HHS Public Access

Author manuscript

J Cardiovasc Pharmacol. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

J Cardiovasc Pharmacol. 2021 August 01; 78(2): 319–327. doi:10.1097/FJC.0000000000001057.

The 5-HT_7 receptor restrains 5-HT-induced 5-HT_{2A} mediated contraction in the isolated abdominal vena cava

Romina Gonzalez-Pons, BS[*], **Kiera McRae, BS**[*], **Janice M Thompson, BS**, **Stephanie W. Watts, PhD**

Department of Pharmacology and Toxicology, Michigan State University, East Lansing MI 48824

Abstract

Though discovered as a vasoconstrictor, 5-hydroxytryptamine (5-HT, serotonin) infused into man and rodent reduces blood pressure. This occurs primarily through activation of 5-HT_7 receptors and, at least in part, venodilation. Vascular mechanisms by which this could occur include direct receptor activation leading to vasodilation and/or suppression of contractile 5-HT receptor activation. The present study tests the hypothesis that the 5-HT_7 receptor restrains activation of the 5-HT_{2A} receptor. A sub hypothesis is whether agonist-induced activation of the 5-HT_7 receptor -- independent of constitutive activity -- is necessary for this restraint. The isolated abdominal aorta and vena cava from the normal male Sprague-Dawley rat was our model. Studies used real time PCR and a pharmacological approach in the isolated tissue bath for measurement of isometric tone. While 5-HT_{2A} receptor mRNA expression in both aorta and vena cava was significantly larger than that of the 5-HT_7 receptor mRNA, the 5-HT_7/5-HT_{2A} receptor mRNA ratio was greater in the vena cava (0.30) than in the aorta (0.067). 5-HT_7 receptor antagonism by SB266970 and DR 4458 increased maximum contraction to 5-HT in the isolated vein by over 50% *vs* control. The 5-HT_{2A} receptor agonists TCB-2 and NBOH were more potent in the aorta compared to 5-HT but less efficacious, serving as partial agonists. By contrast, these same three agonists caused no contraction in the vena cava isolated from the same rats up to a 10 μM agonist concentration. Antagonism of the 5-HT_7 receptor by SB269970 did not increase either the potency or efficacy of TCB-2 or NBOH. These data support that the 5-HT_7 receptor itself needs to be stimulated to reduce contraction and that there is little constitutive activity of the 5-HT_7 receptor in the isolated abdominal vena cava.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important hormone, conserved in species through the ages[1]. In contrast to its discovery as a vasoconstrictor[2], we discovered that chronic 5-HT infusion (week-long) reduces blood pressure of a normal, freely moving rat[3,4]. Our studies came after published reports that supported the ability of acute administration (minutes) of the 5-HT precursor 5-hydroxyryptophan [5,6], 5-HT [7,8] or the 5-$HT_{1A/7}$ receptor agonist 5-carboxamidotryptamine (5-CT) [9, 10] reduced blood pressure of normotensive

Correspondence addressed to: Stephanie W Watts PhD, 1355 Bogue Street Rm B445, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824-1317, wattss@msu.edu, Telephone: 517 353 3724.

[*]contributed equally to this work

rats. The acute ability of 5-HT to reduce blood pressure also occurred in genetic and experimental models of high blood pressure/hypertension [11–14]. Because of the potential therapeutic importance of this finding, we are dedicated to determining the mechanism(s) by which 5-HT-induced hypotension occurs. Pharmacological approaches strongly support that activation of the 5-HT_7 receptor mediates the hypotension caused by 5-HT infusion [15–17]. This is in part mediated by venodilation. Specifically, 5-HT causes a 5-HT_7 receptor-dependent relaxation in isolated veins and venodilation *in vivo* [16, 17, 18, 19]. Arteries of a parallel diameter size do not dilate either *in vitro* or *in vivo*. The 5-HT_7 receptor is largely accepted as being coupled to G_s, activation of which initiates a signaling event (adenylate cyclase stimulation) consistent with mediating vascular relaxation upon receptor stimulation.

We recently created the 5-HT_7 receptor KO rat as a tool to further test the hypothesis that the 5-HT_7 receptor mediated 5-HT-induced hypotension in the rat[20]. Indeed, our experimental findings were consistent with this hypothesis. The 5-HT_7 receptor KO rat showed no hypotension to chronic infusion with 5-HT [21]. Moreover, isolated veins from the KO rat did not relax to 5-HT or 5-CT. Important to the present study, a function of the 5-HT_7 receptor we had not predicted was revealed in this initial study of the 5-HT_7 receptor KO rat. Specifically, 5-HT-induced maximum contraction in isolated veins was profoundly *increased* in the KO *vs* WT. By contrast, 5-HT-induced maximum contraction in isolated arteries was not changed in the KO vs WT. The elevated contraction, unmasked by loss of the 5-HT_7 receptor, was mediated by the 5-HT_{2A} receptor because the 5-$HT_{2A/2C}$ receptor ketanserin antagonized this unmasked contraction. By comparison, NE-induced contraction in these same veins from the KO *vs* WT was not different: both NE potency and NE maximum contraction were similar between the KO vs WT. We interpreted these data to suggest that the 5-HT_7 receptor masks 5-HT_{2A} receptor-induced activation. This idea has merit given the observation that *in vivo*, the pressor response to 5-HT administered acutely was dose-dependently elevated in the 5-HT_7 receptor KO vs WT, paralleling the unmasking of the contraction observed *in vitro* [21].

The present study formally tests the hypothesis that the 5-HT_7 receptor restrains activation of the 5-HT_{2A} receptor and does this in a vein but not artery. A sub hypothesis and important nuance is the necessity of the 5-HT_7 receptor being *activated* to cause such restraint. This is of specific interest relative to the 5-HT_7 receptor because of the numerous reports describing this receptor having constitutive activity[22–31]. If the 5-HT_7 receptor in the vein is constitutively active, agonist stimulation would not be necessary for this receptor to exert a biological effect. We continue to use the abdominal aorta and vena cava from the normal male Sprague-Dawley rat as our model. The abdominal vessels are of the size that we could derive sufficient tissues from one animal to serve as a vehicle and antagonist-incubated tissues. Additionally, outcomes in the artery and vein from the same animal could be compared directly, as well as compared to studies done our 5-HT_7 receptor KO rat[21].

Methods

Animals

The Michigan State University Institutional Animal Use and Care Committees (IACUC) approved all protocols. Male Sprague Dawley rats (225–250 g, 8–12 weeks of age, Charles

River Laboratories) were the focus of this study given that males and females display qualitative and quantitative similar responses to infused 5-HT, and relaxation/contraction in isolated vessels[21], including a significantly enhanced contraction in the isolated abdominal vena cava in the 5-HT_7 receptor KO rat when compared to that of the WT [21].

Tissue preparation

Rats were anesthetized with pentobarbital (60–80 mg/kg i.p.) and the abdominal vena cava and aorta were dissected out together. The abdominal aorta (Ab A) was cannulated on a wire on a silastic coated dish filled with physiological salt solution (PSS) containing (mM): NaCl 130; KCl 4.7; KH_2PO_4 1.18; $MgSO_4 \cdot 7H_2O$ 1.17; $NaHCO_3$ 14.8; dextrose 5.5; $CaNa_2EDTA$ 0.03, $CaCl_2$ 1.6 (pH 7.2). The abdominal vena cava (Ab VC) was embedded in the fat around the artery and was removed first under a microscope. The remaining Ab A was cleaned of perivascular adipose tissue and cut into rings. The Ab VC was then cannulated on the wire, cleaned of perivascular adipose tissue and used in one of the protocols described below. The endothelium was left intact in all vessels.

Real-Time RT-PCR

From fat-cleaned Ab A and Ab VC, total RNA was isolated using the RNeasy Fibrous Tissue Mini Kit (Qiagen, cat. # 74707) and reverse transcribed with the High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, cat. # 4368814). Standard real-time RT-PCR was done with a QuantStudio 7 Flex Real Time PCR System (Applied Biosystems) and PerfeCTa Fast Mix II (Quanta Bio, cat. # 95119–012). Rat primers were purchased from ThermoFisher Scientifi : 5-HT_{2A} (cat. # Rn00568473_m1), 5-HT_7 (cat. # Rn00576048_m1), and calibrator control (beta-actin) (cat. # Rn00667869_m1). PCR conditions were: 95°C for 20 seconds followed by 40 cycles of (95°C, 1 sec; 60°C, 20 sec). No template controls (NTC) were run for each primer set.

Isolated tissue bath

Endothelium-intact rings of Ab A and Ab VC (3–5 mm wide) were mounted in warmed (37°C) and aerated (95% O_2, 5% CO_2) tissue baths (30 mL PSS) on Grass isometric transducers (FT03; Grass instruments, Quincy, MA, USA), connected to an ADInstruments PowerLab (ADInstruments, Colorado Springs, CO, USA) for measurement of isometric contraction. Tissues were placed under optimal resting tension (Ab A – 4 grams; Ab VC – 1 gram) and allowed to equilibrate for 1 hr before an initial challenge with a maximal concentration of norepinephrine (NE; 10 μM). After this challenge, tissues were washed until tone returned to baseline.

Serotonergic agonists.—Increasing concentrations of serotonin receptor agonists were added to the bath in a cumulative fashion (10^{-10} – 10^{-5} M). Agonists tested include (primary selectivity in parentheses): 5-HT, TCB-2 (5-HT_{2A}) or NBOH-2C-CN (NBOH; 5-HT_{2A}). At the end of each experiment, NE (10 μM) was added to be sure that tissues were still viable.

Serotonergic antagonists: Tissues were incubated with either vehicle (water, 0.01% DMSO), MDL 11,939 (100 nM; 5-HT_{2A}), SB269970 (1 μM; 5-HT_7) or DR4485 (50 nM;

5-HT$_7$) for 60 minutes prior to generating a cumulative concentration response curve to a serotonergic agonist.

Test of 5-HT$_7$ receptor stimulation on NE contraction: Tissues were, after initial challenge of NE, washed and then a full concentration response curve to NE (10^{-9} – 3×10^{-5} M) performed. Tissues were washed to baseline, and rested another 30 minutes. Tissues then incubated with either vehicle (water) or the 5-HT$_{1A/7}$ receptor agonist 5-CT (1 μM) for 10 minutes. 5-CT, at this concentration and within this time, caused abdominal vena cava relaxation that is 5-HT$_7$ receptor-dependent[19]. A NE concentration response curve was repeated. Data for only the 2nd NE curves (vehicle-incubated *vs* 5-CT-incubated) are reported.

Materials

5-HT creatinine sulfate and norepinephrine hydrochloride were obtained from Sigma Chemical Company (St. Louis, MO USA). 5-CT, SB269970, DR4485, TCB-2, NBOH and MDL 11,939 were purchased from Tocris (R & D systems, Minneapolis, MN, USA).

Statistical analysis

All quantitative data are reported as means ± SEM for number of animals in parentheses. N represents the number of biological replicates (e.g. individual animals). 5-HT receptor mRNA expression is expressed relative to beta actin. 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). 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. pK$_B$ values, the apparent antagonist dissociation constant for an antagonist, were quantified 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.

Results

Vena cava expresses a higher ratio of 5-HT$_7$ to 5-HT$_{2A}$ receptor mRNA *vs* aorta

5-HT$_{2A}$ and 5-HT$_7$ receptor mRNA were measured and quantitatively compared in the Ab VC and Ab A. Figure 1 depicts that 5-HT$_{2A}$ receptor mRNA was expressed in both abdominal vessels (**1A**: aorta; **1B**; vena cava). By contrast, 5-HT$_7$ mRNA was not detected in the Ab A (figure 1A). This was different from the Ab VC in which a small but

reproducible magnitude of 5-HT$_7$ receptor mRNA was observed in a large sample size (N>10 different rats).

Pharmacological antagonism of the 5-HT$_7$ receptor increased maximum contraction to 5-HT in vena cava but not aorta

Two 5-HT$_7$ receptor antagonists with different structures were tested for their ability to unmask 5-HT-induced contraction in the isolated vessels. SB269970 (figure 2B) increased the maximum contraction in the isolated Ab VC from 41±6 to 72±5 % of NE contraction at the highest concentration of 5-HT given. Potency of 5-HT in the Ab VC was not statistically significant between vehicle (-log EC$_{50}$ [M] = 6.75±0.45) and SB269970-incubated tissues (6.50±0.30). By contrast, SB266970 modestly reduced the potency of 5-HT in the paired Ab A (Vehicle = 6.22±0.15; SB269970 = 5.97±0.16) and did not increase maximum contraction to 5-HT (figure 2A). A chemically different 5-HT$_7$ receptor antagonist, DR4485, was next used. Figure 3A shares a representative tracing of the Ab VC contraction to a maximum concentration of NE (inset, left side) and the 5-HT-induced contraction in the presence of vehicle and DR4485. Quantified in figure 3B, DR4485, similar to SB269970, revealed a greater 5-HT-induced maximum contraction in the Ab VC and also modestly but significantly increased the potency of 5-HT (Vehicle = 5.96±0.03; DR 4485 = 6.32±0.14; $p < 0.05$ for a one-way Students unpaired t test). Thus, when 5-HT is used as an agonist, inhibition of the 5-HT$_7$ receptor results in a greater 5-HT$_{2A}$ receptor contraction.

Use of 5-HT$_{2A}$ receptor agonists

We next tested whether 5-HT$_{2A}$ receptor-mediated contraction would be elevated by 5-HT$_7$ receptor blockade alone. This was done by using two different 5-HT$_{2A}$ receptor agonists, TCB-2 and NBOH, as contractants in the presence of 5-HT$_7$ receptor blockade. In this experiment, the 5-HT$_7$ receptor is not purposefully activated because the agonists used are not known to nor designed to have affinity for the 5-HT$_7$ receptor. Thus, this experiment tests whether pharmacological blockade of the 5-HT$_7$ receptor alone and independent of stimulation is sufficient to unmask the contractile activity of the 5-HT$_{2A}$ receptor.

Prior to this, the 5-HT$_{2A}$ receptor agonism of these two agonists needed to be validated. We compared contraction of TCB-2 and NBOH to that of 5-HT. In the isolated Ab A, TCB-2 threshold for contraction was at a lower concentration than 5-HT and was a partial agonist, reaching only 35% of the maximum caused by 5-HT (figure 4A). The potency of TCB-2 could not strictly be calculated because contraction did not attain a maximum; contraction above 10 μM TCB-2 cannot be blocked by 5-HT$_{2A}$ receptor antagonism (not shown) and should be considered non-specific. Using the estimate of the response to 1 μM TCB-2 as a maximum, the -log EC$_{50}$ value [M] would be equal or greater than ~6.5. NBOH was also more potent than 5-HT and a partial agonist, causing less than 20% of the maximum contraction to 5-HT. Contraction to NBOH was biphasic, diminishing above a 1 μM concentration. For NBOH, an -log EC$_{50}$ value of 7.75±0.12 can be calculated using the 1 μM response as a maximum.

In the Ab VC, all three agonists up to a 10 μM concentration did not cause concentration-dependent contraction, and potency values could not be calculated (figure 4B). Notably,

the lack of contraction to 5-HT in these specific tissues differs from the 5-HT-induced contraction in Ab VC shown in figures 2 and 3. Above 10 μM, TCB-2 caused a contraction. These same tissues contracted to NE given at the end of the agonist curve [Initial NE contraction (milligrams) = 517.7±69.1; final contraction = 462.6±63.4; p> 0.05 by two tailed paired Student's t test). Thus, the Ab VC can contract, just not to these serotonergic agonists.

The ability of TCB-2 and NBOH mediate contraction through activation of a 5-HT_{2A} receptor was tested by determining whether the specific 5-HT_{2A} receptor antagonist MDL-11,939 rightward shifted agonist-induced contraction. Indeed, MDL-11,939 rightward shifted contraction to both TCB-2 (figure 5A) and NBOH (figure 5B) in the Ab A. The apparent antagonist dissociation constant (pK_B [M]) for MDL-11–939 against NBOH was 8.64±0.10 and estimated for TCB-2 as 8.71±0.12. The quantitatively similar pK_B values for these two different agonists support that the modest contraction stimulated by them is likely 5-HT_{2A} receptor-dependent. MDL 11,939 did not block the contraction to TCB-2 at concentration above 10 μM in the Ab VC (not shown). Thus, effects of TCB-2 at this high concentration are likely not 5-HT_{2A} receptor mediated.

Pharmacological antagonism of the 5-HT_7 receptor did not increase maximum contraction to TCB-2 nor NBOH in abdominal vena cava

Having established that contraction caused by TCB-2 and NBOH was mediated by activation of 5-HT_{2A} receptors, we examined the ability of the 5-HT_7 receptor antagonist SB269970 to unmask contraction in the Ab VC to these agonists. Contraction to TCB-2 below 10 μM was modestly but not significantly elevated in the presence of SB269970 (figure 6A). Similarly, contraction to NBOH was not revealed by SB269970 antagonism. Out of six different tissues that comprised this experimental group only one demonstrated contraction to NBOH in the presence of SB269970 (figure 6B). All tissues in the experiment testing SB269970 antagonism against NBOH contracted to NE at the end of the experiment, supporting that the observation of a lack of contraction to NBOH in the presence of 5-HT_7 receptor antagonism is specific to this agonist.

Pharmacological agonism of the 5-HT_7 receptor did not modify NE-induced contraction

In a final set of experiments, we tested whether activation of the 5-HT_7 receptor could reduce contraction to NE. If so, this would support this 5-HT receptor, when activated, as potentially having significant influence over normal vascular tone (e.g. NE mediated). 5-CT was used as a 5-$HT_{1A/7}$ receptor agonist known to relax the Ab VC through activation of the 5-HT_7 receptor[19]. Incubation (10 min) with 5-CT did not reduce either the potency (-log EC_{50} [M]; Vehicle = 6.20±0.08; 5-CT = 6.12±0.05; p>0.05 one way Student's t test) or maximum (% NE [10 μM] contraction; Vehicle = 134±20; 5-CT = 131±24, p>0.05 one way Student's t test). Thus, activation of the 5-HT_7 receptor is not sufficient to reduce contraction to NE, data which supports restricted functions of the 5-HT_7 receptor to mediating serotonergic receptor changes in vascular tone.

Discussion

Cardiovascular diseases (CVDs) have, for decades, been the number one killer of adults. Though treatments for diseases have helped reduce CVD mortality, CVDs, such as hypertension and heart failure, continue to need an expanded portfolio of therapeutics. This need is epitomized by the lack of effective therapies for congestive heart failure and knowledge that control of blood pressure is regressing[32]. The difficulties in treating these diseases may occur for many reasons. Lack of compliance in medication consumption is one, for example. In the specific instance of blood pressure regulation, it must be considered that we have not yet discovered mechanism(s) of blood pressure regulation that could be exploited for new therapeutics. Our laboratory is dedicated to understanding the mechanisms by which 5-HT – an untapped hormone as far as cardiovascular disease is considered - reduces blood pressure. The 5-HT_7 receptor mediates 5-HT-induced fall in blood pressure, and the present study determines whether and how this receptor restrains activation of the 5-HT_{2A} receptor. The 5-HT_{2A} receptor is well established as a receptor which mediates systemically stimulated elevations in blood pressure through, in part, arterial contraction[2].

Ab VC express a higher level of 5-HT_7/5-HT_{2A} mRNA.

We provide the first molecular evidence that there is a significant difference in the ratio of 5-HT_7/5-HT_{2A} receptor mRNA in the isolated Ab VC *vs* Ab A. These calculated ratios are 0.067 for the artery and 0.30 for the vein. There is no doubt that expression of the 5-HT_7 receptor mRNA is significantly lower than that of the 5-HT_{2A} receptor in both vessels. However, if these transcripts are equivalently translated, then the vein has a higher 5-HT_7/5-HT_{2A} receptor ratio. It would be ideal to test this idea at the level of the receptor protein. However, we have not identified a 5-HT_7 receptor antibody, though many have been tested, that specifically identifies said receptor in either immunohistochemical or Western analyses[20]. This is a long-standing problem with G protein coupled receptors[33]. We have not examined the 5-HT_{2A} receptor protein to the same degree but have similar experience in not being able to use available 5-HT_{2A} receptor antibodies with confidence. This remains a severe limitation to the field and will be discussed more later.

5-HT_7 receptor antagonism unmasks 5-HT but not TCB-2 or NBOH-induced vena cava contraction

The most important finding relative to our hypothesis is that antagonism of the 5-HT_7 receptor amplified 5-HT- but neither TCB-2[34] nor NBOH[35]-induced venous contraction. We interpret these data to mean that to restrain 5-HT-induced contraction, the 5-HT_7 receptor must be stimulated. This leads to an important idea within the area of research for the 5-HT_7 receptor. Multiple studies have described significant constitutive activity of the 5-HT_7 receptor[22–31]. The 5-HT_7 receptor is pre-coupled to G_s, resulting in the receptor being active in the absence of an agonist. This could be the case in the Ab VC and would mean that agonist-induced activation of the 5-HT_7 receptor would not be necessary for causing a biological effect. However, two findings suggest this is not the case. First, when SB269970 at the 1 μM concentration was added to the isolated vena cava, we observed no change in tone and would expect a contraction if constitutive activity were present. This is also true for DR4485. SB269970 has been reported to act as a quasi-full inverse

agonist in Chinese hamster ovary cells expressing human recombinant 5-HT_7 receptors[29] and in HEK-293F cells expressing rat or human 5-HT_7 receptors[31]: Little is known about DR4485 displaying inverse agonism. Second, antagonism of the 5-HT_7 receptor alone was not sufficient to elevate contraction to TCB-2 and NBOH. We cannot yet determine if the elevated contraction to 5-HT, observed in both vena cava from the 5-HT_7 receptor KO rat and the normal rat exposed to 5-HT_7 receptor antagonists, is simply removal of relaxant receptor. This is one possible explanation for the outcomes observed. This explanation does not invoke interaction of the 5-HT_{2A} receptor (mediating contraction) and 5-HT_7 receptor (mediating relaxation) at any level other than physiological. No matter the means of interaction – physical or physiological - activation of the 5-HT_7 receptor suppressed the outcome of the 5-HT_{2A} receptor. Importantly, 5-HT has a significantly higher affinity (1–5 nM) for the 5-HT_7 receptor than for the 5-HT_{2A} receptor (200–500 nM; https://pdsp.unc.edu/databases/kidb.php). Circulating concentrations of 5-HT (free, not platelet stored) are in the range to activate the 5-HT_7 receptor endogenously (~40 nM) but not the 5-HT_{2A} receptor[3]. Thus, this receptor interaction is of physiological and potentially therapeutic relevance.

We observed an inherent variability in the maximum contraction to 5-HT in the isolated vena cava. Comparison of responses to 5-HT in figure 2, figure 3 and figure 4 shows significant differences in the maximum contraction, from near zero in figure 4 to close to 50% in figures 2 and 3. Importantly, these studies were carried out by three different individuals, all of whom observed this variability. The animals used in all these experiments came from the same vendor, 5-HT was from the same supplier and same lot, so it is difficult to conclude causes of such a difference outside of inherent variability. Moreover, different segments of the Ab VC were randomized to experimental groups, making it unlikely that there are anatomical differences in 5-HT receptor expression. More careful studies need to be done to ifurther nvestigate this idea. Similarly, there was variability in the response of the isolated vena cava of the 5-HT_7 receptor WT rat, made on a Sprague-Dawley background. We can speculate that humans might also share such a variability, with a potential impact on how individuals might regulate blood pressure through the 5-HT_7 receptor. Several reports have described variants in the 5-HT_7 receptor gene in humans with alcoholism[36] and cortisol levels in African American adults[37]. We could find no studies that investigated associations of 5-HT_7 receptor variants with aspects of the cardiovascular system.

Do the 5-HT_{2A} and 5-HT_7 receptor physically interact?

One goal is to determine if these 5-HT receptors colocalize or even heterodimerize, as has been found for the 5-HT_{1A} and 5-HT_7 receptor[38]. Such experiments would allow determination of whether the two receptor subtypes physically associate, one way they could interact to produce results observed in the vena cava. We lack, unfortunately, selective antibodies directed toward the 5-HT_7 and 5-HT_{2A} receptors such that colocalization, co-immunoprecipitation or other protein-protein interactions could be investigated. Cells, transfected to express tagged versions of these receptors, could be investigated but such a study would not be applicable to the physiological relevance of this interaction. As such, study at the vessel level is required.

Why is understanding this receptor interaction important? Clinical relevance

Our ultimate goal is to determine mechanisms by which 5-HT reduces blood pressure. We can take advantage of this mechanism(s) for treatment of cardiovascular diseases, some of which elude therapeutic management. We were not the first to demonstrate that 5-HT can reduce blood pressure. This qualitative finding goes back to some of the studies done during the discovery of 5-HT [39]. However, we, in combination with other labs, have helped focus the mechanisms of 5-HT-induced hypotension to activation of the 5-HT_7. Venous dilation, as mediated by the 5-HT_7 receptor, may play a role in hypotension but is unlikely to be the only mechanism responsible. Venous dilation cannot explain the fall in total peripheral resistance observed during the fall in blood pressure[18]. Total peripheral resistance is determined primarily by the caliber and tone of small arteries and arterioles. The literature is mixed with respect to whether 5-HT causes dilation in small (< 100-micron diameter) arteries and arterioles[40, 41]. But, it is in these smaller vessels that we hypothesize the interaction between the 5-HT_7 receptor and 5-HT_{2A} receptor could play a decisive role in control of total peripheral resistance. Our focus remains on the actions of the 5-HT_7 receptor because both genetic loss and pharmacological blockade of this receptor completely abolished the ability of 5-HT to reduce blood pressure.

This work has its greatest clinical relevance in the potential treatment of heart failure for two reasons. First, as venoconstriction is a common feature of heart failure[42], a 5-HT_7 receptor agonist used therapeutically would reduce, selectively, venoconstriction and preload to the heart. Second, because activation of the 5-HT_7 receptor reduces blood pressure, a 5-HT_7 receptor agonist would also reduce afterload. The lack of effect of 5-CT on NE-induced contraction is one piece of evidence that suggests such a treatment might have minimal effect with sympathetically mediated vascular tone, important for not inciting reflex mediated changes. Plasma levels of 5-HT have been reported as elevated in decompensated heart failure *vs* stable patients, but the reasons behind this – as well as whether this is beneficially or detrimentally compensatory – are not known [43].

Limitations

We recognize several limitations of this study. First, we focused on one pair of arteries and veins, the Ab VC and Ab A, not extending to other pairs of arteries and veins. However, the superior mesenteric vein and artery are similar in that the 5-HT_7 mediates 5-HT-induced relaxation in the vein but not the artery[19]. Our present findings may translate to these vessels, but this will have to be investigated. 5-HT-stimulated venous relaxation, as mediated by the 5-HT_7 receptor at least in part, has been observed in several different veins of different species[44–53]. The 5-HT_7 receptor could be constitutively active within other circulations. Though not studied here, the activated 5-HT_7 receptor may also contribute to reduction of blood pressure through anti-inflammatory actions[54]. Finally, this work in rodents is relevant given that the 5-HT_7 receptor mRNA and function has been observed in human vessels[55, 56].

Conclusion

5-HT-induced contraction of the isolated Ab VC was increased upon blockade of the 5-HT_7 receptor, the mRNA of which is more greatly expressed in the Ab VC compared to its paired Ab A. There was no evidence of constitutive activity of the 5-HT_7 receptor in the vein, and pharmacological blockade alone was insufficient to increase contraction mediated by specific 5-HT_{2A} receptor agonists. This work, illustrated in figure 7, is important because it delves into an interaction that may have therapeutic relevance, namely how the 5-HT_7 receptor restrains 5-HT_{2A} receptor mediated activity.

Acknowledgments

Sources of support: NIH HL151413

Disclosure of Funding: NIH HL151413

Abbreviations

5-HT	5-hydroxytryptamine, serotonin
DR4485	6-Chloro-2a-[4-[4-(4-chlorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]butyl]-2a,3,4,5-tetrahydrobenz[*cd*]indol-2(1*H*)-one hydrochloride
LY272015	1-[(3,4-Dimethoxyphenyl)methy]-2,3,4,9-tetrahydro-6-methyl-1*H*-pyrido[3,4-*b*]indole
NBOH	NBOH-2C-CN; 4-[2-[[(2-Hydroxyphenyl)methyl]amino]ethyl]-2,5-dimethoxybenzonitrile hydrochloride
SB269970	(2*R*)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine
TCB-2	(4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine

References

1. Azmitia EC. Modern views on an ancient chemical: serotonin effects on cell proliferation, maturation and apoptosis. Brain Res Bull. 200156: 413–424. [PubMed: 11750787]
2. Watts SW, Morrison SF, Davis RP, Barman SM. Serotonin and blood pressure regulation. Pharmacol Rev. 201264:359–388. [PubMed: 22407614]
3. Diaz J, Ni W, King A, Fink GD, Watts SW. 5-hydroxytryptamine lowers blood pressure in normotensive and hypertensive rats. J Pharmacol Exp Ther2008325:1031–1038. [PubMed: 18305011]
4. Tan T, Watts SW, Davis RP. Drug delivery, enabling technology for drug discovery and development. IPRECIO micro infusion pump: programmable, refillable and implantable. Front Pharmacol. 20112:44. [PubMed: 21863140]
5. Ding XR, Stier CT, Itskovitz HD. Serotonin and 5-hydroxytryptophan on blood pressure and renal blood flow in anesthetized rats. Am J Med Sci. 1989297:290–293. [PubMed: 2785758]
6. Echizen H, Freed CR. Long-term infusion of L-5-hydroxytryptophan increases brain serotonin turnover and decreases blood pressure in normotensive rats. J Pharmacol Exp Ther. 1981220:579–584.

7. Centurion D, Glusa E, Sanchez-Lopez A, Valdivia LF, Saxena PR, Villalon CM. 5-HT_7, but not 5-HT_{2B}, receptors mediate hypotension in vagosympathectomized rats. Eur J Pharmacol. 200419:239–242.
8. De Vries P, De Visser PA, Heiligers JPC, Villalón CM, Saxena PR. Changes in systemic and regional haemodynamics during 5-HT_7 receptor mediated depressor responses in rats. Naunyn-Schmied Arch Pharmacol. 1999359:331–338.
9. Balasubramaniam G, Lee HS, Mah SC. Antihypertensive effect of chronic iv administration of 5-carboxamidotryptamine in spontaneously hypertensive rats. Eur J Pharmacol. 1983l237:207–213.
10. Balasubramaniam G, Lee HS, Mah SC. 5-carboxamidotryptamine attenuates the development of deoxycorticosterone acetate-salt in rats. Eur J Pharmacol. 1995276:183–190. [PubMed: 7781688]
11. Dalton DW, Feniuk W, Humphrey PP. An investigation into the mechanisms of the cardiovascular effects of 5-hydroxytryptamine in the conscious normotensive and DOCA-salt hypertensive rats. J Auton Pharmacol. 19866:219–228. [PubMed: 3771594]
12. Baron A, Riesselmann A, Fregly MJ. Reduction in the elevated blood pressure of Dahl Salt sensitive rats treated chronically with L-5-hydroxytryptophan. Pharmacology. 199142:15–22. [PubMed: 1829234]
13. Fregly MJ, Lockley OE, and Sumners C. Chronic treatment with L-5-hydroxytryptophan prevents the development of DOCA-salt induced hypertension in rats. J Hypertens. 19875:621–628. [PubMed: 2963066]
14. Itskovitz HD, Werber JL, Sheridan AM, Brewer TF, Stier CT. 5-hydroxytryptophan and carbidopa in spontaneously hypertensive rats. J Hypertens. 19897:311–315. [PubMed: 2786023]
15. Terrón JA. Role of 5-ht7 receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat. Br J Pharmacol. 1997121: 563–571. [PubMed: 9179401]
16. Seitz BM, Orer H, Krieger-Burke T, Darios E, Thompson J, Fink GD, Watts SW. 5-HT causes splanchnic venodilation, Am J Physiol Heart Circ Physiol. 2017313:H676–H686. [PubMed: 28626072]
17. Seitz BM, Krieger-Burke T, Fink GD, Watts S W. Serial measurements of splanchnic vein diameters in rats using high frequency ultrasound, Frontiers in Pharmacology; experimental Pharmacology and Drug Discovery, 201610.3389/fphar.2016.00116
18. Davis RP, Pattison J, Thompson JM, Tiniakov R, Scrogin KE, Watts SW. 5-hydroxytryptamine (5-HT) reduces total peripheral resistance during chronic infusion: direct arterial mesenteric relaxation is not involved. BMC Pharmacol. 201212:4 doi: 10.11186/1471-2210-12-4. [PubMed: 22559843]
19. Watts SW, Darios ES, Seitz BM, Thompson JM. 5-HT is a potent relaxant in rat superior mesenteric veins. Pharmacol Res Perspect. 20153:e00103. Doi: 10.1002/prp2.103.
20. Demeriva EY, Xie H, Flood ED, Thompson JM, Seitz BM, Watts SW. Creation of the 5-hydroxytryptamine receptor 7 knockout (5-HT7 KO) rat as a tool for cardiovascular research, Physiol Gen. 201951:290–301.
21. Seitz BM, Demireva EY, Xi H, Fink GD, Krieger-Burke T, Burke WM, Watts SW. 5-HT does not lower blood pressure in the 5-HT knockout rat. Physiol Gen. 201951:302–310.
22. Andressen KW, Ulsund AH, Krobert KA, Lohse MJ, Bunemann M, Levy FO. Related GPCRs couple differently to Gs: preassociation between G protein and 5-HT_7 serotonin receptor reveals movement of Galphas upon receptor activation. FASEB J. 201832:1059–1069 [PubMed: 29079700]
23. Gellynck E, Heyninck K, Andressen KW, Haegeman G, Levy FO, Vanhoenacker P, Van Craenenbroeck K. The serotonin 5-HT_7 receptors: two decades of research. Exp Brain Res. 2013230:555–568. [PubMed: 24042216]
24. Guseva D, Wirth A, Ponimaskin E. Cellular mechanisms of the 5-HT_7 receptor mediated signaling. Front Behav Neurosci. 20148:306. Doi: 10.3389/fnbeh.2014.00306. [PubMed: 25324743]
25. Hobson RJ, Geng J, Gray AD, Komuniecki RW. SER-7b, a constitutively active Galpha S coupled 5-HT_7 like receptor expressed in the Caenorhabditis elegans M4 pharyngeal motorneuron. J Neurochem. 200387:22–29. [PubMed: 12969249]
26. Krobert KA, Levy FO. The human 5-HT_7 receptor splice variants and constitutive activity and inverse agonist effects. Br J Pharmacol. 2002135:1563–1571. [PubMed: 11906971]

27. Kvachnina E, Dumuis A, Wlodarczyk J, Renner U, Cochet M, Richter DW, Ponimaskin E. Constitutive Gs-mediated but not G12-mediated, activity of the 5-Hydroxytryptamine 7 receptor is modulated by the palmitoylation of its C-terminal domain. Biochim Biophy Acta. 20091793:1646–1655.
28. Liu P, Yin Y-L, Wang T, Hou L, Wang X-X, Wang M, Zhao G-G, Shi Y, Xu HE, Jiang Y. Ligand induced activation of Erk ½ signaling by constitutively active Gs coupled 5-HT receptors. Acta Pharmacol Sin. 201940:1157–1167. [PubMed: 30833707]
29. Mahe C, Loetscher E, Feuerbach D, Muller W, Seiler MP, Schoeffter P. Differential inverse agonist efficacies of SB-258719, SB-258741 and SB-269970 as human recombinant serotonin 5-HT_7 receptors. Eur J Pharmacol. 2004495:97–102. [PubMed: 15249157]
30. Purohit A, Smith C, Herrick-Davis K, Teitler M. Stable expression of constitutively activated mutant 5-HT6 and h5HT7 serotonin receptors: Inverse agonist activity of antipsychotic drugs. Psychopharmacol. 2005179:461–469.
31. Romero G, Pujol M, Pauwels PJ. Reanalysis of constitutively active rat and human 5-HT7(a) receptors in HEK-293F cells demonstrates lack of silent properties for reported neutral antagonists. Naunyn Schmiedebergs Arch Pharmacol. 2006374:31–39. [PubMed: 16967291]
32. Egan BM, Li J, Sutherland SE, Rakotz M. Hypertension control in the US 2009 to 2018: Rapidly reversing years of Progress. 2020 MP07, Council on Hypertension.
33. https://www.abcam.com/primary-antibodies/challenges-behind-g-protein-coupled-receptor-gpcr-antibodies
34. McLean TH, Parrish JC, Braden MR, Marona-Lewicka D, Gallard-Godoy A. Nichols ED. 1-aminomethylbenzocycloalkanes conformationally restricted hallucinogenic phenethylamine analogues as functionally selective 5-HT2A receptor agonists. J Med Chem. 200649:5794–5803. [PubMed: 16970404]
35. Jensn AA, McCorvy JD, Leth-Petersen S, Bundgaard C, Liebscher G, Kenakin TP, Brauner-Osborne H, Kehler J, Kristensen JL. Detailed characterization of the in vitro pharmacological and pharmacokinetic properties of N-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenylehtylamine (25CN-NBOH), a highly selective and brain-penetrant 5-Ht2A receptor agonist. J Pharmacol Exp Ther. 2017361:441–453. [PubMed: 28360333]
36. Hauser SR, Hedlund PB, Roberts AJ, Sari Y, Bell RL, Engleman EA. The 5-HT_7 receptor as a potential target for treating drug and alcohol abuse. Front Neurosci. 2015 doi:10.3389/fnins.2014.00448.
37. Swanson G, Mille S, Alyahyai A, Wilson B, Saadatmand F, Lee C, Dunston G, Abbas M. Genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African American young adults. F1000Reearch20176:19, last updated 17May 2019.
38. Renner U, Zeug A, Woehler A, Niebert M, Dityatev A, Dityateva G, Gorinski N, Guseva D, Abdel-Galil D, Frohlich M, Doring F, Wischmeyer D, Richter DW, Neher E, Ponimaskin EG. Heterodimerization of serotonin receptors 5-HT_{1A} and 5-HT_7 differentially regulates receptor signaling and trafficking. J Cell Sci. 2012125:2486–2499. [PubMed: 22357950]
39. Page IH, McCubbin JW. The variable arterial pressure response to serotonin in laboratory animals and man. Circ Res. 19531:354–362. [PubMed: 13059881]
40. Struyker-Boudier HA, le Noble JL, Le Noble FA, Messing MW, van Essen H. Hypertension, the microcirculation and serotonin. Clin Physiol Biochem. 1990 8 S 3:28–39.
41. Wilmoth FR, Harris PD, Miller FN. Differential serotonin responses in the skeletal muscle microcirculation. Life Sci. 198434:1135–1142. [PubMed: 6708721]
42. Wood JE, Litter J, Wilkins RW. Peripheral venoconstriction in human congestive heart failure. Circulation13:524–527. [PubMed: 13356407]
43. Selim AM, Sarswat N, Kelesidis I, Iqbal M, Chandra R, Zolty R. Plasma serotonin in heart failure: possible marker and potential treatment target. Heart Lung Circ201726:442–449. [PubMed: 27746058]
44. Chand N. 5-Hydroxytryptamine induces relaxation of goat pulmonary veins: evidence for the noninvolvement of M and D-tryptamine receptors. Br J Pharmacol. 198172:233–237. [PubMed: 7214093]

45. Cocks TM, Arnold PJ. 5-Hydroxytryptamine (5-HT) mediates potent relaxation in the sheep isolated pulmonary vein via activation of 5-HT_4 receptors. Br J Pharmacol. 1992107:591–596. [PubMed: 1422600]
46. Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS. Mediation by 5-hydroxytryptamine$_{2B}$ receptors of endothelium-dependent relaxation in rat jugular vein. Br J Pharmacol. 1995114:400–404. [PubMed: 7881740]
47. Gupta P. An endothelial 5-HT receptor that mediates relaxation in guinea-pig isolated jugular vein resembles the 5-HT_{1D} receptor. Br J Pharmacol. 1992108:703–709.
48. Ishine T, Bouchelet I, Hamel E, Lee TJ. Serotonin 5-HT(7) receptors mediate relaxation of porcine pial veins. Am J Physiol Heart Circ Physiol. 2000278:H907–H912. [PubMed: 10710360]
49. Leung E, Walsh LK, Pulida-Rios MT, Eglen RM. Characterization of putative 5-ht7 receptors mediating direct relaxation in Cynomolgus monkey isolated jugular vein. Br J Pharmacol. 1996117:926–930. [PubMed: 8851512]
50. Sumner MJ, Feniuk W, Humphrey PP. Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava. Br J Pharmacol. 198997:292–300. [PubMed: 2541857]
51. Trevethick MA, Feniuk W, Humphrey PP. 5-hydroxytryptamine-induced relaxation of neonatal porcine vena cava in vitro. Life Sci. 198435:477–486. [PubMed: 6205240]
52. Tsuru H, Nakai S, Uchiyama T, Teranishi Y. Endothelium-independent relaxant effect of 5-hydroxytryptamine (5-HT) on the isolated rabbit facial vein. J Smooth Muscle Res. 199834:101–110. [PubMed: 9972519]
53. Zhang L, Dyer DC, Fiscus RR, Grujic Z, Wang X. 5-Hydroxytryptamine induces endothelium-independent relaxations of sheep pulmonary vein: role of cyclic nucleotide. Eur J Pharmacol. 1995280:335–337. [PubMed: 8566103]
54. Guseva D, Holst K, Kaune B, Meier M, Keubler L, Glage S, Buettner M, Bleich A, Pabst O, Bachmann O, Ponimaskin EG. Serotonin 5-HT_7 receptor is critically involved in acute and chronic inflammation of the gastrointestinal tract. Inflamm Bowel Dis. 201420:1516–1529. [PubMed: 25072499]
55. Ullmer C, Schmuck K, Kalkman HO, Lubbert H. Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett. 1995370:215–221. [PubMed: 7656980]
56. Schoeffter P, Ullmer C, Bobirnac I, Gabbiani G, Lubbert H. Functional, endogenously expressed 5-hydroxytryptamine −5-ht-7 receptors in human vascular smooth muscle cells. Br J Pharmacol. 1996117:993–994. [PubMed: 8882587]

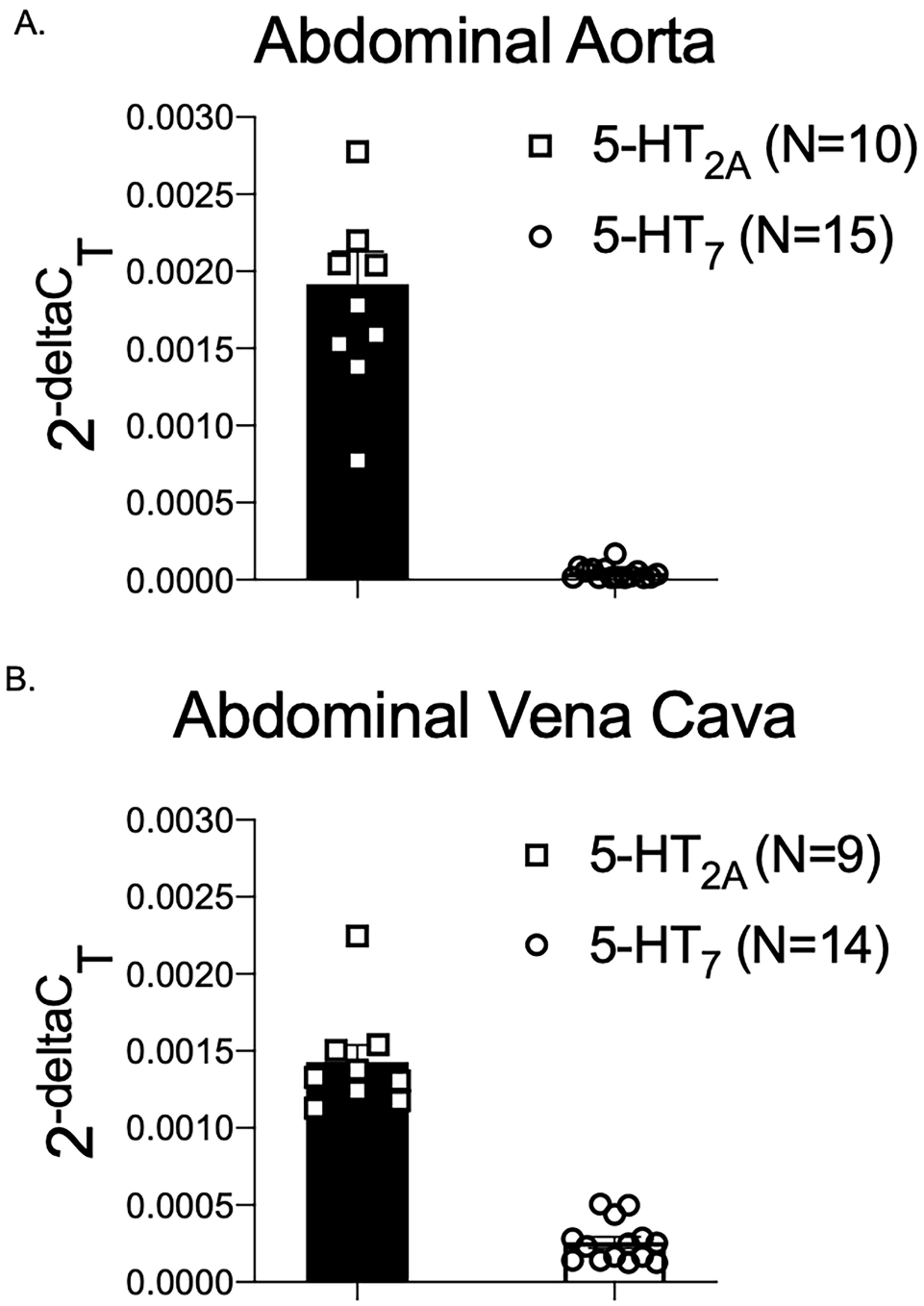

Figure 1.

Real time RT-PCR expression of 5-HT_{2A} (square) and 5-HT_7 (circle) receptor mRNA in abdominal aorta (A) and vena cava (B) isolated from male Sprague Dawley rats. Bars represent means±SEM for number of points scattered around mean, reported as biological replicates (N).

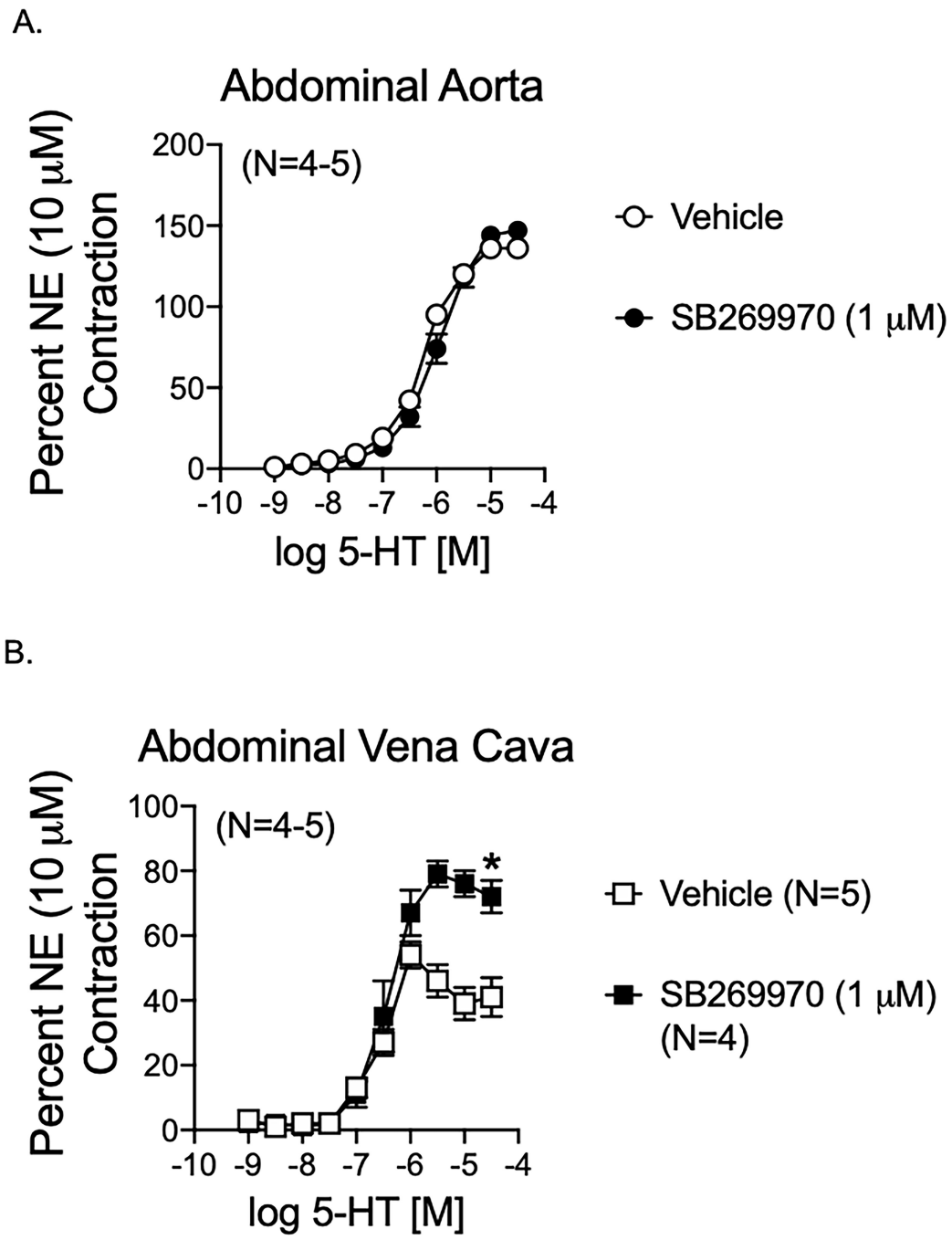

Figure 2.

Concentration-dependent contraction stimulate by 5-HT in the absence (vehicle) and presence of the 5-HT_7 receptor antagonist SB269970 (1 μM) in isolated Ab A (A) and Ab VC cava (B) from the male Sprague Dawley rat. Points represent means±SEM for the number of biological replicates reported as N. * signifies statistically significant differences ($p<0.05$, unpaired t test) between maximum responses of vehicle- and SB269970-incubated tissues.

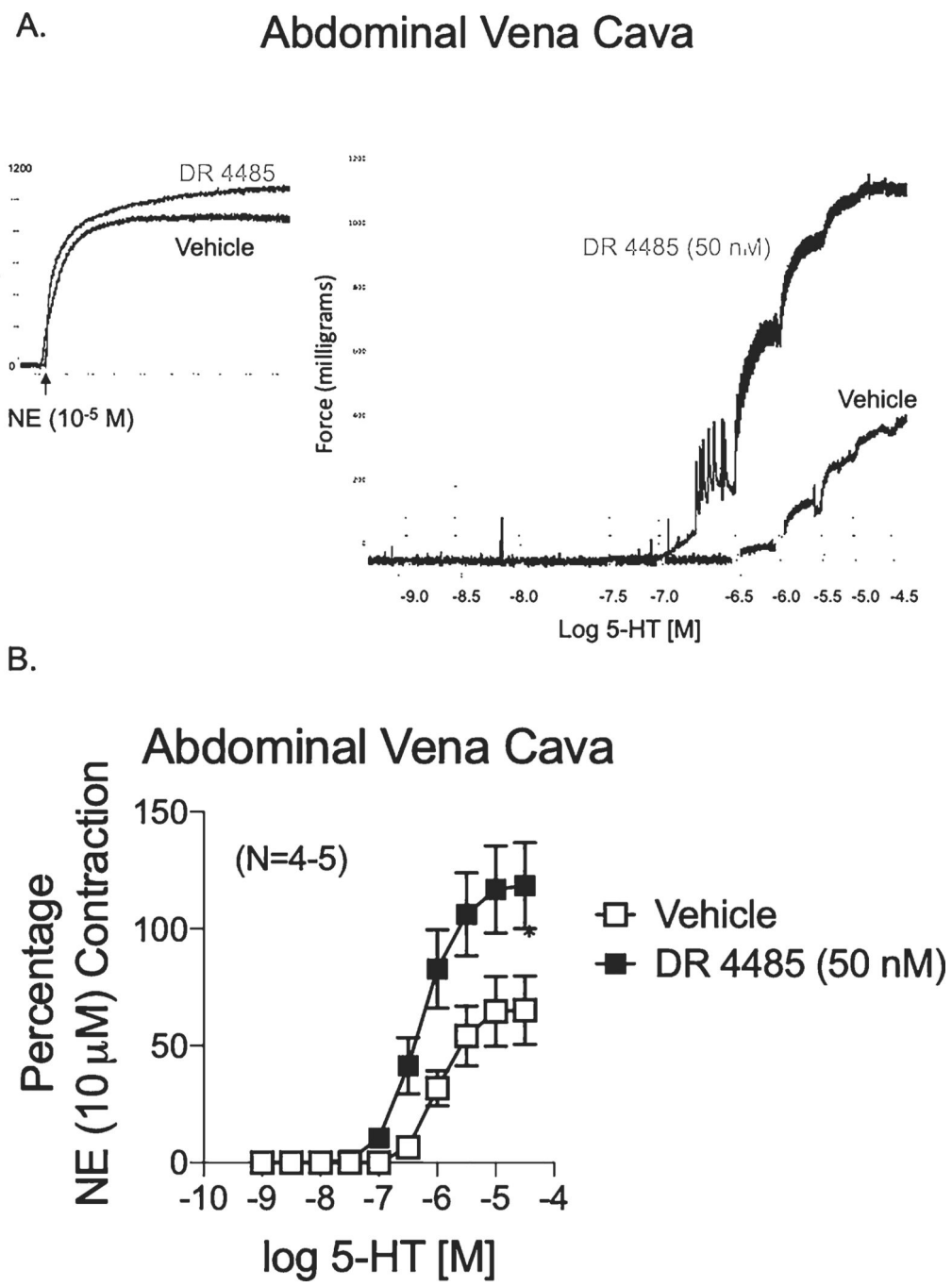

Figure 3.

A. Representative tracing of 5-HT-induced contraction in isolated rat Ab VC exposed to vehicle (black) or 5-HT_7 receptor antagonist DR 4485 (gray; 50 nM). B. Concentration-dependent contraction stimulate by 5-HT in the absence (vehicle) and presence of the 5-HT_7 receptor antagonist DR 4485 (50 nM) in Ab VC isolated from the male Sprague Dawley rat. Points represent means±SEM for the number of biological replicates reported as N. * signifies statistically significant differences ($p<0.05$, unpaired t test) between maximum responses of vehicle- and DR 4485-incubated tissues.

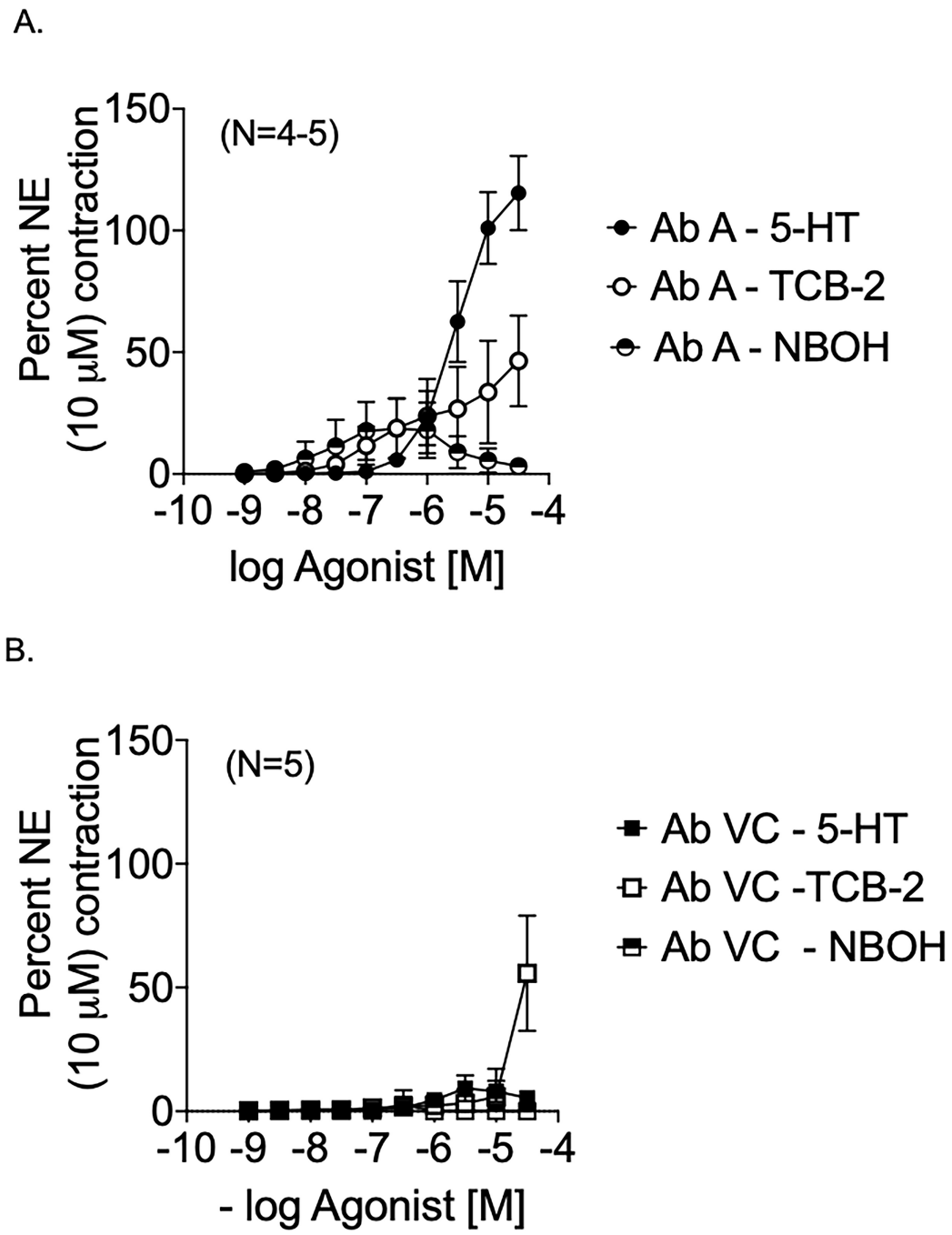

Figure 4.

Concentration dependent contraction caused by 5-HT, the 5-HT_{2A} receptor agonist TCB-2 and 5-HT_{2A} receptor agonist NBOH in isolated Ab A (A) and Ab VC (B) from male Sprague Dawley rats. Points represent means±SEM for the number of biological replicates reported as N.

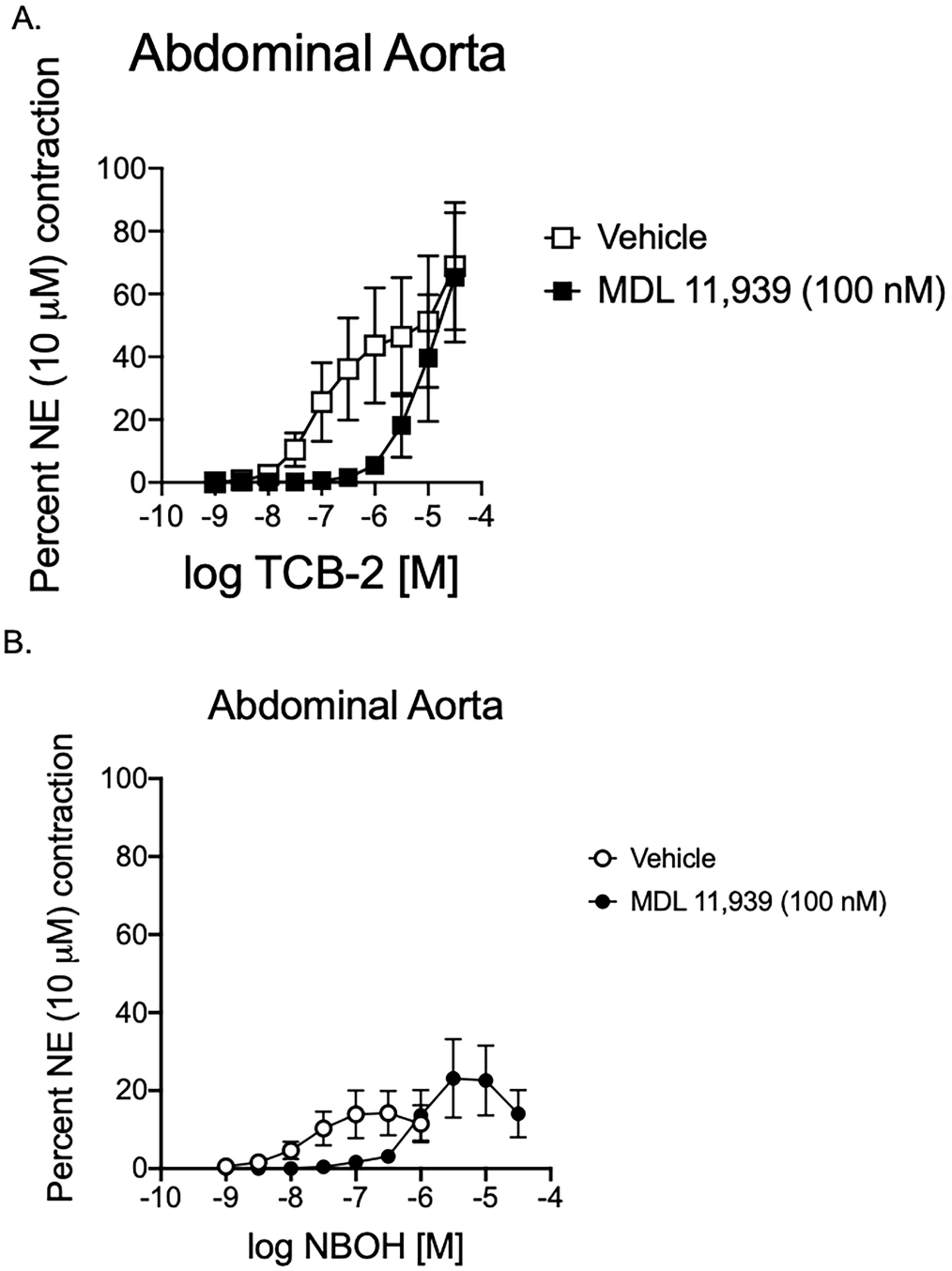

Figure 5.

Antagonism of TCB-2 (A) and NBOH (B)-stimulated Ab A contraction by the 5-HT_{2A} receptor antagonist MDL 11,939 (100 nM). Points represent means±SEM for the number of biological replicates reported as N. Tissues exposed to vehicle or MDL 11939 came from the same rat.

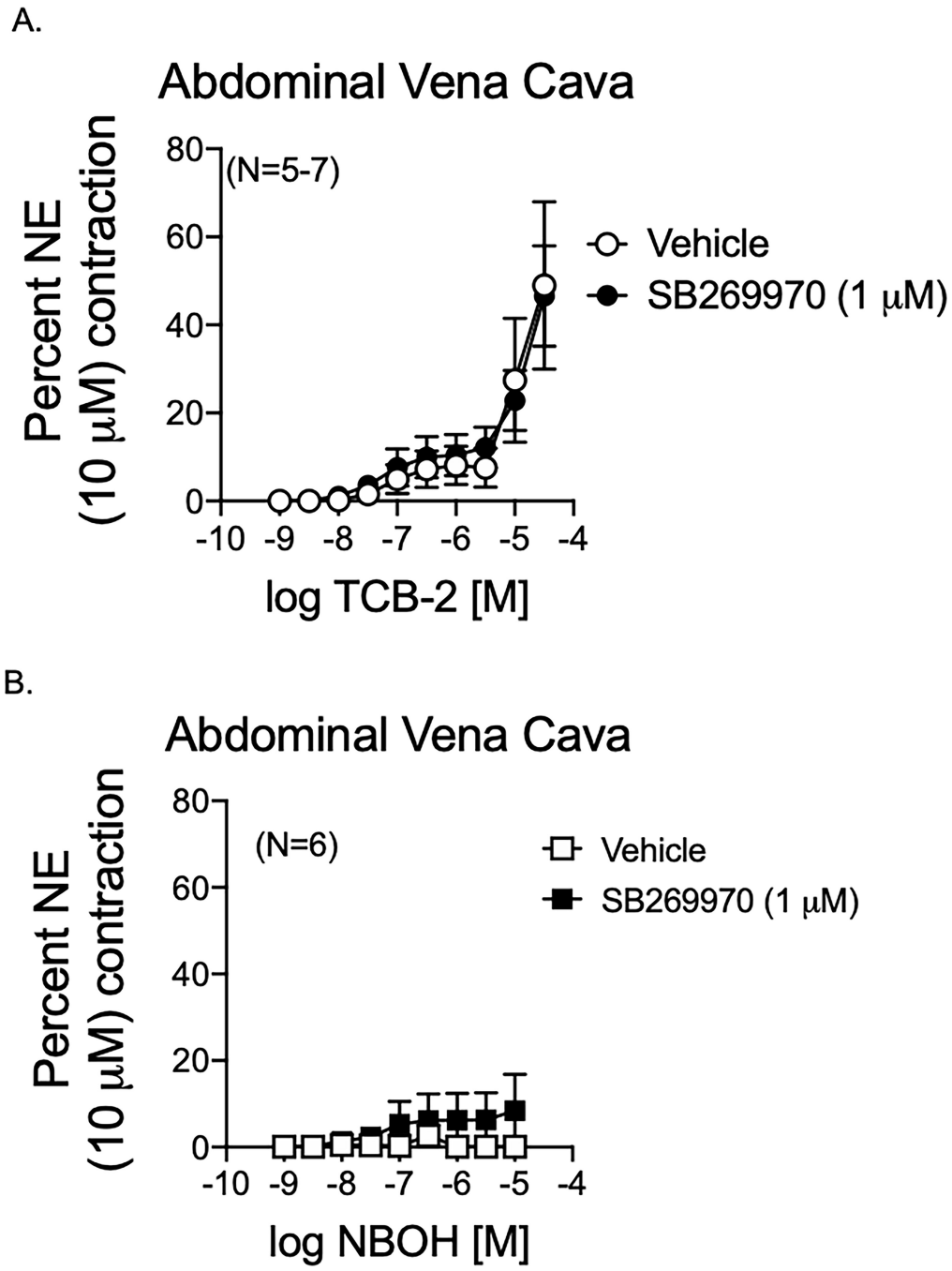

Figure 6.

Lack of effect of the 5-HT_7 receptor antagonist SB296670 (1 μM) on TCB-2 (A) and NBOH (B)-stimulated Ab VC contraction. Points represent means±SEM for the number of biological replicates reported as N. Tissues exposed to vehicle or SB269970 came from the same rat.

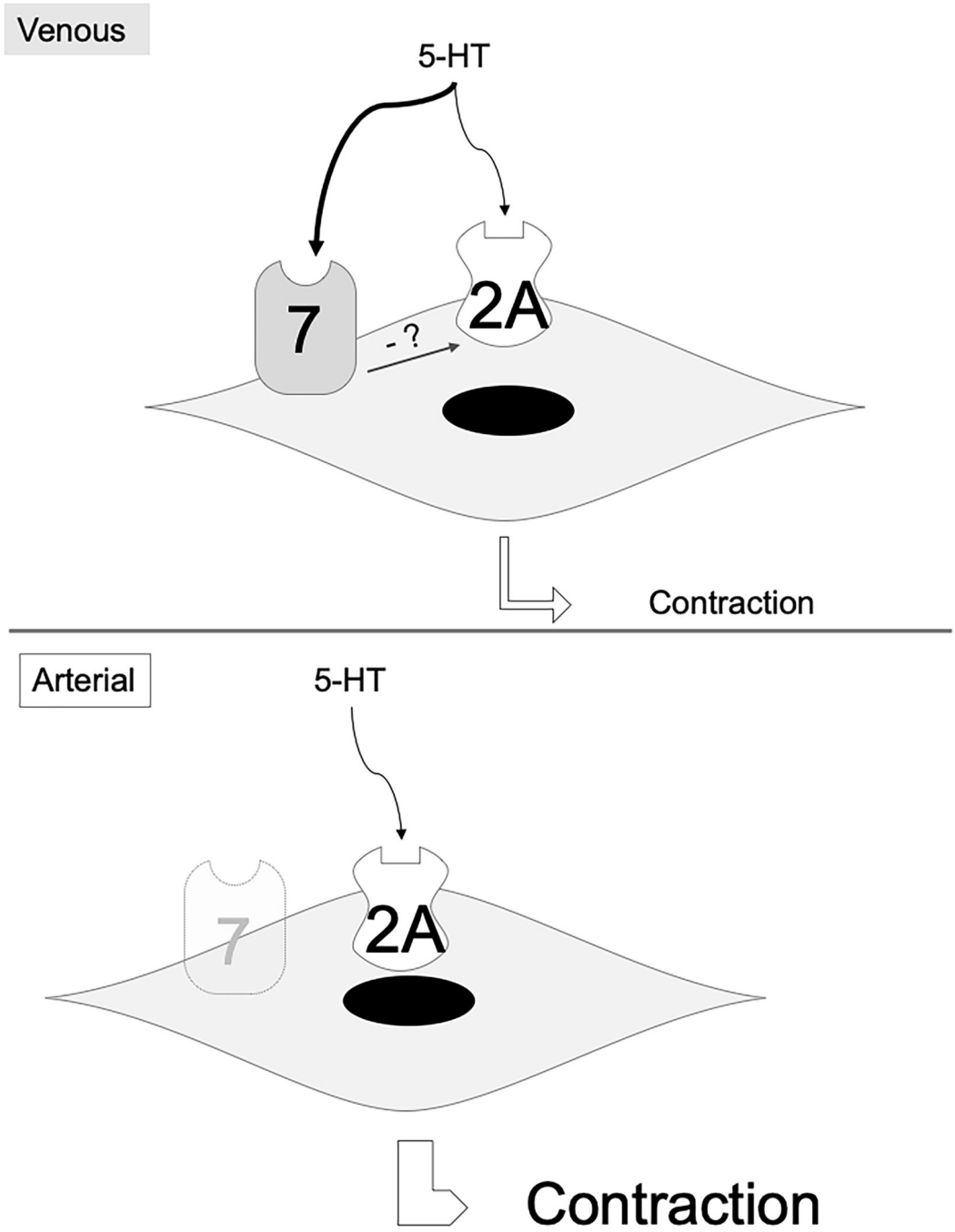

Figure 7.

Depiction of determined differences in 5-HT$_7$ receptor utilization between veins and arteries or veins without a functional 5-HT$_7$ receptor. 7 = 5-HT$_7$ receptor; 2A = 5-HT$_{2A}$ receptor. The thickness of the arrows denotes the higher affinity 5-HT has for the 5-HT$_7$ vs 5-HT$_{2A}$ receptor. The 5-HT$_7$ receptor is depicted in shadow on the arterial diagram given the lack of evidence of expression and function of this receptor.