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# Characterization of the contractile 5-HT receptor in the renal artery of the normotensive rat

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**d) Abbreviations:**

| | |
|---|---|
| BW723C86 | □methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine hydrochloride |
| CP93129 | 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-b]pyridin-5-one dihydrochloride |
| 5-CT | 5-carboxamidotryptamine |
| DOI | 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane |
| LNNA | $N^{w}$-nitro-L-arginine |
| LY53857 | 6-methyl-1-(1-methylethyl)-ergoline-8b-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate |
| LY272015 | 6-methyl-1,2,3,4-tetrahydro-1-[3,4-dimethoxyphenyl)methyl-9H-pyrido[3,4b]indole] hydrochloride |
| LY344864 | (R)-N-[3-Dimethylamino-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluorobenzamide |
| mCPBG | 1-(m-chlorophenyl)-biguanide |
| mCPP | 1-(m-chlorophenyl)piperazine |
| 5-MeOT | 5-methoxytryptamine |
| (+/-)-8-OH-DPAT | (+/-) 8-hydroxy-2-(di-n-propylamino)tetralin |
| RS127445 | 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine |

**e) Recommended Section:** Cardiovascular

## Abstract

Our goal was to characterize the 5-HT receptor(s) mediating contraction in the isolated right renal artery, testing the hypothesis that the 5-$HT_{2A}$ receptor would be the primary and likely only 5-HT receptor involved in contraction. Contraction of arteries was investigated in isolated tissue baths and expression of 5-HT receptors measured using immunohistochemical and Western analyses. Compared to endothelium-denuded rat aorta, a tissue with an established 5-$HT_{2A}$ receptor, endothelium-denuded renal artery contracted to 5-HT with a 10-fold greater potency. Suprisingly, the 5-$HT_{2B}$ receptor agonist BW723C86 caused a concentration-dependent contraction that was antagonized by the 5-$HT_{2B}$ receptor antagonist LY272015 and non-selective 5-$HT_2$ receptor antagonist LY53857. Correlation of –log $EC_{50}$ values with binding affinities ($pK_i$) indicated that contraction of the renal artery elicited by 13 different agonists was likely consistent with activation of a 5-$HT_{2A}$ ($r = 0.928$) and 5-$HT_{2B}$ ($r=0.843$) receptor. 5-HT-induced contraction was shifted by the 5-$HT_{2A}$ receptor antagonist ketanserin (3, 10 nM) and the 5-$HT_{2B}$ receptor antagonist LY272015 (10, 50 nM). Higher than expected concentrations of the 5-$HT_{2A}$/5-$HT_{2B}$ receptor antagonist LY53857 were needed to antagonize 5-HT-induced contraction and the 5-$HT_{2B}$ receptor antagonist RS127445 was virtually inactive. Western and immunohistochemical analyses of the renal artery validated the presence of 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor protein. These results suggest that the renal artery possesses a complex 5-HT receptor population, including ketanserin- and LY272015-sensitive receptors. This unique pharmacology may reflect differences in 5-HT receptor coupling between tissues or heterogeneity in the subtype(s) of 5-HT receptors expressed in the renal artery.

5-HT has an interesting history of involvement in modifying arterial tone, a parameter relevant to blood pressure (Turla and Webb, 1989; Martin, 1994). We have recently begun investigation of the renal artery as the kidney is unarguably relevant to determination of blood pressure. 5-HT has been suggested to be made within the kidney (Hafdi *et al*., 1996) and localized to cells of the kidney (Lincoln *et al*., 1990). In the isolated rat renal artery, it is established that 5-HT causes a concentration-dependent contraction but the receptor mechanism by which this occurs is unknown and may be species-dependent.

In the isolated main renal artery of the rabbit, 5-HT causes contraction through activation of a silent 5-$HT_{1B/1D}$ receptor (Hill *et al.*, 2000; Choppin and O'Connor, 1994) and causes conduit renal arteries in the conscious rabbit to spasm (Wright and Angus, 1987). In the dog, 5-HT reduces renal blood flow *via* activation of a 5-$HT_2$-like receptor (Takahashi *et al.,* 1992; Blackshear *et al.*, 1991). In human renal arteries, 5-HT causes a contraction associated with marked oscillations (Ueda *et al.,* 1982). In many but not all isolated rat arteries, it is the 5-$HT_{2A}$ receptor that is primarily responsible for 5-HT-induced contraction, evidenced by the effectiveness of agonists such as DOI and alpha-methyl-5-HT, and antagonism of contraction by ketanserin and MDL 100907. Many of these studies were performed prior to development of drugs that were selective for the 5-$HT_{2B}$ receptor; thus, the potential involvement of this receptor in renal arterial contraction is unknown. This issue is of particular interest as evidence supports that the 5-$HT_{2B}$ receptor, while present in arterial smooth muscle, does not mediate 5-HT-induced contraction under normal, non-hypertensive conditions (Watts, 1997; Watts and Fink, 1999; Banes and Watts, 2002, Russell *et al*., 2002).

We hypothesized that the 5-$HT_{2B}$ receptor would not be involved in mediating contraction to 5-HT in the renal artery from the normal rat and that, as has been observed in other rat arteries, the 5-$HT_{2A}$ receptor was primarily responsible for mediating 5-HT-induced contraction. We present here studies that potentially negate this hypothesis. While we have observed that the 5-$HT_{2A}$ receptor certainly plays a major role in 5-HT-induced contraction, the renal artery possesses what appears to be a functioning 5-$HT_{2B}$ -like receptor in the normal rat, or a 5-HT receptor complement with significant differences in receptor/effector coupling when compared to other arteries. This study serves to demonstrate two ideas. First, that the pharmacology of the 5-HT receptor(s) mediating contraction in the isolated renal artery is complex and second that the 5-$HT_{2B}$ receptor, or similar receptor, may be differently coupled in normal tissues.

## Methods

*Animal Use*

Male Sprague-Dawley rats (0.225 – 0.250 kg; Charles River, Portage, MI, USA) were used. All rats were given free access to standard pelleted rat chow (Harlan/Teklad 8640 rodent diet).

*Blood Pressure Measurement*

Systolic blood pressures of rats were determined in the conscious state by the tail cuff method (pneumatic transducer, Narco, TX, USA).

*Isolated Smooth Muscle Contractility Measurement*

Rats were deeply anesthetized with pentobarbital (60 mg $kg^{-1}$, i.p.) to the point of a loss of eyelid reflex and lack of withdrawal from painful stimuli. Aorta, right renal artery and stomach fundus were placed in physiologic salt solution consisting of (in mM) NaCl, 130; KCl, 4.7; $KH_2PO_4$, 1.18; $MgSO_4$-$7H_2O$, 1.17; $CaCl_2$-$2H_2O$, 1.6; $NaHCO_3$, 14.9; dextrose, 5.5; and $CaNa_2EDTA$, 0.03; pH 7.2. Aorta and renal artery were cleaned of fat and connective tissue, cut into helical strips and rubbed with a moistened cotton swab to remove the endothelial cell layer. Two strips from the inner most section of the stomach fundus were dissected. Strips were mounted as longitudinal preparations and placed on stainless steel holders in tissue baths (30, 50 mL) for isometric force recordings using Grass polygraphs and transducers (Astro-Med, West Warwick, RI, USA) or PowerLab® for the Macintosh (ADInstruments, Dover, NH, USA). Tissues were placed under optimum resting force (1500 mg for aorta, 500 mg for renal artery,

4000 mg for stomach fundus; determined in preliminary experiments). Muscle baths were filled with warmed (37˚C), aerated (95% $O_2$, 5% $CO_2$) physiological salt solution. Tissues equilibrated for one hour with frequent exchanges of warmed salt solution. After this hour, arteries were challenged with a maximal concentration of □ adrenergic receptor agonist phenylephrine (10 uM) and stomach fundus with KCl (67 mM). Phenylephrine and KCl were washed out and tissue tone returned to baseline. In arterial tissue, functional integrity of the endothelial cells was evaluated by testing relaxation caused by acetylcholine (1 uM) in strips contracted with a half-maximal concentration of phenylephrine (10-100 nM). In all experiments, the endothelial cell layer was functionally removed as evidenced by the lack of relaxation (5±3 % relaxation) caused by acetylcholine. One of the following protocols was then followed.

*Use of 5-HT Receptor Agonists*

In each tissue, two 5-HT receptor agonists ($10^{-9}$ - $10^{-4}$ M) were used to generate cumulative concentration response curves in isolated arteries. These agonists were randomized and separated by a wash period of 1 hour, with a minimum of 10 buffer exchanges, with the exception of BW723C86 and □lpha-methyl-5-HT. Both these agonists were difficult to wash out, so when they were administered as the first agonist, the tissues were not used to generate a second agonist curve. However, there were tissues in which the response to BW723C86 and □lpha-methyl-5-HT was generated as a second agonist. In comparing responses of first and second agonists, we could not detect a difference in potency or maximum response to individual agonists tested, and thus these data are pooled.

*Use of 5-HT Receptor Antagonists*

Tissues were exposed to either the appropriate vehicle (water or 0.1 % dimethylsulfoxide) or antagonist and incubated for one hour without washing. A cumulative concentration response curve to 5-HT or BW723C86 ($10^{-9}$ – $10^{-4}$ M) was then performed.

*Western Analysis*

Tissues were isolated directly from the animal, cleaned and placed directly into liquid nitrogen. In liquid nitrogen, tissues were ground to a powder and ice-cold homogenation buffer added [125 mM Tris (pH 6.8), 4% SDS, 20% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM orthovanadate, 10 ug/ml aprotinin, 10 ug/ml leupeptin]. Homogenates were vortexed, sonicated briefly and transferred to a plastic centrifuge tube and spun at 4$^{o}$C to pellet debris. Supernatant was separated from the pellet and analyzed for protein concentration (BCA protein kit, Sigma Chemical Co., St. Louis, USA). Fifty micrograms of total protein were heated at 100 $^{o}$C for 5 minutes with standard 4:1 sample buffer. Proteins were separated on 1 mm-thick, 10% SDS polyacrylamide gels using a Mini Bio-Rad III apparatus. Membranes were blocked for three hours in 4% chick egg ovalbulmin [4 $^{o}$C, Tris buffered saline (TBS)-0.1% Tween + 0.025% $NaN_3$). Primary antibody (0.5 ug/ml, mouse antibody for 5-$HT_{2B}$ receptor, Pharmingen, San Diego, CA, USA) was incubated with blots overnight at 4 $^{o}$C. Blots were then rinsed thrice in Tris-buffered saline (TBS) + Tween (0.1%) with a final rinse in TBS and incubated with horseradish peroxidase-linked anti-mouse secondary antibody (1:10,000, Amersham Laboratories, Arlington Heights, IL, USA) for 1 hour at 4

ºC with rocking. ECL® reagents (Amersham Life Sciences, Arlington Heights, IL, USA) were used to visualize bands. Gels were stained with Gel Code Blue® (Pierce, Rockford, IL, USA) to verify protein loading and blots were reprobed with smooth muscle □lpha-actin primary antibody (1:1000; Oncogene Research Products, Boston, MA, USA) to ensure equal protein loading.

*Immunohistochemical Analysis*

Renal arteries were snap frozen in OCT compound and stored at –70$^{o}$C until use. Arterial sections (8 □□□□□□) were cut and air dried (overnight, room temp). Samples were cold acetone fixed, washed 3 times with phosphate buffered saline, and endogenous peroxidase blocked [0.3% $H_2O_2$ in phosphate buffered saline (PBS) for 30 minutes]. Sections were blocked for non-specific binding for two hours in PBS containing 1.5% of competing serum. In a humidified chamber, samples then incubated 24 hours with antibody (5-$HT_{2A}$ receptor, Santa Cruz, CA; 4 $^{o}$C, 5 ug/ml with 1.5% blocking serum in PBS) or antibody neutralized with 5-fold excess of competing peptide. The remaining steps were carried out in accordance to the manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Sections were washed 3 times with PBS and incubated with a peroxidase-conjugated secondary antibody (30 minutes, room temp). Samples were washed and incubated with Vectastain® ABC Elite reagant (30 minutes, room temp) followed by 3,3'-diaminobenzidine (DAB)/$H_2O_2$. Reaction was stopped with washing, sections air dried, hematoxylin-stained, mounted and photographed using a Spot 2 digital camera on a Leica light microscope with filters.

*Materials*

Drugs were made daily and in water unless otherwise specified. 5-HT creatinine sulfate, BW723C86, alpha-methyl-5-HT, mCPP, (+/)-8-OH-DPAT, 2-methyl-5-HT, 5-methoxytryptamine, (+/-)DOI, 5-CT, phenylephrine hydrochloride, acetylcholine chloride, ketanserin tartrate (dimethylsulfoxide), LY53857 were purchased from Sigma RBI (St. Louis, MO, USA). LY344864 was a gift from Eli Lilly and company (Indianapolis, IN, USA), CP93129 from Pfizer (Groton, CT, USA), (+)norfenfluramine from SRI International (Menlo Park, CA, USA), sumatriptan from Glaxo (Stevenage, Hertfordshire, UK) and RS127445 (dimethylsulfoxide) from Roche (San Francisco, CA, USA). The 5-$HT_{2B}$ receptor antibody was obtained from Pharmingen (San Diego, CA, USA) while 5-$HT_{2A}$ receptor antibody and competing peptide was purchased from Santa Cruz (Santa Cruz, CA, USA). VECTOR kits (Vector, Burlingame, CA, USA) were used for immunohistochemical analysis.

*Data Analysis*

Data are presented as means $\pm$ standard error of the mean for the number of animals. Contraction is reported a percentage of response to phenylephrine (10 uM) or KCl (67 mM KCl). –log $EC_{50}$ values (negative logarithm of the agonist concentration necessary to produce a half-maximal response [M]) were determined using non-linear regression analysis in GraphPad Prism® (San Diego, CA, USA). Apparent antagonist dissociation constants (apparent $K_B$ values, reported as $pK_B$ ) were calculated using the the method of Arunlakshana and Shield (1959). When comparing two groups, an

unpaired Student's t test was used or, in concentration response curves, ANOVA with repeated measures. ANOVA followed by Student Newman Keuls post hoc test was performed when comparing three or more groups. In all cases, a p value less than or equal to 0.05 was considered statistically significant and is indicated with a *. Band density was quantified using the public domain program NIH Image (v 1.62).

## Results

*Comparison of 5-HT-induced Contraction in Rat Aorta and Right Renal Artery*

Figure 1 demonstrates a difference between 5-HT-induced contraction in the thoracic aorta and main right renal artery dissected from the same normotensive Sprague-Dawley rats (systolic blood pressure of 120±2 mm Hg). The rat thoracic aorta is an established model of 5-$HT_{2A}$ receptor-mediated contraction, as evidenced by the potency of 5-HT and the repeated demonstration that antagonists which block contraction have a high affinity for the 5-$HT_{2A}$ receptor (for review, see Martin, 1994). 5-HT possessed a 10-fold greater potency in contracting the renal artery (-log $EC_{50}$ value [M]= 7.03±0.02) compared to aorta (6.08±0.08).

*Use of 5-HT Receptor Agonists in Rat Renal Artery*

Figures 2A and 2B depict some of the cumulative concentration response curves derived from testing 13 different 5-HT receptor agonists. Data for agonists for which curves are not shown can be found in table 1. These have been divided into those with higher potency and higher maximum response (figure 2A) and those with lower potency and lower maximum response (figure 2B). The following rank order of potency was observed and is detailed in table 1: DOI > 5-HT = 5-methoxytryptamine> mCPP = $\alpha$-methyl-5-HT = BW723C86 > (+)norfenfluramine > 2-methyl-5-HT > CP93129 = 5-CT > (+/-)8-OH-DPAT > sumatriptan > LY344864 (inactive). Because the 5-$HT_{1F}$ receptor agonist LY344864 was inactive, this suggests that 5-$HT_{1F}$ receptors do not play a role in renal arterial contraction. Notably, the 5-$HT_{2B}$ receptor agonist BW723C86 caused a concentration-dependent contraction in the renal artery, though with lower maximum

response than that of 5-HT (figure 2A), and could be antagonized by LY272015 and LY53857 (figure 3), though not in a strictly competitive manner. Additionally, +norfenfluramine and mCPP, two agonists with appreciable affinity for the 5-$HT_{2B}$ receptor, contracted the rat renal artery.

The –log $EC_{50}$ values listed in table 1 were correlated with binding affinities derived from radioligand binding experiments performed in rat tissues and reported in the investigator-driven PSDP Drug database (http://pdsp.cwru.edu/pdspf.asp). These binding affinities include measures of affinity of 12 of the 13 agonists tested with the 5-$HT_{1B}$, 5-$HT_{1F}$, 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors. These receptors were chosen as these are the primary receptors implicated in promoting arterial smooth muscle contractility. Correlations of –log $EC_{50}$ values [M] were significant for the 5-$HT_{2A}$ (r = 0.928) and 5-$HT_{2B}$ receptor binding affinities (r=0.843). Correlations with the 5-$HT_{1B}$ and 5-$HT_{1F}$ receptor were not significant or negative.

*Use of 5-HT Receptor Antagonists in Rat Renal Artery*

To validate the involvement of the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors in 5-HT-induced contraction, we tested the ability of 5-HT receptor antagonists with known affinity for the 5-$HT_{2A}$ and/or the 5-$HT_{2B}$ receptor to inhibit renal arterial 5-HT-induced contraction. Figure 4 demonstrates that the 5-$HT_{2A}$/5-$HT_{2C}$ receptor antagonist ketanserin (3, 10 nM; $pK_B$ =9.9$\pm$0.02) and 5-$HT_{2B}$ receptor antagonist LY272015 (10 nM; $pK_B$ =8.59$\pm$0.03) significantly shifted 5-HT-induced contraction in a parallel and competitive fashion. The $pK_B$ value determined for ketanserin was significantly higher than expected when anticipating interaction with 5-$HT_{2A}$ receptors. The concentration of

LY272015 used (10 nM) was sufficiently low to be assured that it does not interact with 5-$HT_{2A}$ receptors (Cohen *et al.*, 1996), and rightward shifted 5-HT-induced contraction in the isolated stomach fundus with a $pK_B$ value of 9.73±0.05 (data not shown). Interestingly, a higher concentration of LY272015 (50 nM) was unable to further shift 5-HT-induced contraction. However, combination of ketanserin (3 nM) and LY272015 (10 nM) caused a shift that was greater than that caused by either antagonist alone (figure 4B), indicating the two receptor antagonists likely block different receptor populations.

The non-selective 5-$HT_2$ receptor antagonist LY53857 caused a non-parallel and non-competitive antagonism of 5-HT-induced contraction (figure 5). The $pEC_{50}$ of 5-HT and slope of the linear portion of the curves were as follows: vehicle $pEC_{50}$ = 6.88±0.03, slope=60.64±4.47; 10 nM LY53857 $pEC_{50}$ = 6.64±0.04, slope=45.9±0.78; 30 nM LY53857 $pEC_{50}$ = 6.45±0.07, slope = 33.33±1.85; 100 nM LY53857 $pEC_{50}$= 6.01±0.06, slope=36.53±1.67. From the 5-HT concentration of $3x10^{-7}$ M and above, all concentrations of LY53857 significantly reduced contraction when compared to vehicle (marked with a * and arrow). A different 5-$HT_{2B}$ receptor antagonist, RS127445 (figure 6A), was unable to inhibit 5-HT-induced contraction in concentrations demonstrated in our laboratory to shift competitively 5-HT-induced contraction in the isolated rat stomach fundus, a tissue with a *bona fide* 5-$HT_{2B}$ receptor ($pK_B$ = 9.78±0.13; figure 6B). The concentration of RS127445 had to be raised to 1 uM to inhibit 5-HT-induced contraction significantly (figure 6A).

*Verification of the Presence of 5-HT$_{2A}$ and 5-HT$_{2B}$ Receptor Protein in Rat Renal Artery*

We next determined whether both 5-HT receptor proteins were present in the rat renal artery. Two immunoreactive 5-HT$_{\mathbf{2B}}$ receptor bands in both renal and aortic homogenates were identified in Western analyses (figure 7A) at approximately 55 and 110 kDa in size; the band in between these two was observed only spuriously. Positive controls of aortic homogenates from sham normotensive and hypertensive rats were used, where a higher expression of the 5-HT$_{\mathbf{2B}}$ receptor in the aorta of the rats made hypertensive by the nitric oxide synthase inhibitor LNNA was expected and observed (confirmed here, lower 55 kDa band densitometry sham = 5142 arbitrary units, LNNA = 9500; published in Russell *et al*., 2002). The same amount of total protein (50 micrograms) was loaded for the renal artery and aorta. The 5-HT$_{\mathbf{2B}}$-immunoreactive protein in renal artery homogenates was expressed at a considerably lower density as compared to the aorta. We estimate that, per microgram total protein, the renal artery expresses approximately 12% that of the normal aorta (616$\pm$81 arbitrary densitometry units calculated for the lower band only as this is likely the monomeric form of the 5-HT$_{2B}$ receptor). As antibodies useful in Western analyses for the 5-HT$_{\mathbf{2A}}$ receptor are not currently available, we took an immunohistochemical approach to demonstrate the presence of this receptor in the renal artery. Figure 7B demonstrates that immunohistochemically-detectable and specific 5-HT$_{\mathbf{2A}}$ receptor immunoreactivity was observed in the smooth muscle of the renal artery (compare first to second panel). Thus, these studies demonstrate that immunoreactive 5-HT$_{\mathbf{2A}}$ and 5-HT$_{\mathbf{2B}}$ receptor are present in renal artery.

## Discussion

Work by investigators has demonstrated that 5-HT acts as a pressor in the isolated perfused kidney (McGregor and Smirk, 1970; Collis and Vanhoutte, 1977; Tuncer and Vanhoutte, 1993) and constricts renal vasculature. Outside of these works, the role of 5-HT in the kidney is not well understood or studied, especially the receptor mechanism by which 5-HT exerts its effects. We present data characterizing the 5-HT receptor(s) in the right renal artery of the normotensive rat.

### Expected Pharmacology

We took a classical pharmacological approach to determine the receptor(s) through which 5-HT stimulated renal arterial contraction. A minimum of two receptor populations have the potential to mediate 5-HT-induced contraction. This is evidenced by 1) the rank order of agonists in contracting the artery; 2) the correlation of potency values with literature-derived rat receptor binding affinities; 3) the antagonism exerted by carefully chosen concentrations of antagonists selective for different 5-$HT_2$ receptors; and 4) the biochemical identification of proteins recognized by antibodies specific for the 5-$HT_{2A}$ or 5-$HT_{2B}$ receptor. It is fair to acknowledge the limitations of potency correlations with radioligand binding affinities as agonists, alone, cannot be used to characterize a receptor. Demonstration of equilibrium conditions and lack of measurable desensitization are necessary, and we have not proven these definitively occur in our system. Moreover, agonist potency is not purely defined by affinity. As elegantly discussed by Kenakin (2002), efficacy and potency are associated in complex and interesting manners. Even given these limitations, these correlations

allow us to gather agonist data as a whole and evaluate the role of particular 5-HT receptors in contraction. The 5-$HT_{1B}$ and 5-$HT_{1F}$ receptors were excluded, and the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors appear to play the strongest role in mediating contraction. Importantly, mRNA for both the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor in rat renal artery has been reported (Ullmer *et al.*, 1995) and the kidney is one of the sites in which the human 5-$HT_{2B}$ receptor mRNA was found to be highly expressed (Bonhaus *et al*., 1995). The role of these two 5-HT receptor subtypes deserves comment.

First, 5-HT was more potent in the renal artery than the aorta, suggesting that the mechanisms by which 5-HT contracts the two arteries is different. On the basis of affinity alone, the potency of 5-HT in contracting the renal artery was not consistent with interacting with a classical 5-$HT_{2A}$ receptor which, using [$^3$H]-ketanserin as a radioligand, ranges anywhere from 500 nM to 2950 nM in the rat cortex (http://pdsp.cwru.edu/pdspf.asp). Similarly, micromolar affinity values for 5-HT have been observed when [$^3$H]-spiperone was used as a radioligand. One can speculate that the 5-$HT_{2A}$ receptors in the renal artery may be more efficiently coupled to their signalling elements than are 5-$HT_{2A}$ receptor in the aorta, or the renal artery expresses a unique 5-$HT_{2A}$ receptor for which 5-HT has a greater than normal affinity. Without a selective 5-$HT_{2A}$ receptor agonist which, to our knowledge, is currently unavailable, this is difficult to test. However, another explanation is that a different receptor for which 5-HT possesses a higher affinity is present, and this may be a 5-$HT_{2B}$-like receptor for which 5-HT possess a 300-fold higher affinity than the 5-$HT_{2A}$ receptor in the rat (Wainscott *et al.,* 1993).

The ability of the 5-$HT_{2B}$ receptor agonist BW723C86, currently the only relatively selective 5-$HT_{2B}$ receptor agonist available, to cause contraction of the renal artery lends significant support to the idea of a 5-$HT_{2B}$ receptor being involved in 5-HT-induced contraction. This is further supported by the fact that other agonists with affinity for the 5-$HT_{2B}$ receptor [mCPP, DOI, +norfenfluramine (Fitzgerald *et al.*, 2000; Rothman *et al.*, 2000)] also contracted the renal artery. Moreover, the 5-$HT_{2B}$ receptor antagonist LY272015 reduced not only the contraction to 5-HT but to BW723C86 as well. Finally, immunoreactive 5-$HT_{2B}$ receptor protein was present, though expressed at low levels compared to the aorta. However, it appears as if activation of 5-$HT_{2B}$ or 5-$HT_{2B}$-like receptor mediates only a modest portion of contraction to 5-HT as 10 nM LY272015 was maximal in its ability to antagonist 5-HT-induced contraction while ketanserin caused parallel and large shifts in contraction.

**Unexpected Pharmacology**

We found that antagonists which were predicted to shift a 5-$HT_{2B}$ receptor-mediated response, as based on their reported radioligand binding affinity, did not do so. LY53857 has nearly equimolar affinity for the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor (Wainscott *et al.,* 1993) and was predicted to produce a large and parallel rightward shift in 5-HT-induced contraction. This is true if one considers interaction of LY53857 with the 5-$HT_{2A}$ or 5-$HT_{2B}$ receptor alone, and figure 5 demonstrates this was not observed. Moreover, high concentrations of LY53857 (100 nM) were necessary to antagonize contraction to BW723C86. The $pK_B$ value of the 5-$HT_{2B}$ receptor antagonist LY272015 in the renal artery (8.59$\pm$0.03) was different from that in the

fundus (9.73±0.05). This suggests that the renal 5-HT receptor and fundus receptor are not the same in terms of the affinity LY272015 possesses for the receptors. Thus, the renal receptor may not be a classical 5-$HT_{2B}$ receptor. The lack of similarity may also be influenced by the complement of 5-HT receptors present in the renal artery or indicative of tissue heterogeneity in receptor pharmacology.

It is not only the response to LY53857 that was unexpected but the relative inability of RS127445 to block 5-HT-induced contraction. This compound has been well described as a 5-$HT_2$ receptor antagonist (Bonhaus *et al.*, 1999), and in our hands exerts 5-$HT_{2B}$ receptor antagonism as evidenced by a competitive shift in 5-HT-induced contraction in the rat stomach fundus. Thus, it is reasonable to reiterate that the fundus 5-HT receptor is different from the renal 5-HT receptor(s). Blockade by neutral antagonists should not be dependent on coupling efficiency and we know of no evidence that suggests that either LY272015, LY53857 or RS127445 exert any inverse agonism. Thus, the receptor or receptor unit must be of a different character.

Another explanation for these findings, which is purely speculative, is that the combined presence of the 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor may modify the ability of receptor antagonists to inhibit contraction. Such receptor interaction and consequent modification of receptor pharmacology has been reported for endothelin receptors (Adner *et al.,* 2001, Lodge *et al.,* 1995, Watts *et al.*, 2002) and more recently for the 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors (Xie *et al.*, 1999). In this interaction, receptor proteins heterodimerize to form a pharmacologically distinct and functional unit. We are currently unable to test the idea that the 5-$HT_{2A}$ and 5-$HT_{2B}$ interact physically because antibodies for the 5-$HT_{2A}$ receptor that are amenable to

immunoprecipitation/coimmunoprecipitation and Western studies are not available. Thus, the idea of 5-$HT_{2A}$ and 5-$HT_{2B}$ receptor interaction must remain a speculation.

**Perspectives**

Some of these questions may not be answered until the 5-HT receptor(s) is/are cloned from the renal artery, or until the issue of receptor interaction and efficiency is addressed. The 5-$HT_{2B}$ receptor in the rat stomach fundus was cloned in 1992 (Foguet *et al.,* 1992; Kursar *et al.,* 1992), pharmacologically described in 1993 (Wainscott *et al.,* 1993) and is a member of the heptahelical superfamily. The human and rat 5-$HT_{2B}$ receptor differ in terms of the affinity possessed by ketanserin for each receptor (Wainscott *et al.,* 1996). Regardless of the outcome of such studies, there is sufficient evidence to suggest the presence of a functional 5-$HT_{2B}$-like receptor in the rat renal artery that is part of the unique pharmacology of the 5-HT receptors in the renal artery.

These findings are physiologically relevant given the relatively sparse research performed in understanding the role of 5-HT in control of renal function. The finding of a unique pharmacology may present a similarly unique means by which to affect renal arterial function. 5-HT, as does m-CPP, increases perfusion pressure in the *in situ* autoperfused rat kidney (Moran *et al.,* 1997). It is important to note that the two receptors identified in the renal artery -- the 5-$HT_{2A}$ and 5-$HT_{2B}$ or 5-$HT_{2B}$ -like -- also serve the demonstrable function of 5-HT-mediated cellular mitogenesis, growth and tissue formation. Studies in the vasculature demonstrate the ability of the 5-$HT_{2A}$ receptor to mediate 5-HT-induced mitogenesis (Grewal *et al.*, 1999), as measured by

[$^3$H]thymidine uptake, and work using the 5-$HT_{2B}$ receptor knockout mouse underscores the importance of this receptor to the normal formation of the heart (Nebigil *et al.,* 2000) and gastrointestinal system (Fiorica-Howells *et al.*, 2000). The 5-$HT_{2B}$ receptor has also been described as necessary for development of pulmonary hypertension induced by hypoxia (Launay *et al.,* 2002) and for the pathology associated with fenfluramine-induced damage of cardiac valves (Fitzgerald *et al.,* 2000). While not studied here, both receptor subtypes could potentially contribute to renal arterial narrowing and thus contribute to renal disease.

In summary, these findings present evidence of a complex pharmacology of 5-HT receptors mediating contraction to 5-HT in the normal right renal artery of the rat. While the pharmacology of this receptor is curious, the finding of such a receptor(s) response raises questions as to whether 5-HT receptor coupling is tissue-dependent, whether 5-HT receptor heterogeneity exists or whether coexistence of two 5-$HT_2$ receptor subtypes enables a unique pharmacology.

## References

Adner M, Shankley N and Edvinnson L (2001) Evidence that ET-1 but not ET-3, S6b, $ET_A$ receptor mediated contractions in isolated rat mesenteric arteries are modulated by co-activation of $ET_B$ receptors. *Br. J. Pharmacol.,* **133**, 927-935.

Arunlakshana O and Schild HD (1959) Some quantitative uses of drug antagonists. *Br. J. Pharmacol.,* **14**: 48-58.

Banes AB and Watts SW (2002) Upregulation of arterial serotonin1B and 2B receptors in DOCA-salt hypertension. *Hypertension*, **39**, 394-398.

Blackshear JL, Orlandi C and Hollenberg NK (1991) Constrictive effect of serotonin on visible renal arteries: a pharmacoangiographic study in anesthetized dogs. *J. Cardiovasc. Pharmacol.,* **17**, 68-73.

Bonhaus DW, Bach C, DeSouza A, Salazaar FH, Matsuoka BD, Zuppan P, Chan HW and Eglen RM (1995) The pharmacology and distribution of human 5-hydroxytryptamine$_{2B}$ (5-$HT_{2B}$) receptor gene products: comparison with 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors. *Br. J. Pharmacol.,* **115**, 622-628.

Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M, Van Natta K, Chang LK, Pulido-Rios T, Webber A, Leung E and Martin GR (1999)

RS-127445: a selective high affinity, orally bioavailable 5-$HT_{2B}$ receptor antagonist. *Br. J. Pharmacol.,* **127**, 1075-1082.

Choppin A and O'Conner SE (1994) 5-Hydroxytrytpamine1D-like receptors mediate contraction of partially depolarized rabbit renal arteries. *J. Pharmacol. Exp. Ther*., **270**, 650-655.

Cohen ML, Schenck KW, Mabry TE, Nelson DL and Audia JE (1996) LY272015, a potent, selective and orally available 5-$HT_{2B}$ receptor antagonist. *J. Ser. Res*., **3**, 131-144.

Collis MG and Vanhoutte PA (1977) Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circ. Res*., **41**, 759-767.

Fiorica-Howells E, Maroteaux L and Gershon MD (2000) Serotonin and the 5-$HT_{2B}$ receptor in the development of enteric neurons. *J. Neurosci.,* **20**, 294-305.

Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, Sun J-H, Link JR, Abbaszade I, Hollis JM, Largent BL, Hartig PR, Hollis GF, Meunier PC, Robichaud AJ and Robertson DW (2000) Possible role of valvular serotonin 5-$HT_{2B}$ receptors in the cardiopathy associated with fenfluramine. *Mol. Pharmacol.,* **57**, 75-81.

Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman O, Stuhmer W and Lubbert H (1992) Cloning and functional expression of the rat stomach fundus serotonin receptor. *EMBO J*., **11**, 3481-3487.

Grewal JS, Mukhin YV, Garnovskaya MN, Raymond JR and Greene EL (1999) Serotonin 5-$HT_{2A}$ receptor induces TGF-β1 expression in mesangial cells via ERK: proliferative and fibrotic signals. *Am. J. Physiol. Renal,* **276**, F922-F930.

Hafdi Z, Couette S, Comoy E, Prie D, Amiel C and Friedlander G (1996) Locally formed 5-hydroxytryptamine stimulates phosphate transport in cultured opossum kidney cells and in rat kidney. *Biochem. J.,* **320**, 615-621.

Hill PB, Dora KA, Hughes AD and Garland CJ (2000) The involvement of intracellular $Ca^{2+}$ in 5-HT (1B/1D) receptor-mediated contraction of the rabbit isolated renal artery. *Br. J. Pharmacol.,* **130**, 835-842.

Kenakin T (2002) Efficacy at G-protein-coupled receptors. *Nat. Rev. Drug Discov*., **1**, 103-110.

Kursar JD, Nelson DL, Wainscott DB, Cohen ML and Baez M (1992) Molecular cloning, functional expression and pharmacological characterization of a novel

serotonin receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus. *Mol. Pharmacol.*, **42**, 549-557.

Launay JM, Herve P. Peoc'h HK, Tournois C, Callebert J, Nebigil CG, Etienne N, Drouet L, Humbert M, Simonneau G and Maroteaux L (2002) Function of the serotonin 5-hydroxytryptamine$_{2B}$ receptor in pulmonary hypertension. *Nature Medicine*, **8(10),** 1129-1135.

Lincoln J, Loesch A and Burnstock G (1990) Localization of vasopressin, serotonin and angiotensin II in endothelial cells of the renal and mesenteric arteries of the rat. *Cell. Tissue Res.,* **259**, 341-344.

Lodge NJ, Zhang R, Halaka NN and Moreland S (1995) Functional role of endothelin $ET_A$ and $ET_B$ receptors in venous and arterial smooth muscle. *Eur. J. Pharmacol.,* **287**, 762-767.

Martin GR (1994) Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. *Pharmac. Ther.,* **62**, 283-324.

McGregor DD and Smirk FH (1970) Vascular reactivity to 5-hydroxytryptamine and hypertension in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **272**, 101-116.

Moran A, Velasco C, Martin ML and Roman LS (1997) Renal vasoconstrictor response to 5-hydroxytryptamine in the in situ autoperfused rat kidney: involvement of angiotensin II and the 5-$HT_2$ receptor activation. *Eur. J. Pharmacol.*, **330**, 205-211.

Nebigil CG, Choi DS, Dierich A , Hickel P, Le Meur M, Messaddeq N, Launay JM and Maroteaux L (2000) Serotonin 2B receptor is required for heart development. *Proc. Natl. Acad. Sci. USA*, **97**, 9508-9513.

Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ and Roth BL (2000) Evidence for possible involvement of 5-$HT_{2B}$ receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*., **102,** 2836-2841.

Russell A, Banes A, Berlin H, Fink GD and Watts SW (2002) 5-Hydroxytryptamine2B receptor function is enhanced in the $N^w$-nitro-L-arginine hypertensive rat, *J. Pharmacol. Exp. Ther*., **303**, 179-187.

Takahashi T., Hisa H and Satoh S (1992) Serotonin-induced vasoconstriction in dog kidney. *J. Cardiovasc. Pharmacol.,* **20**, 779-784.

Tuncer M and Vanhoutte PM (1993) Serotonin releases a vasoconstrictor prostanoid in the kidney of the aging spontaneously hypertensive rat. *Blood Pressure*., **2,** 142-145.

Turla MB And Webb RC (1989) Vascular responsiveness to 5-hydroxytryptamine in experimental hypertension. In *The Peripheral Actions of 5-Hydroxytryptamine*, ed. By J. R. Fozard, pp. 327-353, Oxford University Press, Oxford.

Ueda S, Ikegami K, Yoneumura K and Sakanashi M (1982) A possible mechanism involved in generation of automaticity of isolated human renal artery. *Arch. Int. Pharmacodyn. Ther.,* **256**, 204-211.

Ullmer C, Schmuck K, Kalkman HO and Lubbert H (1995) Expression of serotonin receptor mRNAs in blood vessels. *FEBS Lett.,* **370**, 215-222.

Wainscott DB, Cohen ML, Schenck KW, Audia J, Nissen JS, Baez M, Kursar J D, Lucaites VL and Nelson D (1993) Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine2F receptor. *Mol. Pharmacol.,* **43**, 419-426.

Wainscott, DB, Lucaites VL, Kursar JD, Baez M and Nelson DL (1996) Pharmacological characterization of the human 5-hydroxytryptamine2B receptor : evidence for species differences. *J. Pharmacol. Exp. Ther.*, **276,** 720-727.

Watts SW (1997) The 5-$HT_{2B}$ receptor antagonist LY272015 inhibits 5-HT-induced contraction in the aorta of mineralocorticoid-salt hypertensive rats. *J. Ser. Res.* **4**, 197-206.

Watts SW and Fink GD (1999) The 5-hydroxytryptamine$_{2B}$ receptor antagonist LY272015 is antihypertensive in severely hypertensive deoxycorticosterone acetate salt rats. *Am. J. Physiol. Heart Circ.,* **276**, H944-H952.

Watts SW, Fink GD, Northcott CA and Galligan JJ (2002) Endothelin-1-induced venous contraction is maintained in DOCA-salt hypertension; studies with receptor agonists. *Br. J. Pharmacol.,* **137**, 69-79.

Wright CE and Angus JA (1987) Diverse vascular responses to serotonin in the conscious rabbit: effects of serotonin antagonists on renal artery spasm. *J. Cardiovasc. Pharmacol.*, **10**, 415-423.

Xie Z, Lee SP, O'Dowd BF and George SR (1999) Serotonin 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors form homodimers when expressed alone and heterodimers when co-expressed. *FEBS Lett.,* **456**, 63-67.

## Footnotes

a) This work was supported by NIH HL58489

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## Figure Legends

**Figure 1.** Comparison of 5-HT-induced contraction in endothelium-denuded aorta and right renal artery from the normotensive rat. Points represent means±SEM for the number of animals in parentheses. PE = phenylephrine.

**Figure 2.** Effect of 5-HT receptor agonists in contracting the endothelium-denuded right renal artery of the normotensive rat. Agonists are divided into **A:** agonists that are potent and cause a large maximum contraction and **B:** agonists which are less potent and cause a smaller maximum contraction. Points represent means±SEM for the number of animals in parentheses indicated in parentheses. PE = phenylephrine.

**Figure 3.** Effect of LY272015 and LY53857 on BW723C86-induced contraction in endothelium-denuded right renal artery. Points represent means±SEM for the number of animals indicated in parentheses. * indicate significant differences ($p < 0.05$) from vehicle. PE = phenylephrine.

**Figure 4.** Effect of the 5-HT $_{2A/2C}$ receptor antagonist ketanserin (3, 10 nM; panel A) and 5-HT$_{2B}$ receptor antagonist LY272015 (10, 50 nM; panel B) with or without ketanserin (3 nM) on 5-HT-induced contraction in endothelium-denuded right renal artery. Points represent means±SEM for the number of animals indicated in parentheses. * indicate significant differences ($p < 0.05$) from vehicle; † =

statistically significant differences ($p < 0.05$) from LY272015 (50 nM)-incubated responses. PE = phenylephrine.

**Figure 5.** Antagonism exerted by the non-selective 5-$HT_2$ receptor antagonist LY53857 against 5-HT in the endothelium-denuded right renal artery. Points represent means±SEM for the number of animals indicated in parentheses. * indicate significant differences ($p < 0.05$) from vehicle for all concentrations starting at the arrow. PE = phenylephrine.

**Figure 6.** Antagonism exerted by the 5-$HT_{2B}$ receptor antagonist RS127445 against 5-HT in the endothelium-denuded right renal artery (**A**) and stomach fundus (**B**). Points represent means±SEM for the number of animals indicated in parentheses. * indicate significant differences ($p < 0.05$) from vehicle. PE = phenylephrine.

**Figure 7.** Western analyses demonstrating the presence of an immuno-recognized 5-$HT_{2B}$ receptor in right renal artery (**A**) and immunohistochemical analysis of right renal artery detecting the presence of immunoreactive 5-$HT_{2A}$ receptor (**B**). Experiments representive of four separate experiments performed with samples from different animals. Arbitrary densitometry units were measured using NIH Image 1.62. Arrow points to specific binding in the area of arterial smooth muscle.

**Table 1.** Pharmacological parameters for 5-HT receptor agonist-induced contraction in endothelium-denuded rat renal artery. Values are means±SEM for the number of animals in parentheses. -------- = could not be obtained.

| **Agonist (N=4-7)** | **-log$EC_{50}$ [M]** | **Percentage maximum PE contraction[a]** |
|---|---|---|
| DOI | 8.21±0.09 | 124.7± 8.7 |
| 5-HT | 7.03±0.02 | 124.8± 9.5 |
| 5-Methoxytryptamine | 6.96±0.03 | 146.8±12.4 |
| mCPP | 6.65±0.04 | 68.7± 4.9 |
| □□□□□-methyl-5-HT | 6.64±0.05 | 141.8± 8.4 |
| BW723C86 | 6.50±0.09 | 58.8±12.1 |
| (+)norfenfluramine | 6.41±0.05 | 138.7±13.4 |
| 2-methyl-5-HT | 6.03±0.08 | 165.8±27.0 |
| CP93129 | 5.51±0.07 | 38.1± 7.6 |
| 5-CT | 5.49±0.03 | 107.1± 6.1 |
| (+/-)8-OH-DPAT | 5.20±0.13 | 49.8±13.0 |
| Sumatriptan | 4.14±0.09 | 15.3± 7.8 |
| LY344864 | ---------- | 0± 0 |

[a.] PE = phenylephrine, 10 uM.

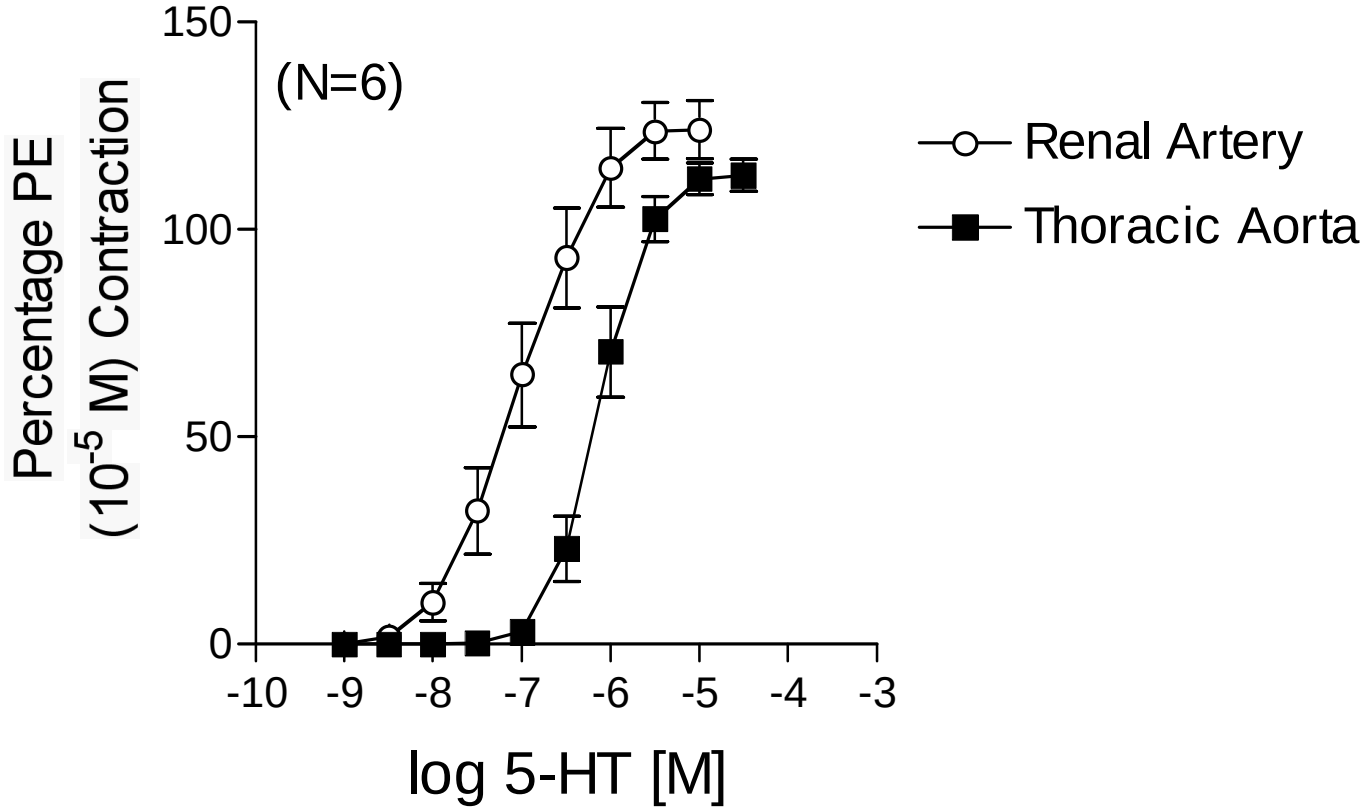
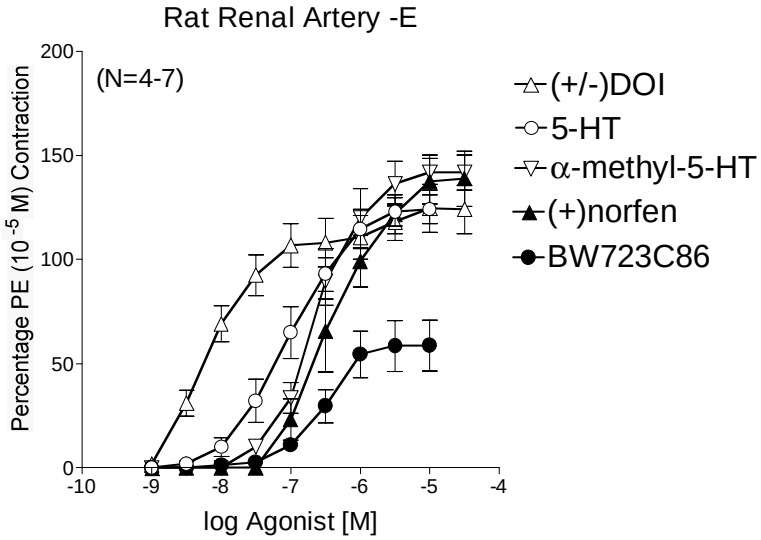

150
100
50
0
(N=6)
Percentage PE
(10-5 M) Contraction
-10
-9
-8
-7
-6
-5
-4
-3
log 5-HT [M]
Renal Artery
Thoracic Aorta

Figure 1

**A.**


Rat Renal Artery -E
(N=4-7)
Percentage PE (10-5 M) Contraction
log Agonist [M]
(+/-)DOI
5-HT
α-methyl-5-HT
(+)norfen
BW723C86

**B.**

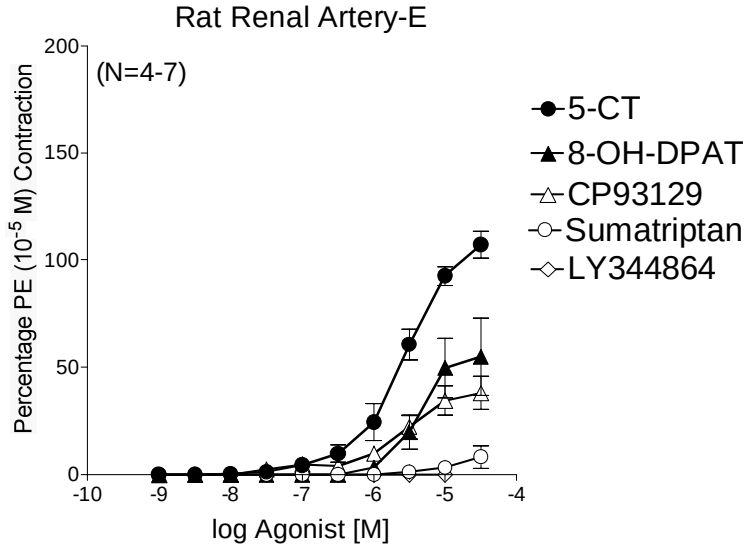

Rat Renal Artery-E
(N=4-7)
Percentage PE (10-5 M) Contraction
log Agonist [M]
5-CT
8-OH-DPAT
CP93129
Sumatriptan
LY344864

Figure 2

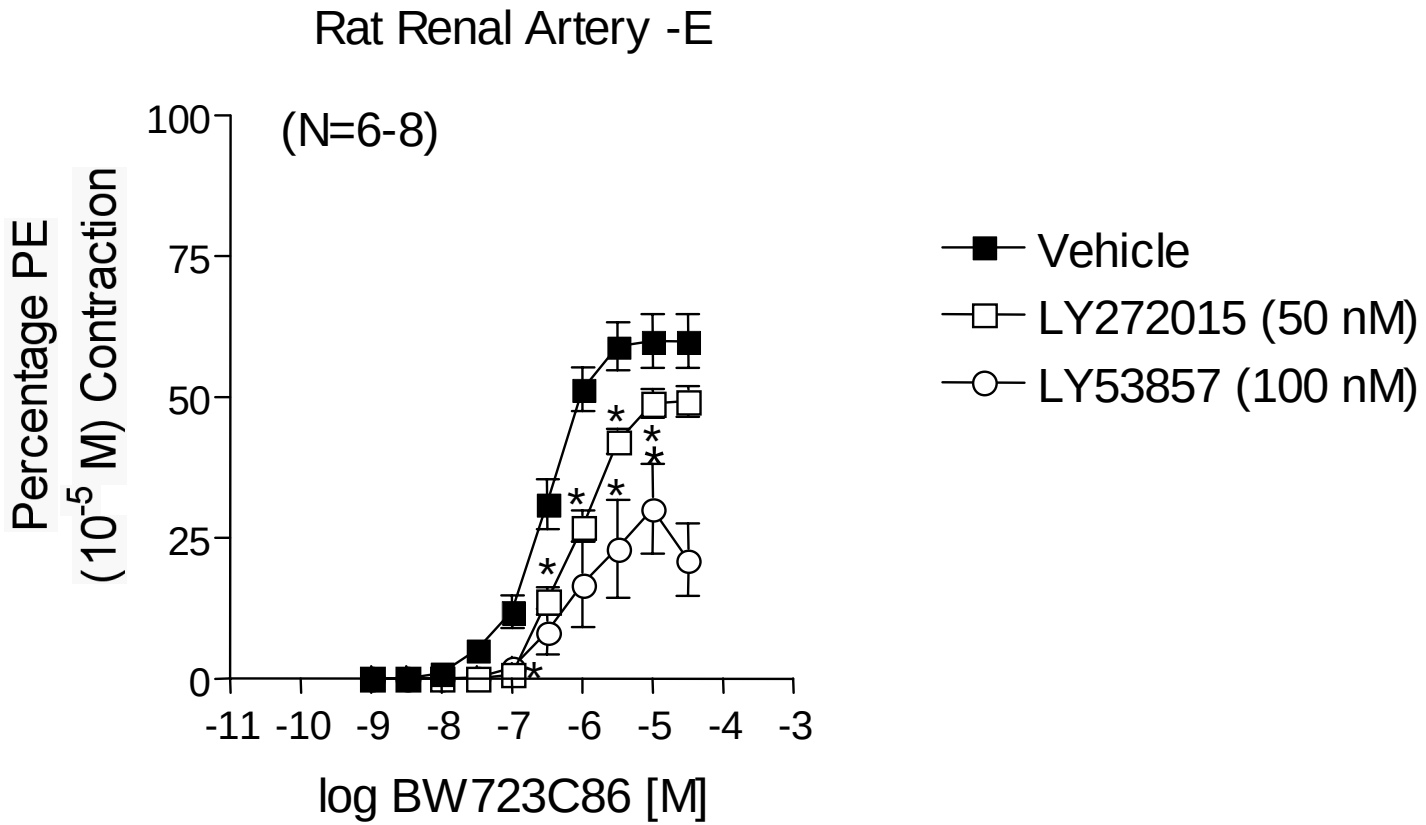
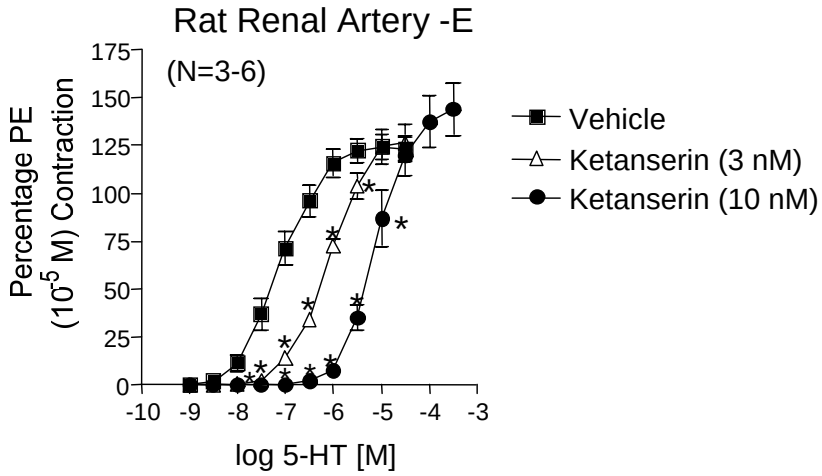

Rat Renal Artery -E
(N=6-8)
Percentage PE
(10-5 M) Contraction
100
75
50
25
0
-11 -10 -9 -8 -7 -6 -5 -4 -3
log BW723C86 [M]
Vehicle
LY272015 (50 nM)
LY53857 (100 nM)

Figure 3

**A.**


Rat Renal Artery -E
(N=3-6)
Percentage PE
($10^{-5}$ M) Contraction
175
150
125
100
75
50
25
0
-10 -9 -8 -7 -6 -5 -4 -3
log 5-HT [M]
Vehicle
Ketanserin (3 nM)
Ketanserin (10 nM)

**B.**

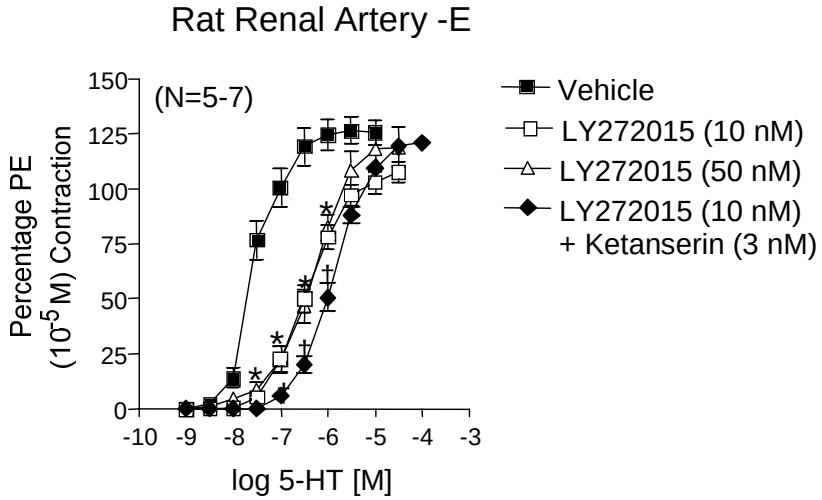

Rat Renal Artery -E
(N=5-7)
Percentage PE
($10^{-5}$ M) Contraction
150
125
100
75
50
25
0
-10 -9 -8 -7 -6 -5 -4 -3
log 5-HT [M]
Vehicle
LY272015 (10 nM)
LY272015 (50 nM)
LY272015 (10 nM)
+ Ketanserin (3 nM)

Figure 4

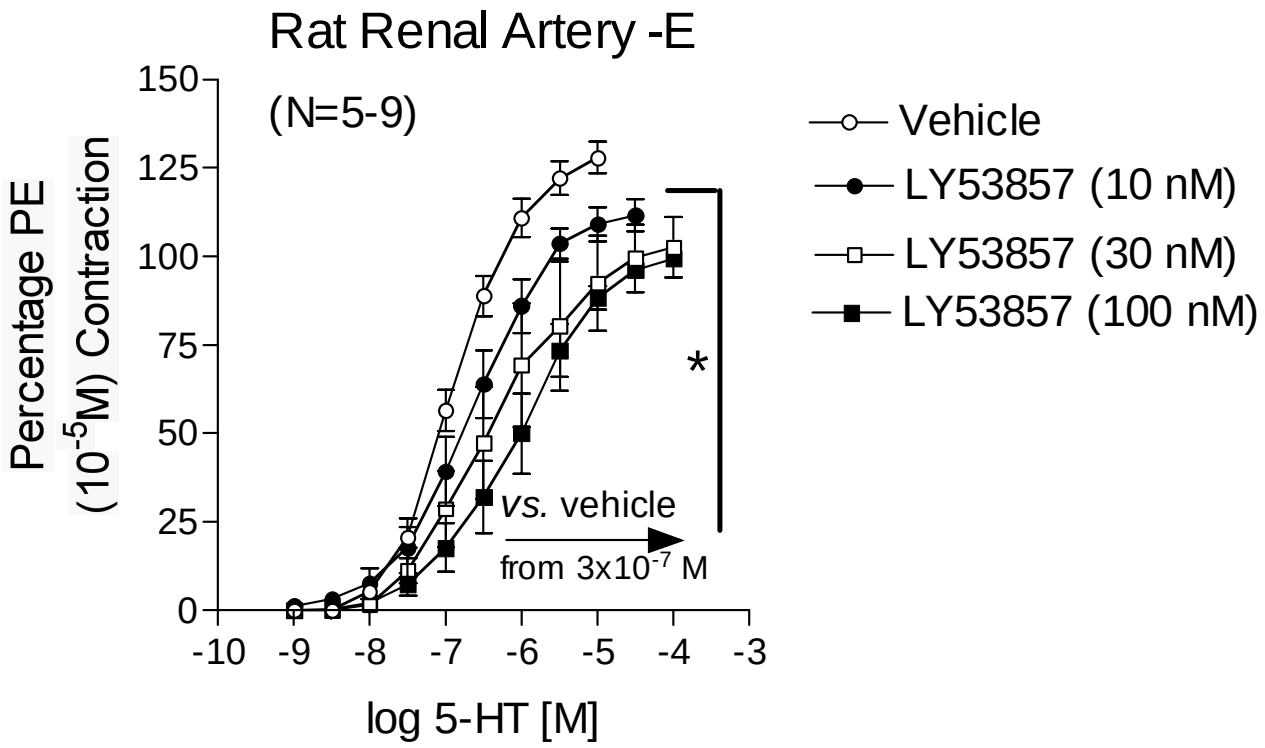

Rat Renal Artery -E
(N=5-9)
Percentage PE
(10-5M) Contraction
150
125
100
75
50
25
0
-10
-9
-8
-7
-6
-5
-4
-3
log 5-HT [M]
vs. vehicle
from 3x10-7 M
*
Vehicle
LY53857 (10 nM)
LY53857 (30 nM)
LY53857 (100 nM)

Figure 5

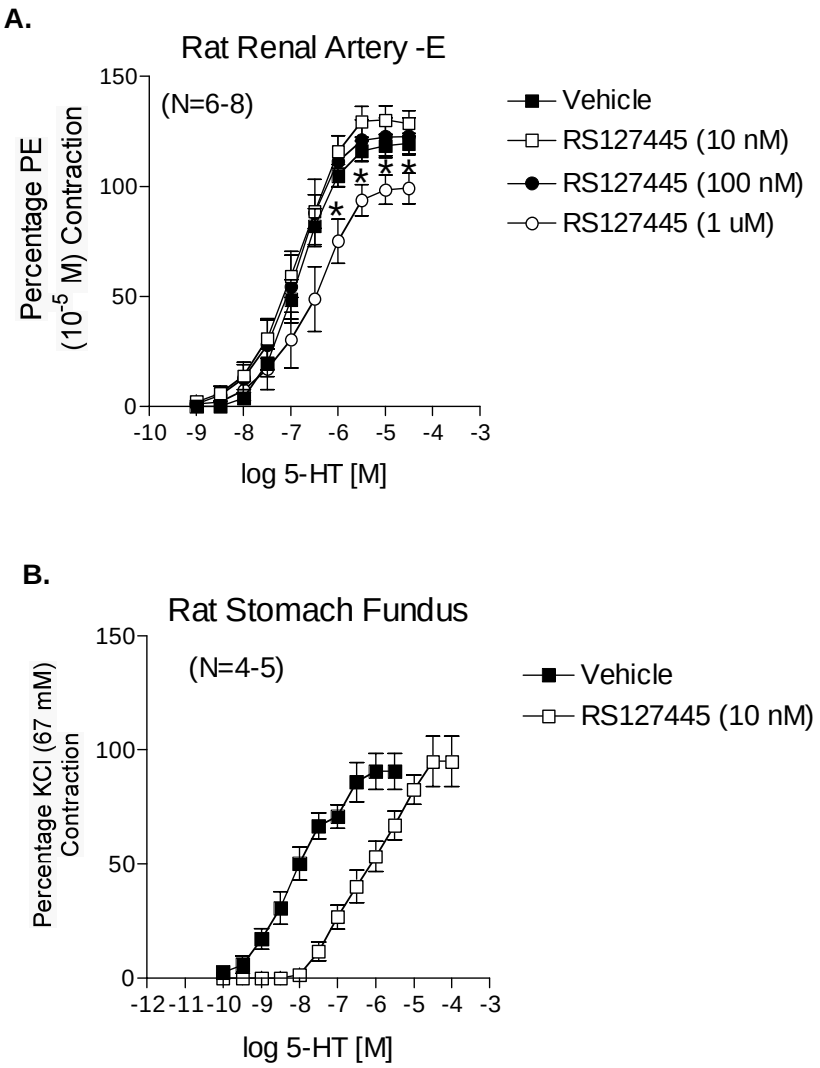
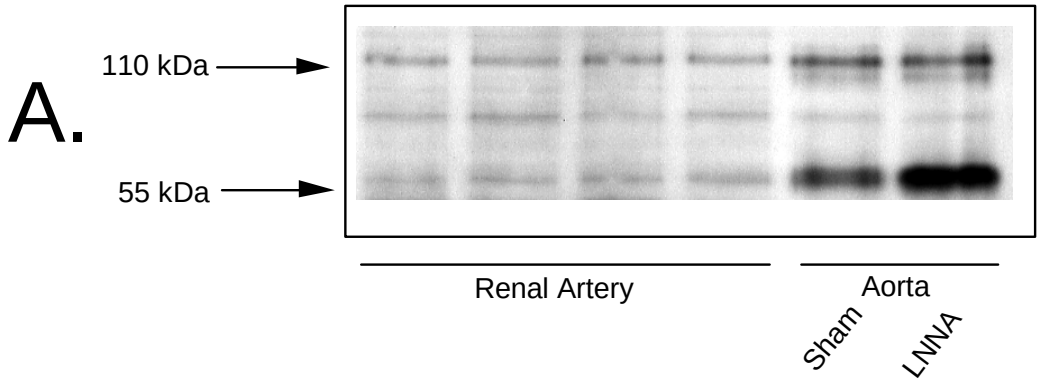

A.
Rat Renal Artery -E
(N=6-8)
Percentage PE
(10-5 M) Contraction
150
100
50
0
-10 -9 -8 -7 -6 -5 -4 -3
log 5-HT [M]
Vehicle
RS127445 (10 nM)
RS127445 (100 nM)
RS127445 (1 uM)
B.
Rat Stomach Fundus
(N=4-5)
Percentage KCl (67 mM)
Contraction
150
100
50
0
-12 -11 -10 -9 -8 -7 -6 -5 -4 -3
log 5-HT [M]
Vehicle
RS127445 (10 nM)

Figure 6


A.
110 kDa
55 kDa
Renal Artery
Aorta
Sham
LNNA

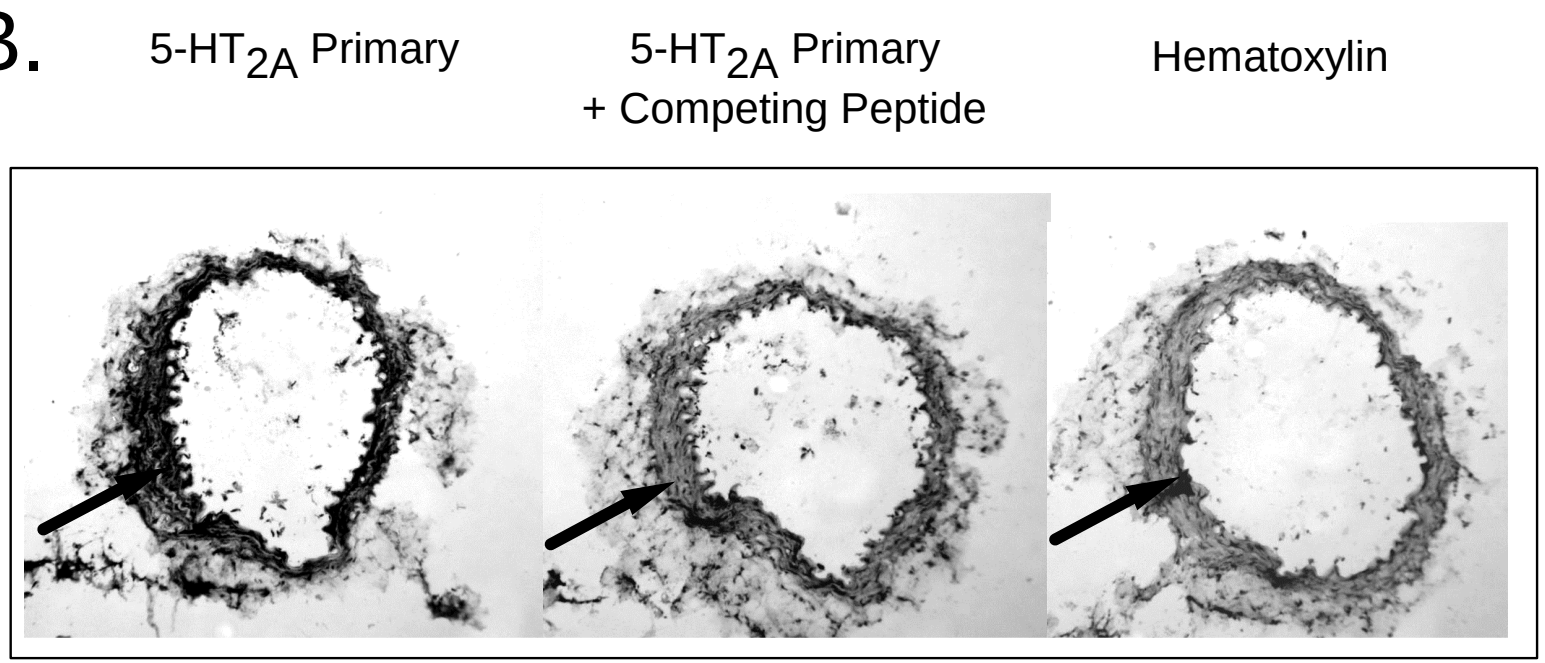

B.
5-HT2A Primary
5-HT2A Primary
+ Competing Peptide
Hematoxylin

Figure 7