of vascular tone and blood pressure are of interest for several reasons. 5-HT possesses low nanomolar affinity for the 5-HT$_7$ receptor such that free circulating 5-HT has the potential to activate this receptor (Watts et al, 2012). The 5-HT$_7$ receptor also possesses interesting properties. It can be allosterically modified (Alberts et al, 2001); has constitutive activity (Andressen et al, 2018; Gellynck et al 2013; Hobson et al, 2003, Krobert et al, 2002; Kvachnina et al, 2009; Mahe et al, 2004; Purohit et al, 2005; Romero et al, 2006); has natural variants that change receptor pharmacology (Bruss et al, 2005; Kiel et al, 2003); can heterodimerize (Renner et al, 2012); and, discussed below, can be biased in its signal transduction.

Independent groups have consistently shown that infusion of 5-HT or its pathway limited precursor 5-hydroxytryptophan can lower blood pressure in animals, primarily the rat, that have either normal or elevated blood pressure (Balasubramaniam 1993, 1995; Baron et al, 1991; Cade and Fregly, 1992; Centurion et al, 2004; Dalton et al, 1986; DeVries et al, 1999; Ding et al, 1989; Echizen and Freed, 1981; Fregly et al, 1987; Itskovitz et al, 1989). Our group has focused on the mechanisms of long term hypotension caused by infusion of 5-HT over the course of a week (Diaz et al 2008). 5-HT, given as such, reduces total peripheral resistance (TPR), invoking involvement of the vasculature (Davis et al, 2012). We now know that 5-HT can relax skeletal muscle arterioles and reduce hindquarter vascular resistance (Jackson et al, 2023; Seitz et al, 2021), and relax veins *in vitro* and *in vivo* (Seitz et al, 2016; 2017; Watts et al, 2015). These events are predominantly mediated by activation of the 5-HT$_7$ receptor, validated by a loss of many of these effects in the 5-HT$_7$ KO rat we created (Demireva et al, 2019; Seitz et al, 2019) or antagonism by SB269970 (Hagan et al, 2000). Our findings follow important work by Terron et al whom first suggested the 5-HT$_7$ receptor could be involved in a hypotensive response to 5-HT (Terron 1997; Terron et al, 2007). As such, the 5-HT$_7$ receptor is an attractive target for developing new therapeutics for combating diseases of elevated total peripheral resistance, such as hypertension.

Only recently has the 5-HT$_7$ receptor, a G protein coupled receptor, been observed to show biased agonism, with activation of Gs and β-arrestin pathways those towards which agonist

may be differently biased (El Khamlichi et al, 2022). Our goal is to determine the ideal way of activating the 5-HT$_7$ receptor to effect a fall in TPR/blood pressure with minimal side effects. Thus, we test here the hypothesis that the 5-HT$_7$ receptor is not β-arrestin biased in its actions on blood pressure/vascular function. We use a vascular model – the isolated abdominal aorta and vena cava – that we've well established as being a vessel with a non-functional (aorta) and functional 5-HT$_7$ receptor (vena cava) (Gonzalez-Pons et al, 2021; Seitz et al, 2019; Watts et al, 2015). We take advantage of the newly created drug serodolin as a β-arrestin biased agonist at the 5-HT$_7$ receptor (El Khamlichi et al, 2022). Using a combination of *in vitro* and *in vivo* methods, our findings strongly support that activation of the 5-HT$_7$ receptor unlikely utilizes β-arrestin pathways to effect the fall in vascular resistance/blood pressure.

2.0   MATERIALS AND METHODS

2.1   Animal approval, use and dissection

Male Sprague Dawley rats were purchased from Charles River Laboratories (Matawan, MI, USA). Previous studies indicate no difference in the cardiovascular functioning of the 5-HT$_7$ receptor between male and female (Seitz et al, 2019). Thus, in the interest of reducing animal use, only males were used. Animals were on a normal diet [Teklad 22/5 Rodent diet (Madison WI, USA)]. Food and drinking water were available ad libitum. Procedures using animals complied with National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011). Procedures used in this study were approved by the MSU Institutional Animal Care and Use Committee (PROTO202000009). Finally, this study was conducted with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (essential 10 and recommended) in mind.

For in vitro work and before tissue removal, rats were given pentobarbital as a deep anesthetic (80 mg kg^{-1}, ip). A bilateral pneumothorax was created prior to vessel dissection. The abdominal vessels [vena cava (AbIVC) and aorta (AbA)] were removed *in toto* and separated from one another under a stereomicroscope and in a Silastic®-coated dish filled with physiological salt solution [PSS in mM: NaCl 130; KCl 4.7; KH$_2$PO$_4$ 1.18; MgSO$_4$ • 7H$_2$O 1.17; NaHCO$_3$ 14.8; dextrose 5.5; CaNa$_2$EDTA 0.03, CaCl$_2$ 1.6 (pH 7.2).].

2.2   Isometric Contractility

Rings of tissues (3–5 mm wide) were placed onto two L-shaped stainless-steel rings. Rings were mounted in warmed (37°C) and aerated (95% O$_2$, 5% CO$_2$) tissue baths (10 ml or 30 ml volume) on Grass isometric transducers (FT03; Grass instruments, Quincy, MA, USA) connected to an 8 channel PowerLab C through an Octet Bridge (ADInstruments, Colorado Springs, CO, USA) or a 4 channel PowerLab connected to a Quad Bridge. Sample type (aortic or vena cava ring) and exposure to vehicle or inhibitor were randomized daily into different tissue baths. Tissues were placed under optimum resting tension (determined in previous experiments: AbA 4 grams; AbIVC: 1 gram) and allowed to equilibrate for one hour with frequent exchange of buffer. At this time, tissues were challenged with a maximum concentration of norepinephrine (10 μM). Tissues were washed to baseline, and

one of the following protocols commenced. Tissues were used in only one of the following protocols.

- *Test of vehicle or serodolin from baseline*: Vehicle (increasing % of DMSO to 0.1% or serodolin) were added in a cumulative fashion (1 nM - 10 μM), waiting at least three minutes before adding the next concentration. If an effect was observed, a plateau was allowed to be achieved before addition of next concentration.

- *Test of serodolin as a relaxant (vena cava only):* AbIVC were contracted with a half maximal concentration of the thromboxane A2 mimetic U46619 (1 μM) or endothelin-1 (ET-1; 1 nM). Once contraction plateaued, either vehicle or serodolin (1 μM) were added to observe whether relaxation ensued.

- *Test of serodolin vs 5-carboxamidotryptamine (5-CT) (vena cava only):* AbIVC were incubated with vehicle (0.01% DMSO) or serodolin (100 nM) for 45 minutes. Tissues were then contracted to a half-maximal concentration of U46619, and 5-CT (1 μM) added to stimulate relaxation.

- *Test of serodolin vs 5-HT*: AbA and AbIVC were incubated with either vehicle (up to 0.1% DMSO) or one concentration of serodolin (10 nM, 100 nM, 1 μM) for one hour without washing. A cumulative concentration response curve to 5-HT ($10^{-9} - 3\times10^{-4}$ M) was then constructed.

2.4 *In vivo* administration of 5-HT, serodolin

The catheter of a telemeter probe (Data Sciences International, Minneapolis, MN, USA) was inserted into a femoral artery to measure blood pressure throughout the experiment. An open catheter was inserted via a femoral vein for drug infusion. A 30-minute baseline period was followed by a 20-minute infusion of 5-HT or serodolin at progressive rates of 5, 25 and 75 μg/min by means of an infusion pump. A 30-minute recovery period was allowed between 5-HT and serodolin infusion.

2.5 Data/Statistical analyses and presentation.

All quantitative data are reported as means α SEM for number of animals in parentheses. N represents the number of biological replicates (e.g. individual animals). For isometric contractile studies, contraction is reported as milligrams (tracing) or as a percentage of initial contraction to a maximum concentration of NE (10 μM). Relaxation is reported as a percentage of a half-maximal contraction to the thromboxane A_2 mimetic U46619 or endothelin −1 (ET-1). Agonist potencies were calculated using a non-linear regression (curve fit) within GraphPad Prism 9.0 (La Jolla, CA, USA) and are reported as –log EC_{50} values [M]. Maximums are reported as the maximal effect achieved. Where a maximal response was not achieved, the actual potency (EC_{50} value) was considered equal or greater than the reported value. The pK_B value or the apparent antagonist dissociation constant for an serodolin at the $5-HT_{2A}$ receptors was calculated using the equation:

$$Log(DR - 1) = log[B] - log\ K_B$$

where DR is the EC_{50} value of agonist in the presence of antagonist/EC_{50} value in the absence of antagonist; [B] is the molar concentration of the antagonist.

Repeated measures two-way ANOVA followed by the Bonferroni post hoc test was used to compare concentration-response curves. In all cases, $p < 0.05$ was considered significant.

2.6 Materials

5-HT hydrochloride, norepinephrine hydrochloride and dimethylsulfoxide were obtained from Sigma Chemical Company (St. Louis, MO USA). 5-CT and SB269970 were purchased from Tocris (R & D systems, Minneapolis, MN, USA). ET-1 was purchased from Echelon Biosciences (Salt Lake City, UT, USA). U46619 was purchased from Cayman Chemical Co (Ann Arbor, MI USA). Serodolin was provided through a Materials Transfer Agreement with Dr. Morisset-Lopez, the CNRS and Orléans University.

3.0 Results

3.1. Serodolin did not change baseline tone of isolated aorta or vena cava

These two tissues – the AbA and AbIVC – were tested for their ability to respond to serodolin given in a cumulative fashion. When compared to a vehicle that carried an equivalent percentage of DMSO, serodolin neither increased nor decreased the baseline tone of either vessel (figure 1). Tissues were alive given their contraction to NE (in figure legend 1).

3.2 Serodolin did not cause relaxation of contracted vena cava

The lack of response from baseline is not surprising given that this would not be expected unless tone had been established. However, when the AbIVC was contracted with half-maximal ET-1 or U46619, serodolin (1 μM) did not cause relaxation (figure 2A). Collectively, these data support serodolin is likely not an agonist that causes relaxation.

3.3 Serodolin antagonized 5-CT-induced relaxation in contracted vena cava.

In the next experiment, serodolin (100 nM) was tested for its ability to antagonize a response recognized as being $5-HT_7$ receptor dependent, 5-CT-induced relaxation. 5-CT caused a relaxation to 30% of U46619-induced contraction (figure 2B; open bar) that was reduced significantly in the presence of serodolin (figure 2B; **shaded bar**). This finding supports serodolin acting as a $5-HT_7$ receptor antagonist in this model.

3.4. Serodolin acted as a $5-HT_{2A}$ and $5-HT_7$ receptor antagonist

In final *in vitro* experiments, increasing concentrations of serodolin (10 nM, 100 nM or 1 μM) were tested for the ability to modify 5-HT-indcued contraction. Figure 3 depicts effects in the AbA (figure 3A) and AbIVC (figure 3B), as well as pharmacological parameters calculated for Table 1. In the aorta, Serodolin caused a concentration dependent rightward shift of 5-HT induced contraction. The antagonist dissociation constant (pKB) calculated for 5-HT in the Ab Awa was 7.57. Contraction in the AbA is largely mediated by the $5-HT_{2A}$ receptor; serodolin is most likely antagonizing the $5-HT_{2A}$ receptor.

The actions of serodolin were different in the AbIVC. Consistent with past results, 5-HT did not cause a concentration-dependent contraction in the AbIVC. Whereas serodolin alone did not modify contraction in the AbIVC (see Figure 2), it potentiated the effect of 5-HT in stimulating contraction at all tested concentrations. The potency of 5-HT to induce contraction was greatest in the presence of 10 nM serodolin, and progressively decreased as increasing concentrations were used. Table 1 shares the pharmacological parameters of these curves in figure 3. In the AbIVC, the increased potency of 5-HT can be explained by serodolin acting as a 5-HT_7 receptor antagonist at a low concentration. With increasing serodolin concentration, 5-HT_{2A} receptor antagonism was observed.

3.5. Serodolin alone did not decrease blood pressure and antagonized 5-HT-induced hypotension

Figures 4 and 5 share data on the effects of serodolin when given *in vivo* to anesthetized male Sprague Dawley rats. Serodolin, infused independently, did not modify blood pressure. By contrast, 5-HT caused the expected hypotension, best observed at the 25 μg/kg dose (figure 4). If this same dose response curve is constructed after the final addition of serodolin (75 μg/kg on board), 5-HT no longer caused a reduction in blood pressure (figure 5). Here, the ability of serodolin to antagonize a 5-HT_7 receptor mediated event *in vivo* is consistent with 5-HT_7 receptor antagonism exerted *in vitro*.

4.0 Discussion

Blood pressure control is regressing in the US and groups of resistant (3 meds do not decrease blood pressure) and refractory (5 meds do not decrease blood pressure) hypertension exist (Acelajado et al, 2019; Egan et al, 2021; Muntner et al, 2022). These daunting facts raise the idea that we do not fully understand how both normal and elevated blood pressures are regulated. As such, we and others have invested in the idea that agonism at the 5-HT_7 receptor could provide a new therapeutic. The present study supports that 5-HT_7 receptor agonists that are not β-arrestin biased are likely the drugs with the best potential of therapeutic efficacy.

4.1 Serodolin shows no agonism but acts as an antagonist

Whether serodolin was tested from baseline or in a contracted tissue, serodolin did not stimulate either direct contraction or relaxation in either the isolated abdominal vena cava or aorta. Importantly, this is not because tissues were not viable. All data are reported as a percentage of an initial contraction (NE) or half-maximal contraction (ET-1/U46619). All tissues contracted to an acceptable magnitude to these agonists such that response allowed their continuance in the protocol. We conclude that serodolin does not have the ability to activate a receptor that would directly influence vascular contraction. This was validated by *in vivo* studies which support little to no direct effect of serodolin on mean arterial pressure.

Rather, our data support that serodolin, in these blood vessels, functions as a 5-HT_7 receptor antagonist at low nM concentrations and at higher concentrations as a 5-HT_{2A} receptor antagonist. The ability to antagonize the 5-HT_7 receptor was particularly evident in the profound enhancement of 5-HT-induced contraction in the AbIVC. This finding is consistent

with previous work which discovered that the 5-HT$_7$ receptor restrained the contractile function of the 5-HT$_{2A}$ receptor in the vena cava (Gonzalez-Pons et al, 2021; Seitz et al, 2019). In these two studies either pharmacological blockade by the antagonist SB269970 (Gonzales-Pons et al, 2021; Hagan et al, 2000) or genetic removal (Seitz et al, 2019) of the 5-HT$_7$ receptor unveiled a 5-HT contraction conducted through the 5-HT$_{2A}$ receptor in the rat abdominal vena cava. The mechanism of how this occurs, such as a physical association of the two 5-HT receptors that is modified with antagonism/loss, is not known. The 5-HT$_7$ receptor can heterodimerize with the 5-HT$_{1A}$ receptor (Renner et al, 2012); we were unable to find reports of the 5-HT$_7$ receptor heterodimerizing with the 5-HT$_{2A}$ receptor.

The ability of serodolin to act as a 5-HT$_7$ receptor antagonist is consistent with its known pharmacology (El Khamlichi et al, 2022). Occupancy of the orthosteric site of the 5-HT$_7$ receptor with no measurable efficacy would permit antagonism of 5-HT-stimulated events.

4.2   5-HT$_7$ biased ligands

Serodolin is among the first described β-arrestin biased ligands. Kim et al published a series of tetrahydroazepine derivatives with β-arrestin bias (Kim et al, 2018). In parallel with El Khamlichi et al, Onyameh published on β-arrestin biased 5-HT$_7$ receptor agonists (2022).

The intent of production of these molecules was largely focused on effects in the central nervous system, including endpoints such as sleep time, grooming and pain. Here, we use (one of) them in a different way: to examine whether the 5-HT$_7$ receptor, through the β-arrestin pathway, was sufficient to cause vascular relaxation or hypotension. Serodolin caused neither of these events, leading to the conclusion that activation of the β-arrestin pathway through the 5-HT$_7$ receptor is not necessary for producing hypotension. As such, it is most likely activation of Gs through the 5-HT$_7$ receptor that leads to vascular relaxation and hypotension.

There is a small amount of information about the Gs/β-arrestin bias of agonists used to interrogate the 5-HT$_7$ receptor. While important, such information comes with the caveat of bias measures being done in artificial, constructed cells, typically human embryonic kidney or HEK cells, that allow for measure of cAMP (Gs) or β-arrestin trafficking. This makes it impossible to be fully confidentthat the observations made in these artificial systems could be translated to, in our case, the venous smooth muscle cell. In such systems, 5-CT activates cAMP production through activation of the Gs pathway through the 5-HT$_7$ receptor, but may also minimally activate β-arrestin through protein kinase A (El Khamlichi et al 2022). 5-CT is significantly more potent than 5-HT in reducing blood pressure in the rat, in part because it lacks the affinity for the 5-HT$_{2A}$ receptor possessed by 5-HT. Lee et al (2021) calculated the bias factor for 5-HT and the 5-HT$_7$ receptor agonist E-55888, finding that 5-HT was equivalently biased towards Gs and β-arrestin (bias factor 1:1), while E-55888 was more biased towards β-arrestin (bias of 0.34:1). Onyameh et al confirmed that 5-HT is equally biased to Gs and β-arrestin (2022). If these findings translate to the naïve 5-HT$_7$ receptor, then the 5-HT-induced vascular relaxation and hypotension is likely mediated predominantly by Gs stimulation. It will be interesting to compare a 5-HT$_7$ receptor agonist that has pure Gs bias to serodolin.

4.4 Limitations

We did not carry out assays that would demonstrate the ability of serodolin to recruit β-arrestin in any of the cell types of the blood vessel. This was not considered necessary given the significant proof of principle done by El-Khamlichi et al (2022). We have also not compared it to other β-arrestin biased ligands. Again, the present studies are proof of principle for us to now understand the β-arrestin arm of 5-HT$_7$ receptor activation is not necessary for the hypotensive actions of 5-HT. Finally, our conclusions can only be made relative to the rat given that this is the only species tested.

5.0 Conclusions

We conclude that activation of the β-arrestin pathway through the 5-HT$_7$ receptor is not necessary for the relaxant/hypotensive effects of 5-HT (Graphical Abstract). This knowledge allows focus on 5-HT$_7$ receptor ligands that are biased towards Gs for development of cardiovascular therapeutics.

Acknowledgements, Conflicts, Funding and Author Contributions

Data Availability

All data generated during this study are included in this article.

Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5-CT	5-carboxamidotryptamine
ET-1	endothelin-1
SB269970	(2*R*)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine

7.0 References

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Highlights

- 5-HT$_7$ receptor stimulation mediates the hypotension when 5-HT is infused, but the ability of this receptor to function in a biased manner calls to question how 5-HT effects a hypotension: through Gs or β-arrestin?

- Serodolin as a β -arrestin biased agonist at the 5-HT$_7$ receptor was unable to relax isolated vessels nor did it cause a dose-dependent hypotension.

- These findings point to the actions that proceed from 5-HT stimulation of the 5-HT$_7$ receptor to be dependent on Gs signaling.
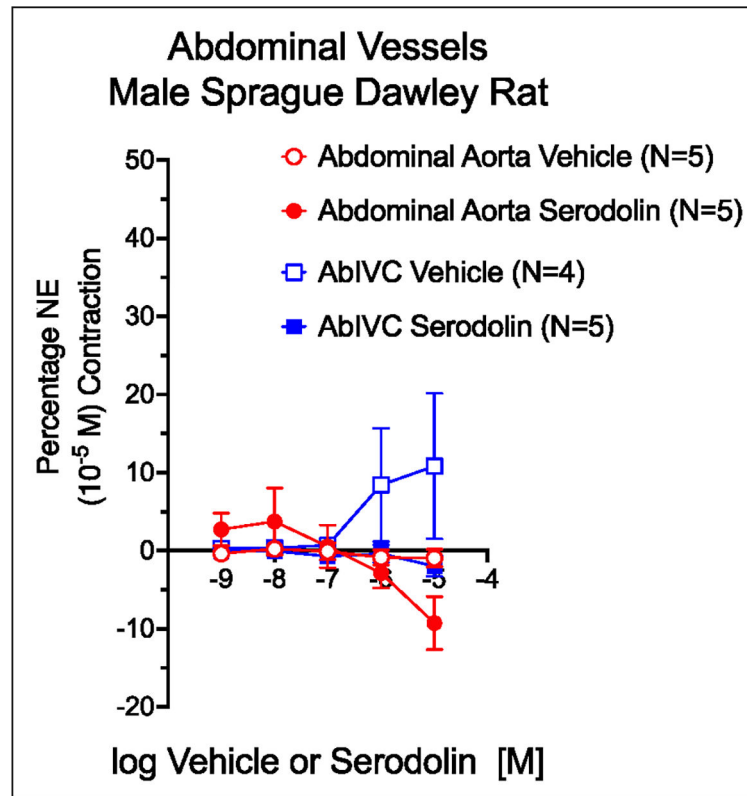
Figure 1.
Effect of vehicle (graded DMSO) and serodolin in the abdominal aorta (red) and Vena Cava
of the male Sprague Dawley rat. Points are means$\pm$SEM for number of animals stated in
parentheses. Contraction to NE (10^{-5} M; in milligrams): Aorta Vehicle = 2249$\pm$196; Aorta
Serodolin = 1739$\pm$115; AbIVC Vehicle = 383$\pm$111; AbIVC Serodolin = 461$\pm$88.
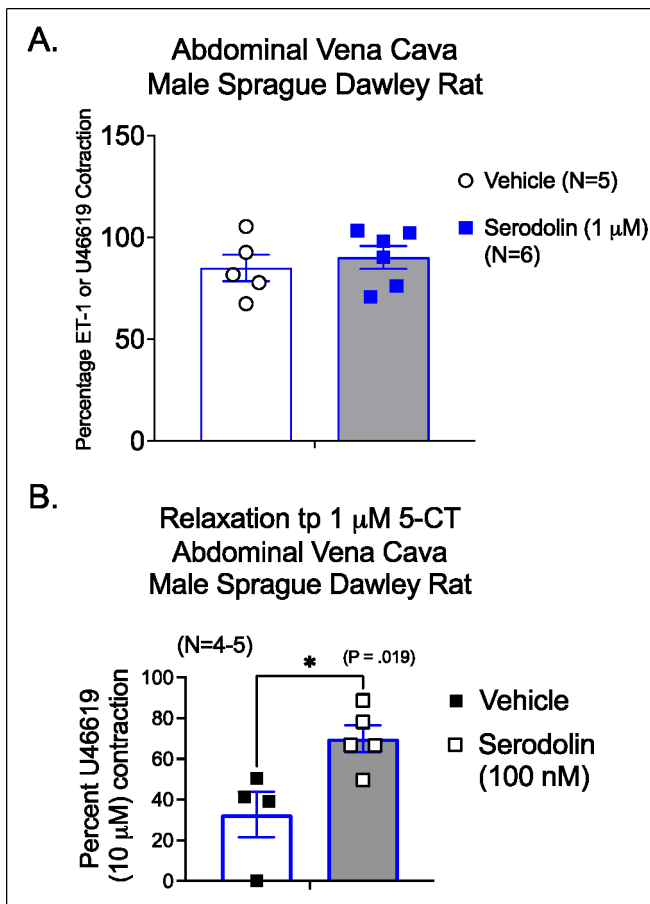
Figure 2.

A. Effect of vehicle or serodolin on the contracted Vena Cava of the male Sprague Dawley rat. ET-1 contraction in milligrams: Vehicle = 447α163; Serodolin = 658α125. **B.** Ability of serodolin (100 nM) to reduce the relaxation caused by 5-CT (1 μM) in the contracted vena cava. ET-1/U46619 contraction in milligrams Vehicle = 294α78; Serodolin = 518α88. *signifies statistically significant differences as determined by an unpaired Students t test. Bars are means+SEM for number of animals stated in parentheses.
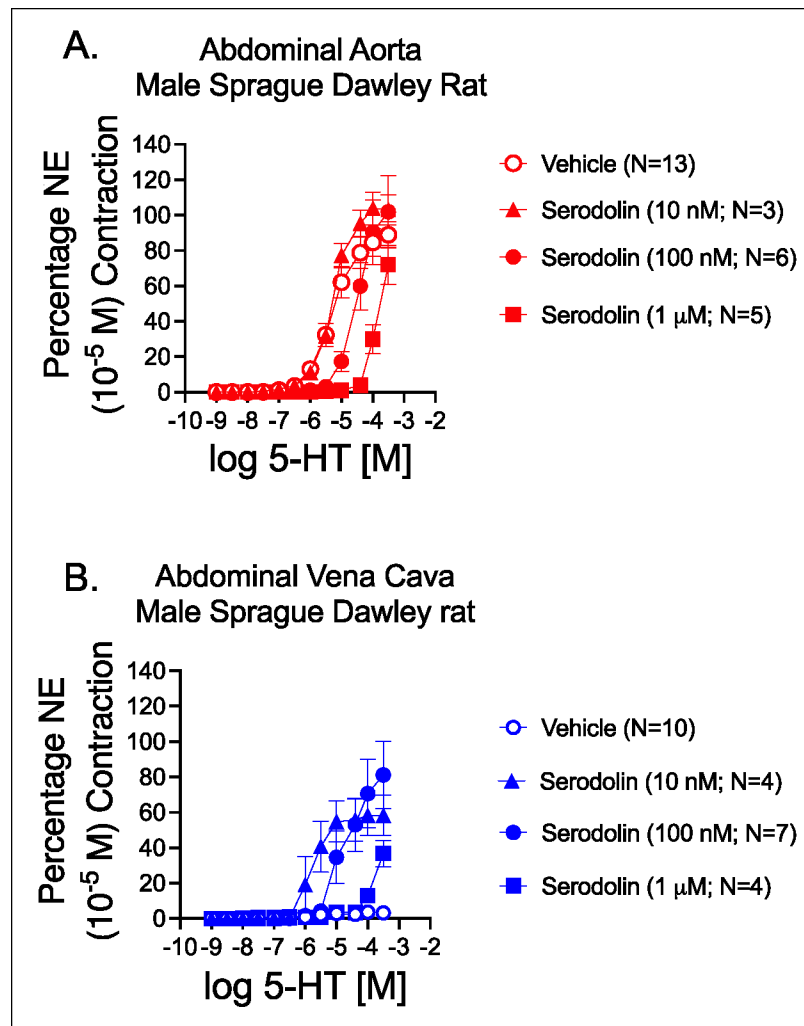
Figure 3.
Effect of vehicle (graded DMSO) or increasing concentrations of serodolin in modifying 5-HT-induced contraction in the Abdominal Aorta (A) and Vena Cava (B) of the male Sprague Dawley rat. Points are means±SEM for number of animals stated in parentheses. (NE maxes in milligrams for aorta were (Vehicle = 2261±235; 10 nM Ser = 1867±220; 100 nM Ser = 2065±163; 1 μM Ser = 1849±85; p>0.05 by one way ANOVA. NE maxes for vena cava were: Vehicle = 393±96; 10 nM Ser = 606±117; 100 nM Ser = 509±106; 1 μM Ser = 260±109; p0.05 by one way ANOVA).
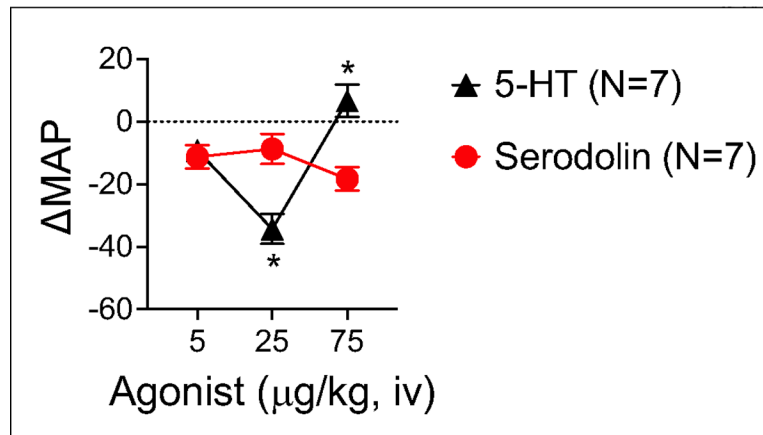
Figure 4.
Comparison of change in mean arterial blood pressure (MAP) caused by infusion of 5-HT and serodolin when given *iv* in a dose-dependent fashion to anesthetized Sprague Dawley male rat. Points represent means±SEM for number of animals in parentheses. * signifies statistical differences calculated through two way ANOVA.
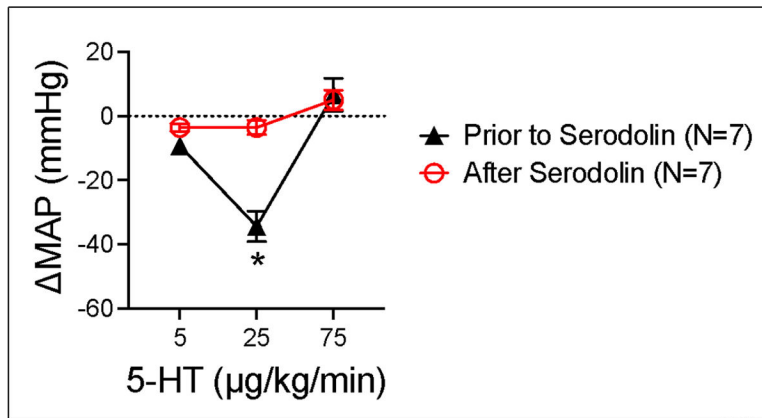
Figure 5.
Antagonism of 5-HT-induced hypotension by serodolin (75 μg/kg) when 5-HT was administered *iv* in a dose-dependent fashion to anesthetized Sprague Dawley male rat. Points represent meansαSEM for number of animals in parentheses. * signifies statistical differences calculated through two way ANOVA.

Table 1.

Potencies and Maximal contraction of 5-HT in vessels incubated with vehicle or serodolin. Potency is reported as $-\log EC_{50}$ [M] and maximums as a percentage of initial NE (10 μM) induced contraction. Reported as means±SEM.

Tissue	Vehicle	10 nM Ser	100 nM Ser	1 μM Ser
Abdominal Aorta ($-\log EC_{50}$)	5.17±0.15 0/13 unstable	5.27±0.04 (0/3 unstable)	4.49±0.07[*] (1/6 unstable)	3.86±0.06[*] (0/5 unstable)
Abdominal Vena Cava ($-\log EC_{50}$)	5.36+0.48 (7/10 unstable)	5.73+0.17 (0/4 unstable)	4.93+0.13 (1/7 unstable)	3.99 (2/4 unstable)
Abdominal Aorta Maximum (%)	89.0±7.2	103.1±8.5	101.8±20.4	71.8±11.1
Abdominal Vena Cava Maximum (%0	3.2±1.7	58.3±11.4[*]	81.2±19.2[*]	36.6±7.7

[*] signifies statistically significant differences (p<0.05) from vehicle as determined by a one way ANOVA followed by Tukey's multiple comparisons.

• Unstable means Graphpad Prism could not calculate a value. The frequency of this occurrence is reported in the table with the denominator equivalent to the number of tissues tested.