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Serotonin Receptors in Rat Jugular Vein: Presence and Involvement in the Contraction

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ABSTRACT

Serotonin (5-hydroxytryptamine; 5-HT) is released during platelet aggregation, a phenomenon commonly observed in blood clot formation and venous diseases. Once released, 5-HT can interact with its receptors in the peripheral vasculature to modify vascular tone. The goal of this study was to perform a detailed pharmacological characterization of the 5-HT receptors involved in the contractile response of the rat jugular vein (RJV) using recently developed drugs with greater selectivity toward 5-HT receptor subtypes. We hypothesized that, as for other blood vessels, the 5-$HT_{1B/1D}$ and 5-HT_{2B} receptor subtypes mediate contraction in RJV alongside the 5-HT_{2A} receptor subtype. Endothelium-intact RJV rings were set up in an isolated organ bath for isometric tension recordings, and contractile concentration-effect curves were obtained for 13 distinct serotonergic receptor agonists. Surprisingly, the 5-HT_{1A} and the mixed 5-$HT_{1A/1B}$ receptor agonists (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronapthalene (8-OH-DPAT) and 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl) (1*H* indole) (RU24969) caused contractions that were antagonized by the 5-HT_{1A} receptor antagonist [*O*-methyl-3*H*]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100135). The contractile curve to 5-HT was shifted to the right by WAY100135, 3-[2-[4-(4-fluoro benzoyl)-piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin; 5-$HT_{2A/C}$ receptor antagonist), and 1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole hydrochloride (LY266097; 5-HT_{2B} receptor antagonist). Ketanserin also caused rightward shifts of the contractile curves to 8-OH-DPAT, RU24969, and the 5-HT_{2B} receptor agonist (α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine) (BW723C86). Agonists for 5-$HT_{1B/1D/1F}$, 5-HT_3, 5-HT_6, and 5-HT_7 receptors were inactive. In real-time polymerase chain reaction experiments that have never been performed in this tissue previously, we observed mRNA expression for the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 receptors, whereas no significant mRNA expression was found for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors. These results support the 5-HT_{2A} receptor as the main subtype targeted by 5-HT to contract the RJV.

Serotonin (5-hydroxytryptamine; 5-HT) was first described as a substance with the ability to increase smooth muscle tone (Vialli and Erspamer, 1937; Rapport et al., 1948). 5-HT is synthesized by the enterochromaffin cells and released into the circulation. In the periphery, platelets are the largest store for 5-HT. It is interesting that our laboratory recently showed that peripheral vasculature has the ability to synthesize, take up, and metabolize 5-HT (Linder et al., 2008; Ni et al., 2008). Moreover, the amount of 5-HT measured in veins is comparatively higher than in arteries, suggesting that peripheral veins also may constitute important stores of the amine (Linder et al., 2008). Thus, by controlling the amount of 5-HT within the peripheral vasculature environment, veins may exert relevant roles in adjusting vascular tone.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); DOI, (±)-2,5-dimethoxy-(−)4-iodo-2,5-dimethoxyphenylisopropylamine; PCR, polymerase chain reaction; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; 8-OH-DPAT, (±)-2-dipropyl-amino-8-hydroxyl-1,2,3,4-tetrahydronapthalene; RU24696, 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl) (1*H* indole); 5-CT, 5-carboxamidotryptamine; BRL54443, 5-hydroxy-3-(1-methylpiperidin-4-yl)-1*H*-indole; BW723C86, α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine; EMD386088, 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole hydrochloride; LP44, 4-[2-(methylthio)phenyl]-*N*-(1,2,3,4-tetrahydro-1-naphthalenyl)-1-piperazinehexanamide hydrochloride; WAY100135, [*O*-methyl-3*H*]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide; GR127935, 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic-acid[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide; LY266097, (1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole hydrochloride); SB269970, (2*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-mentyl-1-piperidinyl)ethyl] pyrrolidine hydrochloride; β2m, β-2-microglobulin; Ct, cycle threshold.

5-HT is among the mediators released during platelet aggregation, a process frequently observed in venous diseases, such as deep vein thrombosis and varicose veins (Ficarelli et al., 2008; Bailey et al., 2009). Although venous diseases may affect and decrease the quality of life in up to a quarter of the adult population (Lim et al., 2008), veins have attracted relatively little attention in terms of studies in the cardiovascular field. Once released, 5-HT can be taken up by peripheral tissues (Linder et al., 2008, 2009; Ni et al., 2008) or interact with its receptors in target tissues to induce its actions. The contraction induced by 5-HT is increased in the human varicose spermatic vein during varicocele, a venous disease (Yildiz et al., 2003).

Traditionally, the physiological actions of 5-HT are mediated by seven families of 5-HT receptors (5-HT_1–5-HT_7), with at least 15 different subtypes (Hoyer et al., 1994). The main 5-HT receptors responsible for modifying vascular tone are the 5-HT_1, 5-HT_2, and 5-HT_7 receptors. In many arterial beds, such as the rat and mouse aorta, the 5-HT_{2A} receptor is the primary receptor mediating 5-HT-induced contraction (McKune and Watts, 2001; Russell et al., 2002). Most of these findings were supported by pharmacological studies showing that agonists such as (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) and α-methyl-5-HT induce arterial contractions that are antagonized by 3-[2-[4-(4-fluoro benzoyl)-piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin). In addition to the 5-HT_{2A} receptor, the 5-$HT_{1B/1D}$ receptor and 5-HT_{2B} receptor are also involved in 5-HT-induced arterial smooth muscle contraction (Kaumann et al., 1993; Morecroft et al., 1999; Banes and Watts, 2002; Gul et al., 2003; Watts and Thompson, 2004).

Previous studies suggest that 5-HT contracts the rat jugular vein mainly by 5-HT_{2A} receptor subtype activation (Cohen et al., 1981; Cushing and Cohen, 1992). However, 5-HT receptors such as the 5-HT_6 and 5-HT_7 receptors were sequenced only after these studies. In addition, despite the development of many more selective 5-HT receptor ligands since the publication of those studies, such drugs have not yet been used to better characterize the 5-HT receptor population present in the rat jugular vein. Understanding the receptor targets of 5-HT in veins may be of relevance for the treatment of venous diseases. Therefore, the present study was undertaken to further characterize the 5-HT receptors subtypes implicated in constriction of the rat isolated endothelium-intact jugular vein, by testing the effects of some of these newer pharmacological tools in classical functional assays of isometric tension carried out in organ baths, allied to SYBR Green-based real-time PCR, a technique not used in this tissue before. We hypothesized that in addition to the 5-HT_{2A} receptor, the 5-$HT_{1B/1D}$ and 5HT_{2B} receptor subtypes also are involved in mediating contraction to 5-HT in the jugular vein from the rat.

Materials and Methods

Animal Use. Male Sprague-Dawley rats (250–300 g; Charles River Breeding Laboratories, Portage, MI) were maintained on a 12-h light/dark cycle with free access to rat chow and water. On the day of the experiment, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and both jugular veins were excised. All procedures involving animal use were performed in accordance with the Institutional Animal Use and Care Committee of Michigan State University.

In Vitro Measurement of Isometric Force Generation in Jugular Vein Rings. After removal of fat and connective tissue, endothelium-intact rings (2–4 mm long) from the proximal jugular vein (before bifurcation) were mounted in an organ chamber for isometric tension recordings and bathed in physiological salt solution (130.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.6 mM $CaCl_2$, 14.9 mM $NaHCO_3$, 0.03 mM EDTA, and 5.5 mM glucose) that was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. The jugular vein rings were set gradually at 1.0-g passive tension (optimum passive tension). After a 1-h equilibration period, vessels were contracted with 60 mM KCl to test tissue viability. Only tissues that contracted 150 mg or more were included in this study. The tissues were rinsed with physiological salt solution to wash out KCl and to return to baseline before being challenged with $PGF_{2\alpha}$ (1 μM). Preliminary data showed that the contraction induced by this concentration of $PGF_{2\alpha}$ is easily reversed upon washout and reproducible when rings are challenged at 30-min intervals (first challenge, 285.5 ± 65.9 mg; second challenge, 364.4 ± 58.2 mg; $n = 6$; $P \geq 0.05$). The integrity of the endothelium was evaluated by the ability of 10 μM acetylcholine (Sigma-Aldrich, St. Louis, MO) to relax $PGF_{2\alpha}$-contracted jugular vein rings. Only rings that relaxed 70% or more to acetylcholine were included in this study. One of the following protocols was then followed.

Response to 5-HT Receptor Agonists. Increasing concentrations of 5-HT, α-methyl-5-HT, 5-methoxytryptamine, 8-OH-DPAT, RU24969, 1-[3-(2-dimethylaminoethyl)-1*H*-indol-5-yl]-*N*-methyl-methanesulfonamide (sumatriptan), 5-CT, BRL54443, BW723C86, 4-amino-*N*-1-azabicyclo[2.2.2]oct-3-yl-5-chloro-2-methoxybenzamide (zacopride), EMD386088, LP44, or DOI were added cumulatively to the organ bath in the concentration range of 1 nM to 10 μM to obtain a concentration-effect curve to the agonist in study. Each jugular vein ring was used to obtain concentration-response curves to two agonists. In most cases, agonist assignment to any given preparation, as well as the order in which each of the curves was to be obtained (at 60-min intervals), was randomized. Because the magnitude of the contractions induced by each of these agonists was similar when used to generate the first or second curve, the data concerning each agonist were pooled. It is important to note, however, that BW723C86 and α-methyl-5-HT were always used as the second agonist due to the difficulty in washing out their effects upon completion of the curve, as described previously (Watts and Thompson, 2004).

Influence of Receptor Antagonists on Contractions Induced by 5-HT Receptor Agonists. To assess the possible contribution of 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/2C}$, 5-HT_{2B}, and 5-HT_7 receptors to contractions induced by 5-HT, concentration-response curves to 5-HT (1 nM–10 μM) were obtained in absence or in presence of their selective antagonists WAY100135 (0.1 and 0.3 μM), GR127935 (3 and 10 nM), ketanserin (30 nM), LY266097 (10 and 30 nM), and SB269970 (10 and 30 nM), respectively.

In most cases, two consecutive curves to 5-HT were obtained at a 1-h interval, with the antagonist being added 30 min after completion of the first control curve and restoration of tension to baseline values (by multiple rinses). In such cases, the antagonist was incubated for 30 min before initiation of the second curve. Control preparations were incubated solely with the corresponding volume of deionized water (the vehicle used to prepare antagonists), to check for potential vehicle effects or spontaneous changes in responsiveness. For ketanserin, concentration-response curves to 5-HT were performed after a 30-min incubation with ketanserin (30 nM) and compared with the responses induced by 5-HT in rings in which ketanserin was omitted.

The same type of protocol (i.e., 30-min incubation with antagonist before the second curve) was also used to evaluate the susceptibility of the contractile effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT and the mixed 5-$HT_{1A/1B}$ receptor agonist RU24969 to antagonism by the 5-HT_{1A} and 5-HT_{2A} receptor antagonists WAY 100135 and ketanserin, respectively. However, because contractions induced by the 5-HT_{2B} receptor agonist BW723C86 are largely resistant to washout, the an-

tagonistic effects of the selective 5-HT_{2B} receptor antagonist LY266097 (10 nM) or ketanserin (30 nM) on concentration-response curves to this agonist were evaluated by comparing single curves obtained for the agonist in distinct preparations incubated (for 30 min) with the antagonist or its vehicle. The concentrations of all antagonists used were chosen based on their affinities for the receptors of interest.

RNA Isolation, Reverse Transcription, and Real-Time PCR. Total RNA from approximately 10-mg sections of endothelium-intact jugular vein was isolated using the MELT total RNA isolation system (Ambion/Applied Biosystems, Austin, TX) and quantified on a NanoDrop spectrophotometer NanoDrop Technologies, Inc. (Wilmington, DE). One microgram of DNase-treated total RNA from each sample was reverse-transcribed using an oligo$(dT)_{12\text{-}18}$ primer, dNTP mix, and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Primers for RNA encoding the rat 5-HT receptor subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 and for rat β-2-microglobulin (β2m) were purchased from SuperArray (Frederick, MD). This gene has been used previously for comparative real-time gene expression studies (Wacker and Godard, 2005; Linder et al., 2008). Quantification of 5-HT receptors and β2m amplification products was performed using the respective primers (0.1 μM) and the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in the 7500 Real-Time PCR System (Applied Biosystems), according to the manufacturer's protocol. For each reaction, the cycle threshold (Ct) value was determined as the cycle number at which the fluorescence value reached the threshold level, which was set above the background fluorescence in the exponential phase of the real-time amplification curves. A dissociation curve was performed at the end of each run to ensure that a single product was amplified.

Materials. The drugs were ultimately diluted in deionized water on the day of the experiment from stock solutions diluted in the appropriate vehicle. 5-HT creatinine sulfate, BW723C86, 8-OH-DPAT, α-methyl-5-HT, 5-methoxytryptamine, DOI, acetylcholine chloride, and ketanserin tartrate were purchased from Sigma-Aldrich. α-Methyl-5-HT, 5-CT, BRL54443, zacopride, RU24696, EMD386088, WAY100135, SB269970, LP44, and GR127935 were purchased from Tocris Bioscience (Ellisville, MO). Sumatriptan was purchased from GlaxoSmithKline (Stevenage, Hertfordshire, UK), and $PGF_{2\alpha}$ was from Cayman Chemical (Ann Arbor, MI). LY266097 was kindly provided by Eli Lilly & Co. (Indianapolis, IN).

Data Analysis. Results concerning intensities of isometric contraction were measured in milligrams of force generated and are shown as percentages of the initial response to 60 mM KCl and expressed as mean ± S.E.M. KCl (60 mM) produced 244.0 ± 9.3 mg of force (n = 54) in the rat jugular vein. Sensitivity to agonists is expressed as pD_2 values ($-\log EC_{50}$) calculated by nonlinear regression in Prism (GraphPad Software Inc., San Diego, CA), where EC_{50} is the effective molar concentration of the agonist that induces 50% of the maximal response. When a maximal contraction was not clearly obtained, the estimated EC_{50} value is possibly smaller than the true EC_{50}. Apparent antagonist dissociation constants (apparent K_B values, reported as pK_B) were calculated using the following formula: $\log K_B = \log (dr\text{-}1) - \log [B]$, where dr indicates the ratio between the EC_{50} values of the agonist obtained in presence and absence of antagonist, and [B] corresponds to antagonist concentration. The relative quantification of the 5-HT receptors to β2m mRNA was expressed as $2^{-\Delta Ct}$, where ΔCt is the difference in cycle threshold between the 5-HT receptor gene and the housekeeping gene β2m. Data were analyzed by Student's t test for paired or unpaired comparisons, as appropriate. Additional statistical analyses were performed using one-way analysis of variance followed by the Student-Newman-Keuls post hoc test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of Serotonergic Receptor Agonists. Figure 1A shows the concentration-effect curves to those 5-HT receptor agonists that are potent and that induced an equivalent or larger maximal contractile response in rat jugular vein compared with 5-HT. Among these agonists are the nonselective 5-HT receptor agonists 5-CT and 5-methoxytryptamine, the nonselective 5-HT_2 receptor agonist α-methyl-5-HT, the partial 5-HT_{2A} receptor agonist DOI, and the mixed 5-HT_{1F}/5-HT_{2A} receptor agonist BRL54443. Figure 1B shows the concentration-effect curve of the 5-HT receptor agonists that are less potent and induced a smaller contraction compared with 5-HT or no contraction in rat jugular vein. The 5-HT_{1A}, 5-$HT_{1A/1B}$, and the 5-HT_{2B} receptor agonists 8-OH-DPAT, RU24696, and BW723C86, respectively, induced a concentration-dependent contraction. Alternatively, sumatriptan (5-$HT_{1B/1D/1F}$ receptor agonist), EMD386088 (5-HT_6 receptor agonist), zacopride (5-HT_3 receptor agonist), and LP44 (5-HT_7 receptor agonist) failed to induce a concentration-effect curve in rat jugular vein rings. LP44 induced a contraction only at the highest concentration tested (10 μM). Parameters of the sensitivity (pD_2) and potency (maximal effect) of these agonists are shown in Table 1.

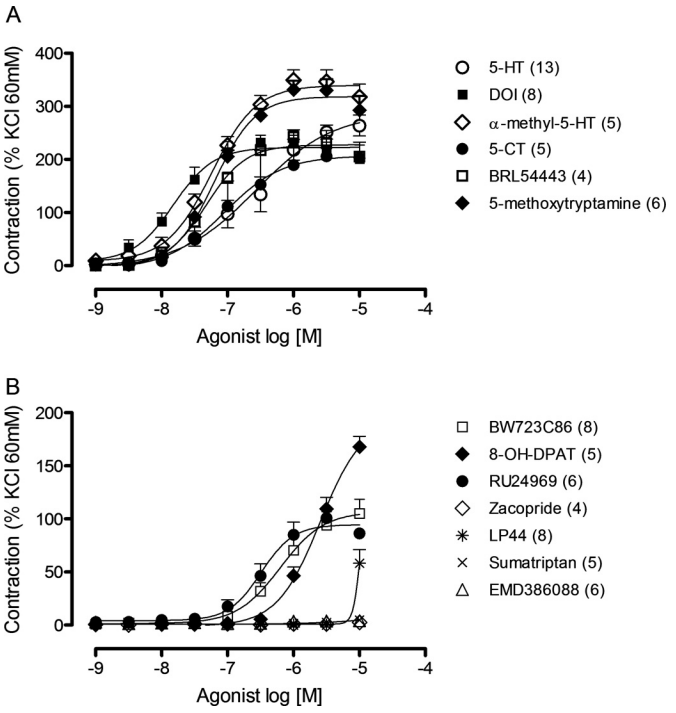

Fig. 1. Effect of 5-HT receptor agonists in contracting endothelium-intact rat jugular vein rings. Concentration-effect curves for 13 5-HT receptor agonists were constructed. Agonists are divided into agonists that are potent and cause a large maximal contraction (A) and agonists that are less potent and cause a smaller maximal contraction than 5-HT (B). Points represent means ± S.E.M. of the contraction expressed as percentage of KCl (60 mM)-induced contraction of (n) experiments.

Blockade by Serotonergic Receptor Antagonists. Figure 2 shows that the concentration-effect curve induced by 5-HT is right-shifted in the presence of the 5-HT_{2A} receptor antagonist ketanserin (30 nM). To further investigate the additional putative 5-HT receptors involved in 5-HT-induced contraction in rat jugular vein, the contraction evoked by 5-HT was evaluated in the presence of antagonists of the 5-HT_{1A} (WAY100135), 5-HT_{1B} (GR127935), 5-HT_{2B} (LY266097), and 5-HT_7 (SB269970) receptors. Figure 3 shows the concentration-effect curve to 5-HT in the absence and in the presence of these different 5-HT receptor

TABLE 1
Pharmacological parameters for 5-HT receptor agonist-induced contraction in endothelium-intact rat jugular vein

Agonist	$pD_2 = -\log EC_{50}$	Maximal Effect	n^a
		mg	
5-HT	6.65 ± 0.17	729 ± 83.4	13
α-Methyl-5-HT	7.23 ± 0.05	777 ± 108.5	5
DOI	7.76 ± 0.12	631 ± 59.1	8
5-Methoxytryptamine	7.19 ± 0.04	627 ± 35.8	6
5-CT	6.52 ± 0.40	502 ± 30.53	5
BRL5443	7.32 ± 0.03	482 ± 12.4	4
8-OH-DPAT	5.58 ± 0.06	329 ± 23.4	5
BW723C86	6.17 ± 0.10	310 ± 47.8	8
RU24969	6.38 ± 0.10	229 ± 23.7	6
LP44	N/A	155 ± 31.8	8
Sumatriptan	N/A	10 ± 3.41	5
Zacopride	N/A	N/A	4
EMD386088	N/A	N/A	6

N/A, could not be obtained.
[a] Number of experiments.

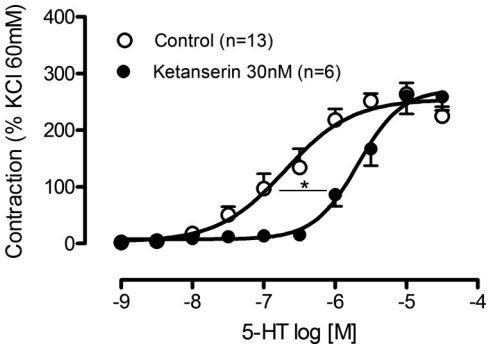

Fig. 2. Effect of 5-HT $_{2A/2C}$ receptor antagonist ketanserin on 5-HT-induced contraction in rat jugular vein. The concentration-effect curve obtained with 5-HT was evaluated in the absence (control) and in the presence of ketanserin (30 nM). Points represent means ± S.E.M. of the contraction expressed as percentage of KCl (60 mM)-induced contraction of (n) experiments. *, $P < 0.05$ versus control.

antagonists. The 5-HT curve was shifted to the right in the presence of WAY100135 (0.3 μM; $pK_B = 6.75 \pm 0.12$) (Fig. 3B) and LY266097 (10 nM and 30 nM; $pK_B = 8.1 \pm 0.06$) (Fig. 3, E and F). The lowest concentration of WAY100135 (0.1 μM) (Fig. 3A) was ineffective in modifying the concentration effect curve to 5-HT in the rat jugular vein. GR127935 (Fig. 3, C and D) and SB269970 (Fig. 3, G and H) had no effect on the contraction induced by 5-HT in rat jugular vein.

Contractions Mediated by 5-HT$_{1A}$ and 5-HT$_{2B}$ Receptors. To give further support to the involvement of the 5-HT$_{1A}$ receptor in the contraction induced by 5-HT in rat jugular vein, the contractions induced by the 5-HT$_{1A}$ and 5-HT$_{1A/1B}$ receptor agonists 8-OH-DPAT and RU24969, respectively, were investigated in the presence of the 5-HT$_{1A}$ antagonist WAY100135 ($pK_B = 6.46 \pm 0.16$ and 6.56 ± 0.07, respectively). The concentration-effect curves obtained with 8-OH-DPAT (Fig. 4A) and RU24969 (Fig. 4B) were shifted to the right in the presence of WAY100135 (0.3 μM). When WAY 100135 was replaced by vehicle, the concentration-effect curves induced by 8-OH-DPAT and RU24969 were comparable with the responses obtained in the absence of any treatment (data not shown).

To give further support to the involvement of the 5-HT$_{2B}$ receptor in contraction, the contraction induced by the 5-HT$_{2B}$ receptor agonist BW723C86 was investigated in the presence of the 5-HT$_{2B}$ receptor antagonist LY266097. The maximal contraction induced by BW723C86 was impaired in the presence of the highest concentration of LY266097 (10 nM) and unaltered in the presence of the lowest concentration of LY266097 (0.5 nM) compared with the contraction induced by BW723C86 in the absence of LY266097 (Fig. 4C). LY266097 did not modify the potency of BW723C86.

Effect of Ketanserin on the Contraction Induced by 8-OH-DPAT, RU24969, and BW723C86. To rule out the participation of 5-HT$_{2A}$ receptors in the contraction induced by the 5-HT$_{1A}$, 5-HT$_{1A/1B}$, and 5-HT$_{2B}$ receptor agonists in the rat jugular vein, 8-OH-DPAT, RU24969, and BW723C86 were tested in the presence of ketanserin. Figure 5 shows that the contraction induced by all the agonists was abolished or dramatically reduced in the presence of ketanserin (30 nM) compared with control curves in which ketanserin was absent.

mRNA Expression of the 5-HT Receptors in Rat Jugular Vein. RNA from endothelium-intact jugular vein was isolated for measurements of 5-HT receptor mRNA expression by real-time PCR. The value at which measurable product was first observed in real-time PCR (cycle threshold values) was 16.4 ± 0.14 cycles for the housekeeping gene β2m in jugular vein ($n = 5$). Figure 6 shows mRNA expression for 5-HT receptor subtypes relative to mRNA expression for β2m. mRNA expression was observed for 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_7$ receptors but not for the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors.

Discussion

This work provides evidence for the presence of the 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_7$ receptors in endothelium-intact rat jugular vein rings. The endothelium was left intact to capture the greatest amount of physiologically relevant data as possible, even given potential confounding results of endothelial cell opposing smooth muscle cell function. By the judicious use of newly developed pharmacological tools, we show that the contraction induced by 5-HT in rat jugular vein is mainly mediated by 5-HT$_{2A}$ receptor activation.

5-HT$_1$ Receptors. From the 5-HT$_1$ receptor family, the subtypes 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1F}$ have been found in blood vessels, as opposed to the 5-HT$_{1A}$ receptor subtype that has been shown to be located at the central nervous system. Among these subtypes, the 5-HT$_{1B}$ receptor is the predominant subtype in mediating contraction of vascular tissues (Bhattacharya et al., 2004). mRNA expression for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors in vascular smooth muscle has been reported previously (Ullmer et al., 1995; Watts et al., 2001). In aorta from normotensive rats, 5-HT$_{1B}$ receptor expression is not associated with contraction (Banes and Watts, 2001). We show here no pharmacological evidence for the involvement of 5-HT$_{1B}$ receptor in the contraction induced by 5-HT in rat jugular vein. These findings were supported by the lack of RNA expression for this receptor subtype in this blood vessel. Functional and/or expression data did not support the involvement of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor subtypes in contracting rat jugular vein rings.

The findings that the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT and the mixed 5-HT$_{1A/1B}$ receptor agonist RU24969 induced

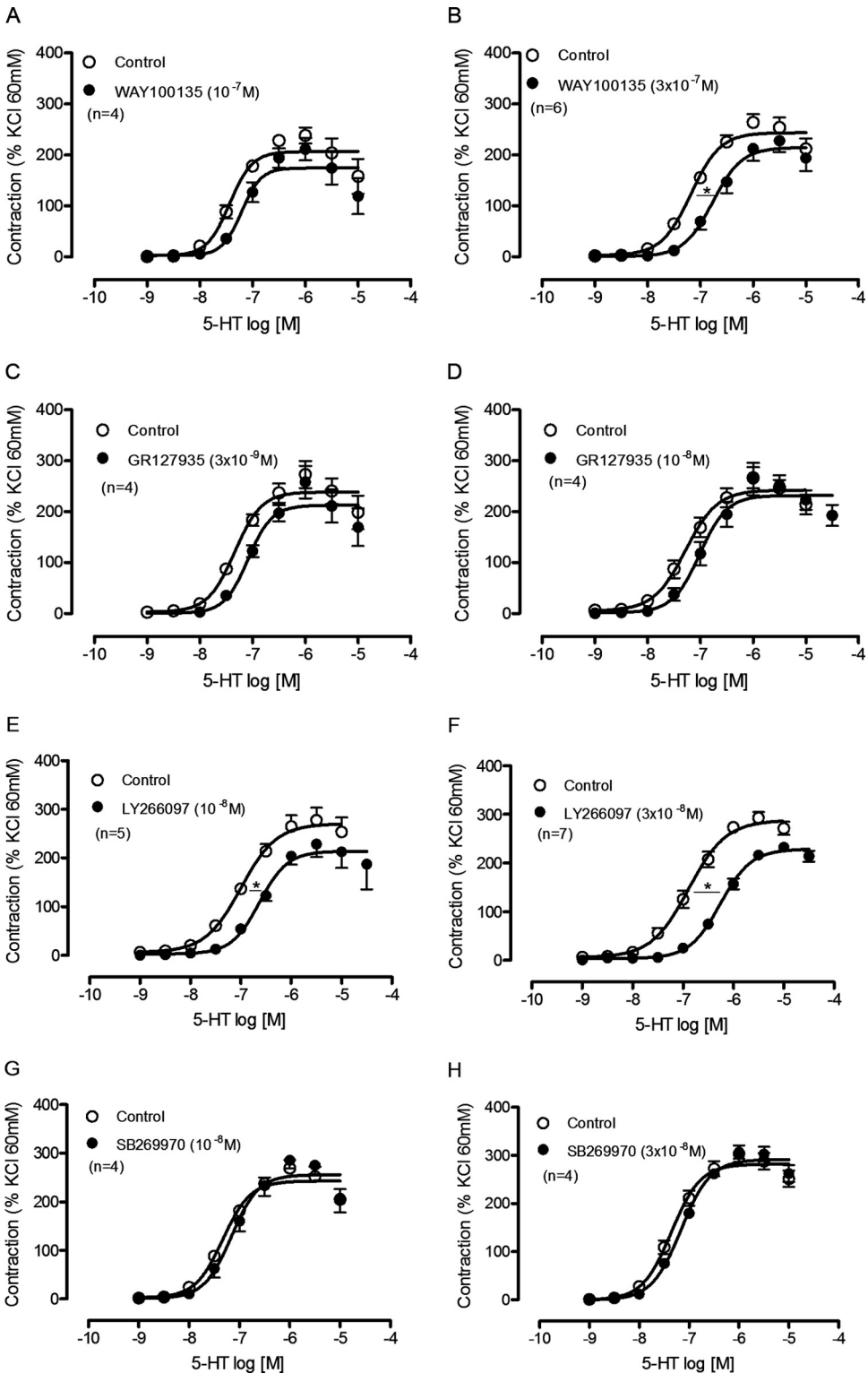

Fig. 3. Effect of 5-HT receptor antagonists on 5-HT-induced contraction of rat jugular vein. Concentration-effect curve to 5-HT was performed in the absence (control) and in the presence of the antagonists for the receptors 5-HT_{1A} (WAY100135; A and B), 5-HT_{1B} (GR127935; C and D), 5-HT_{2B} (LY266097; E and F), and 5-HT_7 (SB269970; G and H). Points represent means ± S.E.M. of the contraction expressed as percentage of KCl (60 mM)-induced contraction of (n) experiments. *, $P < 0.05$ versus control.

contraction of rat jugular vein were unexpected. This would be the first evidence for 5-HT_{1A} receptor mediating contraction of vascular tissue, to our knowledge, despite reports of a central role for 5-HT_{1A} receptor on the control of cardiovascular function (Kuhn et al., 1980) and of the involvement of this receptor in mediating contractions of nonvascular tissues, such as the jejunum circular muscle (Delesalle et al., 2008). However, when we further explored the serotonergic pharmacology of the rat jugular vein, we observed that the contractions induced by the so-called "5-HT_{1A} receptor agonists" were inhibited by the 5-$HT_{2A/2C}$ receptors antagonist ketanserin. It is important to highlight that ketanserin, at the concentration used in the present study, has less than 5% affinity for the rat 5-HT_{1A} receptor (Gozlan et al., 1983; Titeler et al., 1987). In addition, the pD_2 values for 8-OH-DPAT and RU24969 obtained in this study are closer to the

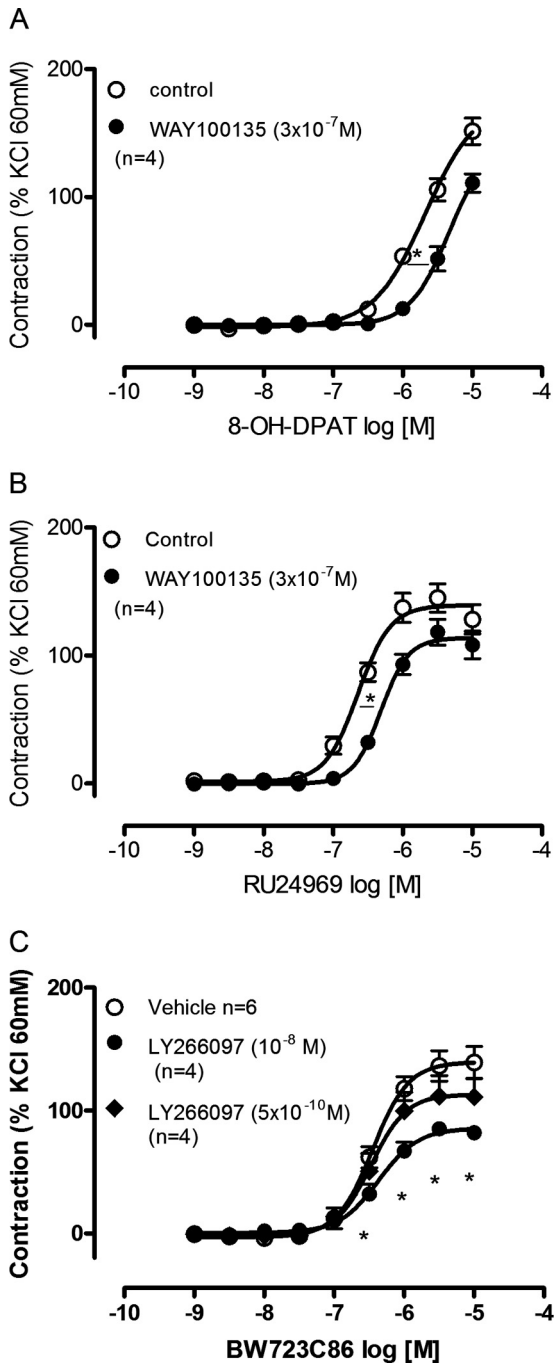

Fig. 4. Effect of the 5-HT_{1A} and 5-HT_{2B} receptor antagonists on the contraction induced by the 5-HT_{1A}, 5-$HT_{1A/1B}$, and 5-HT_{2B} receptor agonists in rat jugular vein. The concentration-effect curves induced by the agonist of the 5-HT_{1A} receptor 8-OH-DPAT (A), the 5-$HT_{1A/1B}$ receptor RU24969 (B), and the 5-HT_{2B} receptor BW723C86 were evaluated in the absence (control) and in the presence of antagonists of the 5-HT_{1A} receptor WAY100135 (3×10^{-7} M) (A and B) and the 5-HT_{2B} receptor LY266097 (10^{-8} M) (C). Points represent means ± S.E.M. of the contraction expressed as percentage of KCl (60 mM)-induced contraction of (n) experiments. *, $P < 0.05$ versus control.

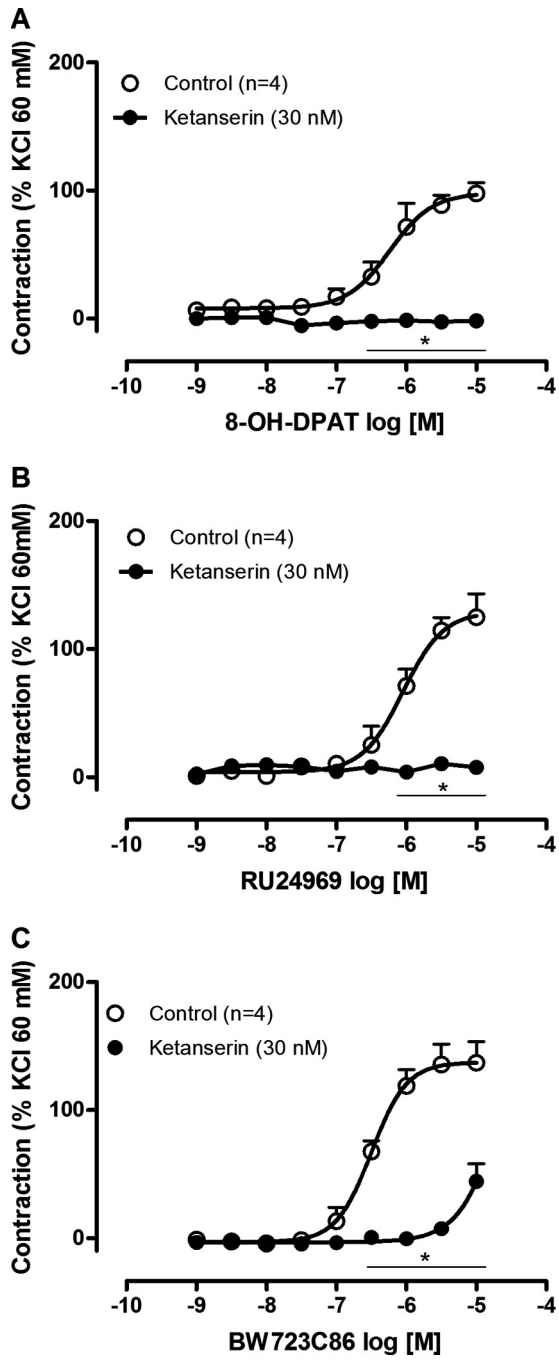

Fig. 5. Effect of ketanserin on the contraction induced by 5-HT receptor agonists in rat jugular vein. The concentration-effect curve induced by 8-OH-DPAT (A), RU24969 (B), and BW723C86 (C) was evaluated in the absence (control) and in the presence of ketanserin (30 nM). Points represent means ± S.E.M. of the contraction expressed as percentage of KCl (60 mM)-induced contraction of (n) experiments. *, $P < 0.05$ versus control.

values obtained for these agonists toward 5-HT_{2A} receptor rather than toward 5-HT_{1A} receptor (Hoyer et al., 1994). Along with the lack of RNA expression for 5-HT_{1A} receptor in the rat jugular vein, our results do not support a role for 5-HT_{1A} receptor in mediating contraction of the rat jugular vein.

5-HT_2 Receptors. The 5-HT_{2A} receptor mediates the contraction induced by 5-HT in several human vascular beds, including the pulmonary, mesenteric, and coronary arteries, and the cutaneous and saphenous veins (Bax et al., 1992; Bodelsson et al., 1992; Cortijo et al., 1997; Nilsson et al., 1999; Gul et al., 2003). In the rat, the 5-HT_{2A} receptor subtype is the main receptor activated by 5-HT to induce contraction in arteries such as aorta and mesenteric artery (Watts, 2002). Through the use of appropriate techniques, we

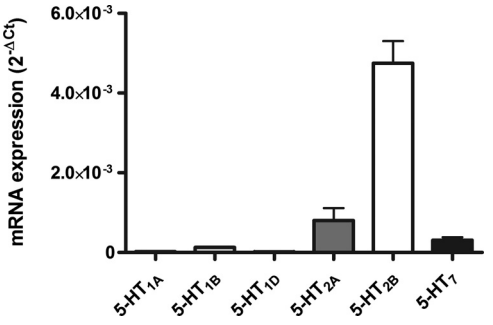

Fig. 6. RT-PCR analysis of 5-HT receptors mRNA expression in rat jugular vein. Data are means ± S.E.M. for $n = 5$ measurements expressed as the change from β2m ($2^{-\Delta Ct}$).

show evidence to support that the contraction induced by 5-HT in the rat jugular vein is mainly due to 5-HT_{2A} receptor activation. These data give further support to a previous report by Cohen et al. (1981). However, compared with these studies performed previously, the present findings were based on the knowledge of newest cloned receptors and made use of newest and more selective drugs toward 5-HT receptor subtypes.

Whereas the involvement of the 5-HT_{2A} receptor on 5-HT-induced contraction of rat jugular vein is shown, we were unable to confirm the involvement of the 5-HT_{2B} receptor as a mediator of 5-HT-induced contraction of rat jugular vein regardless of its expression in this blood vessel. The pD_2 value for the 5-HT_{2B} agonist BW723C86 found here is closer to the value found for 5-HT_{2A} receptor rather than for 5-HT_{2B} receptor (Kitka and Bagdy, 2008). The pK_B value for the 5-HT_{2B} antagonist LY266097 we observed (~8) is similar to the one reported in rat jugular vein (Audia et al., 1996), and it is closer to the pK_B value of this antagonist toward 5-HT_{2A} receptor (7.7) rather than toward 5-HT_{2B} receptor (9.3) (Kitka and Bagdy, 2008). A contraction mediated by 5-HT_{2B} receptor activation was observed in aorta from hypertensive but not from normotensive rats (Russell et al., 2002). Whereas this receptor was detected in endothelium-denuded vascular tissues (Banes and Watts, 2002; Watts and Thompson, 2004), 5-HT_{2B} receptor activation is associated with endothelium-dependent relaxation in porcine vena cava and pulmonary artery, as well as rabbit and rat jugular vein (Watts and Cohen, 1999). In unpublished data, we observed that 5-HT is unable to induce relaxation of rat jugular vein contracted with a prostaglandin mimetic agonist. It is interesting to note that Ellis et al. (1995) showed relaxation induced by 5-HT of contracted rat jugular vein only when the serotonergic receptors involved in contraction were inhibited by ketanserin (Ellis et al., 1995). In preliminary studies, we discovered that removing the endothelial cell from this blood vessel—without destroying the smooth muscle—is enormously difficult. Whether the 5-HT_{2B} receptor is functionally present mediating relaxation (dependent or not on the endothelium) in the rat jugular vein has yet to be investigated because mRNA expression may not always be mirrored by protein expression. Measurements of mRNA expression represent a useful tool to predict protein expression levels. However, a poor correlation between mRNA expression and protein expression can be found (Chen et al., 2002; Guo et al., 2008) as a consequence of a myriad of factors involving post-transcriptional events, post-translational modifications, variations in mRNA half-life and protein stability, as well as technical imperfections (Greenbaum et al., 2003). Further studies are necessary to understand whether the highly expressed mRNA for the 5-HT_{2B} receptor is accompanied by the expression of a functional protein in the rat jugular vein. The 5-HT_{2C} receptor was not given attention, because localization and function for this receptor in the cardiovascular system are still unknown (Villalón and Centurion, 2007).

5-HT_3, 5-HT_6, and 5-HT_7 Receptors. We were unable to show through pharmacological approaches the involvement of 5-HT_3, 5-HT_6, and 5-HT_7 receptors in 5-HT-induced contraction of rat jugular vein. Whereas the 5-HT_3 receptor is involved in cardiovascular responses to 5-HT (Villalón and Centurion, 2007), no reports for the involvement of the 5-HT_6 receptor in the cardiovascular system have been published to our knowledge. A role for the 5-HT_7 receptor in the cardiovascular system has been shown (for review, see Villalón and Centurion, 2007). No reports to date (including ours) have shown contraction induced by 5-HT_7 receptor activation. Conversely, endothelium-independent relaxation mediated by 5-HT_7 receptor has been reported previously (Ishine et al., 2000; Jähnichen et al., 2005). Therefore, we cannot exclude the involvement of the 5-HT_7 receptor in relaxation of rat jugular vein; however, this was not the aim of this study and it has yet to be tested.

In summary, the contractile data in combination with mRNA receptor expression support the conclusion that the 5-HT_{2A} receptor is the main receptor involved in 5-HT-induced contraction in rat jugular vein. However, a minor role for 5-HT_{2B} receptor cannot be ruled out. We are aware that our results intentionally pertain to the whole jugular vein, including smooth muscle and endothelium, according to the original intent of the present investigation. Whether mRNA expression for the 5-HT_{2B} and 5-HT_7 receptors is associated with a functional role, if any, in the rat jugular vein is a question that has yet to be explored.

Perspectives. Venous diseases have been recognized since old times as mentioned by Hippocrates (460–377 B.C.), and they currently may affect a quarter of the adult population (Lim et al., 2008). However, veins occupy only a marginal part of the total research in the cardiovascular field compared with arteries. Examples of venous diseases are the deep venous thrombosis and the human spermatic varicose vein. For the latter, increased vascular reactivity to 5-HT has been reported previously (Yildiz et al., 2003). Alternatively, an injury to the endothelium with a potential involvement of 5-HT may play an important role in the origin of deep venous thrombosis after platelet aggregation. It is interesting to note that the jugular vein can also be the target of a (although rare) septic thrombophlebitis followed by primary oropharyngeal infections, a venous disease known as Lemierre syndrome (McMullan et al., 2004; Hile et al., 2009). Despite the development of animal models for studying venous hypertension (Pascarella et al., 2005) and venous diseases after vein grafts (Schachner et al., 2006), basic research has not yielded the significant advances in venous diseases that have been seen for other areas. We postulate that understanding the 5-HT pharmacology in veins may help navigate through the unknown avenues toward the treatment of venous diseases.

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