Serotonin-Induced Contraction in Mesenteric Resistance Arteries
Signaling and Changes in Deoxycorticosterone Acetate–Salt Hypertension

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Abstract—Large arteries from hypertensive subjects are hyperresponsiveness to 5-hydroxytryptamine (5-HT). We tested the hypothesis that small arteries (225 μ ID) have a profile similar to conduit arteries, including signal transduction mechanisms and the 5-HT receptor subtype(s) mediating arterial contraction in normal and high blood pressure. Aorta and mesenteric arteries from Sprague-Dawley (232±6 μ ID), sham (229±7 μ ID; systolic blood pressure, 120±2 mm Hg), or deoxycorticosterone acetate (DOCA)–salt rats (255±11 μ ID, 192±8 mm Hg) were mounted in a wire-based myograph. In resistance arteries from Sprague-Dawley rats, the 5-HT2A receptor mediated contraction; agonists of the 5-HT1B, 5-HT1D, 5-HT1F, and 5-HT2B receptor were inactive. The tyrosine kinase inhibitor genistein (5 μmol/L, 4.8-fold rightward shift), PD 098,059 (10 μmol/L, 3.2-fold shift), phospholipase C inhibitor NCDC (100 μmol/L), and nifedipine (50 nmol/L) reduced maximum 5-HT–induced contraction in small arteries (4.5% and 53% control, respectively). As in aorta, 5-HT had a decrease in threshold (100-fold lower), increase in potency (11.6-fold leftward shift), and increase in efficacy (140% sham response) in small arteries from DOCA-salt rats compared with sham. Unlike in aorta, 5-HT–induced contraction in DOCA-salt small arteries was shifted competitively by the 5-HT2A receptor antagonist ketanserin (log KB [mol/L] for both sham and DOCA-salt, 9.25±0.1), and contraction to the 5-HT2B agonist BW723C86 was not observed. Thus, the 5-HT2A receptor remains the contractile receptor in hypertension in small arteries. Although similarities were observed for large and small arteries, differences under the condition of DOCA-salt hypertension exist that may determine serotonergic compounds effective in lowering blood pressure.

Key Words: 5-hydroxytryptamine • resistance • deoxycorticosterone

Hyperresponsiveness of blood vessels from hypertensive subjects to contractile agonists has long been known. A pronounced hyperresponsiveness is observed in the tryptophan-derived autacoid serotonin (5-hydroxytryptamine [5-HT]).1–5 The involvement of 5-HT in hypertension is unclear, as studies both support and dispute the importance of this hormone.6–8 A goal of our laboratory is to understand the mechanisms that enable arteries to become hyperresponsive to 5-HT and to determine whether this hyperresponsiveness is important to hypertension.

At this time, there are 7 major subtypes of 5-HT receptors.7,9 In the rat, the 5-HT2A receptor is primarily responsible for mediating contraction to 5-HT in large arteries. This receptor subtype is supplanted by the 5-HT2B receptor as the predominant contractile receptor in large arteries in deoxycorticosterone acetate (DOCA)–salt hypertension.10–14 5-HT has a 300-fold higher affinity for the 5-HT2B compared with the 5-HT2A receptor, whereas the 5-HT2A receptor antagonist ketanserin has a 3000-fold lower affinity for the 5-HT2B receptor.15,16 This change in large arteries has been supported first by a pharmacological profile consistent with 5-HT2B receptor activation in arteries from DOCA-salt rats compared with sham, and second as an increase (≈200 to 250%) in the density of 5-HT2B receptor protein in aorta and superior mesenteric arteries from DOCA-salt rats.10–14,17 Moreover, the 5-HT2B receptor antagonist LY 272015 reduces blood pressure of severely hypertensive DOCA-salt rats.14 Based on these findings, we hypothesized that the 5-HT2B receptor would also be upregulated at the level of resistance arteries. Thus, we used a wire-based myograph to examine whether the 5-HT2B receptor is functionally upregulated in small resistance arteries, arteries responsible for modulation of total peripheral resistance, of DOCA-salt hypertensive rats.

We began with studies that investigate whether the 5-HT2A receptor serves as the primarily contractile receptor in the small arteries of a normotensive rat, as has been published larger arteries.18–23 This was followed by studies investigating the basic signal transduction mechanisms of 5-HT–induced contraction in resistance arteries. With the 5-HT2A receptor established as the primary contractile receptor in the resistance arteries, we then proceeded to investigate 5-HT responsiveness in DOCA-salt hypertension. This particular series of experiments in resistance arteries was performed in parallel with some experiments in the aorta removed from the
same animals. This allowed a direct comparison within an animal as to the receptor mechanism of small and large artery hyperresponsiveness to 5-HT.

Methods

Surgical and Blood Pressure Protocol

Male Sprague-Dawley rats (0.225 kg, Charles River, Portage, Mich) were uninephrectomized under Metofane anesthesia. Half were given a DOCA-impregnated silastic implant (200 mg/kg) and received salt water to drink (1.0% NaCl+0.2% KCl). Sham rats drank tap water. After 4 weeks, systolic blood pressure was measured using a tail cuff.

Isolated Tissue Bath Protocol

Rats were euthanized (pentobarbital, 60 mg/kg IP), and the aorta and/or mesentery (below) was removed. Arteries were dissected into helical strips (0.2×1.0 cm), and the endothelial cell layer was removed. Tissues were placed in physiological salt solution for measurement of isometric contractile force as described previously. Arteries equilibrated for 1 hour before challenge with a maximal concentration of phenylephrine (10 μmol/L). Tissues were washed and rechallenged with a half-maximal concentration of phenylephrine (10 nmol/L), and acetylcholine (1 μmol/L) was added to verify the lack of endothelial cells. Tissues were washed, and cumulative response curves to agonists (10⁻¹ to 3×10⁻⁴ mol/L) were performed. When investigating antagonists or inhibitors, either vehicle (0.1% dimethylsulfoxide) or antagonist/inhibitor equilibrated with tissues for 1 hour before agonist addition was used.

Myograph Protocol

Rats were euthanized, and the small intestine was removed and pinned down in a silastic-filled Petri dish in cold physiological salt solution. Mesenteric arteries (200 to 300 μm ID) were carefully dissected. Two tungsten wires (0.002-cm diameter, California Wire) were threaded through the lumen of the artery using a light microscope. One wire was mounted to a micrometer and the other to a microtransducer connected to a Grass polygraph. Arteries from sham and DOCA-salt animals were mounted in the same experiment, with tissues for 1 hour before agonist addition was used.

Data Analysis

Data are presented as mean±SEM and as a percentage of the initial response to phenylephrine (10⁻⁵ mol/L) for the number of animals indicated in parentheses. agonist EC₅₀ values were calculated using a nonlinear regression analysis using the algorithm \[\text{EC₅₀} = \text{maximum response} / (1 + (\text{agonist concentration} / \text{EC₅₀}))\] (GraphPad Prism). Antagonist dissociation constants (pKᵦ) were calculated using the following formula: \[\log (dr−1) = \log [B] = \log K_a\] where dr indicates the EC₅₀ value of 5-HT after antagonist/EC₅₀ value of 5-HT before antagonist; B, the antagonist concentration.

Chemicals

Solutions of compounds were prepared in deionized water unless indicated otherwise: acetylcholine chloride, DOCA (silastic), 5-HT, NCDC (ethanol), nifedipine (ethanol), and phenylephrine from Sigma Chemical Co; \(\alpha\)-methyl-5-HT, BRL 54443, BW 723C86, daidzein (DMSO), genistein (DMSO), ketanserin (DMSO), PD 098,059 (DMSO), and RU24969 from Sigma RBL, LY 344864, PNU0142633, and sumatriptan were gifts from Lilly Research Laboratories (Indianapolis, Ind), Pharmacia (Kalamazoo, Mich), and GlaxoWellcome (Hertfordshire, UK), respectively.

Results

Response of Normal Mesenteric Resistance Arteries to Serotonergic Agonists

Figure 1 (top) displays response of endothelium-intact mesenteric resistance arteries from normal Sprague-Dawley rats. Bottom, Effect of the inactive tyrosine kinase inhibitor daidzein, inhibitor of MEK activation PD 098,059, L-type calcium channel antagonist nifedipine, general tyrosine kinase inhibitor genistein, and phospholipase C inhibitor NCDC on 5-HT-induced contraction in endothelium-intact mesenteric resistance arteries from normal Sprague-Dawley rats. Values are mean±SEM for the number of animals indicated in parentheses. *P<0.05 vs control response. PE indicates phenylephrine.

Figure 1. Top, Effect of serotonin receptor agonists on contraction of endothelium-intact mesenteric resistance arteries from normal Sprague-Dawley rats. Bottom, Effect of the inactive tyrosine kinase inhibitor daidzein, inhibitor of MEK activation PD 098,059, L-type calcium channel antagonist nifedipine, general tyrosine kinase inhibitor genistein, and phospholipase C inhibitor NCDC on 5-HT–induced contraction in endothelium-intact mesenteric resistance arteries from normal Sprague-Dawley rats. Values are mean±SEM for the number of animals indicated in parentheses. *P<0.05 vs control response. PE indicates phenylephrine.

Figure 1 (top) displays response of endothelium-intact arteries from normal Sprague-Dawley rats to agonists of 5-HT receptors that have been implicated in modulating arterial tone. The average diameter of these arteries was 232±6 μm. 5-HT contracted the resistance arteries in a concentration-dependent manner with a −log EC₅₀ value [mol/L] of 6.15±0.04. The 5-HT₁ receptor agonist \(\alpha\)-methyl-5-HT was slightly less potent (5.82±0.05, P<0.05), whereas the putative 5-HT₄ receptor agonist BRL 54443 was similarly potent to 5-HT (6.23±0.06). BRL 54443 has significant affinity for the 5-HT₃ₐ receptor, \(^{25}\) and we have found that the 5-HT₃ₐ receptor antagonist ketanserin can block BRL 54443–induced contraction in rat and mouse aorta. \(^{26,27}\) This suggests that BRL 54443 is acting as a partial agonist of 5-HT₃ₐ receptors rather than stimulating arterial contraction via 5-HT₄ receptors. Further supporting this suggestion is the fact that a more selective 5-HT₄ receptor agonist, LY 344864, \(^{28}\) did not contract the resistance arteries (Figure 1, top). Agonists for other 5-HT receptors were completely inactive. Sumatriptan (5-HT₁B/F receptor), PNU0142633 (5-HT₁D receptor), RU24969 (5-HT₁₈ receptor), and BW 723C86 (5-HT₂B recep-
tor) were without effect on isometric tension in resistance arteries. These data suggest that the predominant receptor mediating 5-HT-induced contraction is a 5-HT2A receptor, similar to the aorta and superior mesenteric artery.

Effect of Signaling Inhibitors on 5-HT–Induced Contraction in Normal Mesenteric Resistance Arteries

5-HT was tested against a number of signaling inhibitors to determine the mechanism of contraction in resistance arteries. Similar to the aorta,29–31 the L-type calcium channel antagonist nifedipine significantly reduced the maximal contraction to 5-HT (53% reduction), and the phospholipase C (PLC) inhibitor NCDC nearly abolished 5-HT (53% reduction), and the phospholipase C (PLC) inhibitor NCDC nearly abolished 5-HT–induced contraction (Figure 1, bottom). The general tyrosine kinase inhibitor genestein shifted the 5-HT–induced contraction 4.8-fold rightward, whereas PD 098,059 (inhibitor of MEK activation) shifted contraction rightward to a smaller degree (3.2-fold). Daidzein, an inactive analog of genistein, was without effect. These data suggest that 5-HT–induced contraction in the normal mesenteric arteries depends on activation of L-type calcium channels, PLC, and tyrosine kinases, including those important to the Erk MAPK pathway.

Response to 5-HT in Hypertension

We next investigated the response of resistance arteries to 5-HT from DOCA-salt hypertensive animals. Four weeks after surgery, sham animals had a systolic blood pressure of 120±2 mm Hg; DOCA-salt, 192±8 mm Hg (P<0.05). As observed in larger arteries, 5-HT had a decrease in threshold (100-fold lower), increase in potency (11.6-fold leftward shift), and increase in maximal contraction (140% sham maximum) in small arteries from DOCA-salt rats compared with sham. Thus, small arteries from hypertensive rats are hyperresponsive to 5-HT (Figure 2, top). We have evidence to suggest that in large arteries, this hyperresponsiveness is caused by upregulation of a serotonin receptor that is extremely sensitive to 5-HT, the 5-HT2A receptor.10–14 Two hallmarks of 5-HT2B receptor activation in the vasculature are (1) insensitivity of blockade to the 5-HT2A receptor antagonist ketanserin and (2) concentration-dependent contraction to the 5-HT2B receptor agonist BW 723C86. Thus, in the next experiments, we investigated the sensitivity of 5-HT–induced contraction to ketanserin and the ability of BW 723C86 to contract both resistance arteries and aorta from sham and DOCA-salt rats.

Figure 2 (top) depicts the effects of ketanserin on 5-HT–induced contraction in sham and DOCA-salt resistance arteries. Contrary to that expected, ketanserin competitively shifted 5-HT–induced contraction in both sham and DOCA-salt resistance arteries. The resultant −log K_i values (mol/L) were nearly identical (9.25±0.1 for both groups). This contrasts with the inability of ketanserin to cause a competitive shift in aorta from the same DOCA-salt animals (Figure 2, bottom). The response to 5-HT appears biphasic in the aorta of the DOCA-salt rat, and the lower part of the curve (10^{-7} to 3×10^{-7} mol/L) is insensitive to blockade by ketanserin. The upper part of the curve shows a slight shift to the right that is likely indicative of 5-HT2A receptors still playing a role in contractility at high concentrations of 5-HT. These data suggest that the 5-HT2A receptor remains the predominant contractile receptor in DOCA-salt hypertension in small arteries.

Supporting this is a lack of an enhanced response to the 5-HT2B receptor agonist BW 723C86 in resistance arteries of DOCA-salt rats (Figure 3, top). This differs significantly from the dramatically upregulated contraction observed in response to this agonist in the aorta of the same DOCA-salt rats (Figure 3, bottom). Thus, these data suggest that upregulation of the 5-HT2B receptor in DOCA-salt hypertension is not global and appears to not occur in small mesenteric resistance vessels.

Discussion

The role of 5-HT in hypertension has been a controversial subject. It is enticing to believe that 5-HT is involved in hypertension because hyperresponsiveness of arteries from hypertensive humans and animals is profound.1–5 However, studies of 5-HT in hypertension stalled when it was discovered that the 5-HT2A receptor antagonist ketanserin was antihypertensive not because of blockade of the 5-HT2A receptor but because of blockade of the α1 adrenergic receptor.6–8 In addition, it has been argued that 5-HT cannot reach plasma levels that are sufficient to interact with arterial 5-HT receptors. Estimates of anywhere between 12 to 150 nmol/L of free 5-HT have been measured in humans and rats, and these are concentrations that are below the dissociation constant of 5-HT for the receptor normally expressed in arteries, the 5-HT2A receptor (K_d = 3 μmol/L).21 However, we and other investigators have suggested that there is an upregulation in arteries (aorta and superior mesenteric artery) of a receptor for which 5-HT has a significantly higher
5-HT2A receptor in the resistance arteries are qualitatively different from those in the aorta. 5-HT2A receptor-mediated contraction in mesenteric resistance arteries is blocked by ketanserin and is conserved between normotensive and DOCA-salt hypertensive rats. However, the aorta and mesenteric resistance arteries do not display a similar profile of the 5-HT2A receptor. This is supported by the similarity of the 5-HT2A receptor in blocking 5-HT-induced contraction in the aorta and mesenteric resistance arteries. The fact that 5-HT-induced contraction in mesenteric resistance arteries is similarly antagonized by ketanserin as in sham mesenteric resistance arteries suggests that the same receptor subtype is mediating contraction in both vessel types. This was wholly consistent with what has been observed in thoracic aorta and superior mesenteric artery from normotensive rats.

Before this investigation, we first needed to determine the profile of the 5-HT receptor mediating contraction under conditions of normal blood pressure. Using a series of serotonergic receptor agonists, we found that the pharmacological profile of the 5-HT receptor in the resistance arteries was consistent with that of a 5-HT2A receptor. Specifically, this is supported by the similarity of the 5-HT2A receptor agonist α-methyl-5-HT and 5-HT and the apparent dissociation constant of ketanserin in blocking 5-HT-induced contraction. This is wholly consistent with what has been observed in thoracic aorta and superior mesenteric artery from normotensive rats. In neither the resistance arteries nor the aorta do other 5-HT receptors that have been implicated in contraction appear to play a role in directly modulating arterial contractility. This includes the 5-HT1B, 5-HT1D, 5-HT1F, and 5-HT2B receptor subtypes. The signal transduction pathways activated on stimulation of the 5-HT2A receptor in the resistance arteries are qualitatively similar to those observed in the aorta. 5-HT-induced contraction of thoracic aorta can be reduced by the same concentration of inhibitors of L-type calcium channels, PLC, and tyrosine kinase(s). These pathways operate in parallel in the aorta; we have not yet determined if this is the case in the resistance arteries. Nonetheless, it is clear that there are marked similarities in the 5-HT receptor pharmacology and signal transduction in the aorta and mesenteric resistance arteries of normotensive rats.

In the DOCA-salt rat, the aorta and mesenteric resistance arteries remain similar in that both display a hyperresponsive-ness to 5-HT. This is observed as a decrease in threshold, increase in potency, and increase in maximal contraction. However, the similarities stop here. Both the superior mesenteric artery and aorta from the DOCA-salt rat display a pharmacological profile consistent with the 5-HT2B receptor being the primary contractile receptor. In part, this is evidenced by insensitivity to ketanserin and contraction to BW 723C86. In addition, we have recently been able to demonstrate that 5-HT2B receptor protein is increased 2- to 3-fold in arteries from DOCA-salt rats compared with sham rats. However, unlike in the aorta, 5-HT-induced contraction in mesenteric resistance arteries remain sensitive to ketanserin and do not contract to the 5-HT2A receptor agonist BW 723C86. One immediate difference between the aorta and resistance arteries is that we have kept the endothelium intact in the resistance arteries. We have published, however, that the presence of the endothelium does not affect the enhanced response to 5-HT, and so this is an unlikely explanation for our results.

These results suggest that the mechanisms behind the enhanced responsiveness to 5-HT in mesenteric resistance arteries do not include an upregulated functional 5-HT2B receptor, and that other mechanisms serve this hyperresponsive-ness. The fact that 5-HT–induced contraction in DOCA mesenteric resistance arteries is similarly antagonized by ketanserin as in sham mesenteric resistance arteries suggests that the same receptor subtype is mediating contraction in both vessel types. This was truly surprising as we had previously found that the 5-HT2B receptor antagonist LY 272015 significantly reduced the blood pressure of animals that had severe hypertension. There were animals with severe hypertension (systolic blood pressure >250 mm Hg) in our experiments, and the mesenteric resistance arteries of those animals did not contract to BW 723C86. This finding calls into question whether the antihypertensive effects of LY 272015 were arterial in nature and why compounds like ketanserin do not appear to exert an antihypertensive effect via blockade of 5-HT2A receptors. LY 272015 may have exerted its antihypertensive effect centrally or in a systemic site in the body other than the mesenteric resistance arteries. Alternatively, large vessels in the DOCA-salt model could potentially contribute to changes in pulse pressure, an independent risk factor for cardiovascular morbidity and mortality. We have recently discovered that the 5-HT1B receptor is also upregulated in arteries of hypertensive rats and ketanserin possesses low affinity for the 5-HT1B receptor; the affinity of LY 272015 for the 5-HT1B receptor is not established. This is one avenue of research that we are pursuing. In addition, we are unsure whether these mechanisms are applicable to the human condition, as we have not yet investigated contractility in arteries from hypertensive humans.

In summary, important similarities were observed between the aorta and mesenteric resistance arteries. Under conditions of normal blood pressure, the 5-HT2A receptor is the primary
receptor mediating contraction, and the signal transduction pathways linked to this receptor are similar in both vessels. Under conditions of high blood pressure, the 5-HT2B receptor is the primary contractile receptor in the aorta, whereas the 5-HT2A receptor remains the primary contractile receptor in the mesenteric resistance arteries. These differences may help determine which serotonergic compounds could be effective in lowering blood pressure and create the need to reevaluate the antihypertensive mechanism of LY 272015.

Acknowledgments
These studies were supported in part by a grant from the National Institutes of Health (HL58489) and by the Atorvastatin Research Awards Program.

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Hypertension. 2002;39:825-829
doi: 10.1161/hy0302.104668

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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