Enhanced Contraction to 5-Hydroxytryptamine Is Not Due to “Unmasking” of 5-Hydroxytryptamine\textsubscript{1B} Receptors in the Mesenteric Artery of the Deoxycorticosterone Acetate–Salt Rat

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Abstract—5-Hydroxytryptamine\textsubscript{1B} (5-HT\textsubscript{1B}) receptors have been implicated in mediating arterial contraction to 5-HT. Additionally, the 5-HT\textsubscript{1B} receptor has been reported to be “unmasked” by depolarizing stimuli. We hypothesized that 5-HT\textsubscript{1B} receptors in arteries from hypertensive animals, arteries reported to have a depolarized resting membrane potential in smooth muscle cells, are unmasked and participate in the supersensitivity observed to 5-HT in hypertension. We used the isolated tissue bath apparatus and examined the response of superior mesenteric arteries from normotensive sham and hypertensive deoxycorticosterone acetate (DOCA)–salt rats. The 5-HT\textsubscript{1B} agonists CP93129 and sumatriptan (10^{-9} to 10^{-5} mol/L) caused a maximal contraction (50±12% of phenylephrine [10^{-5} mol/L] contraction) in arteries from DOCA-salt rats; no contraction was observed in arteries from normotensive rats. The 5-HT\textsubscript{1B} receptor antagonist GR55562 (100 nmol/L) inhibited both the 5-HT– (4-fold rightward shift) and CP93129-induced (11-fold rightward shift) contractions in mesenteric arteries from hypertensive DOCA-salt rats. In other experiments, arteries from normotensive rats were incubated with 15 mmol/L KCl, as a depolarizing stimulus, and then exposed to 5-HT and CP93129. In the presence of KCl, a small leftward shift to 5-HT was observed. However, the presence of a depolarizing stimulus was unable to produce changes in the 5-HT maximal response to resemble that of arteries from DOCA-salt rats, nor was contraction to CP93129 observed. These data support the conclusions that 5-HT\textsubscript{1B} receptors mediate contraction in mesenteric arteries from hypertensive rats and that this enhanced response to 5-HT is not due to membrane depolarization alone. (Hypertension. 2001;38:891-895.)

Key Words: serotonin ■ mesenteric arteries ■ vasoconstriction ■ deoxycorticosterone

Serotonin (5-hydroxytryptamine, 5-HT) is a molecule that has generated much interest and controversy in cardiovascular research. A finding that is common, but not absolute, is that arteries from animals with hypertension demonstrate supersensitivity to 5-HT.\textsuperscript{1,2} Under normotensive conditions, the 5-HT\textsubscript{1B} receptor has been implicated as the predominant receptor involved in mediating 5-HT–induced contraction in many arteries.\textsuperscript{3,4} Recently, 5-HT\textsubscript{1B} receptors have also been implicated as mediators of 5-HT–induced vasoconstriction. The 5-HT\textsubscript{1B} receptor has been shown to mediate contraction in human pulmonary artery,\textsuperscript{5} rat pulmonary artery,\textsuperscript{6} human coronary artery,\textsuperscript{7} human cerebral artery,\textsuperscript{8} rabbit carotid artery,\textsuperscript{9} rabbit ear artery,\textsuperscript{10} rabbit renal artery,\textsuperscript{11} and rat tail artery.\textsuperscript{12} The 5-HT\textsubscript{1B} receptor appears to be the predominant receptor mediating 5-HT–induced contraction in the human cerebral arteries.\textsuperscript{8} This receptor is a G protein–coupled receptor, using G\textsubscript{i} and G\textsubscript{o} to couple negatively with cAMP modulatory pathways.\textsuperscript{13-15}

Unlike other serotonin receptors, the 5-HT\textsubscript{1B} receptor has been described as a receptor that can be “unmasked” to mediate its contractile effects.\textsuperscript{10} This unmasking of a functionally silent receptor can be caused by a depolarizing stimulus and has been reported to be required in rabbit mesenteric artery,\textsuperscript{16} rabbit renal artery,\textsuperscript{11} rabbit ear artery,\textsuperscript{10} and rat tail artery.\textsuperscript{12} However, this unmasking is not required in all arteries in which the 5-HT\textsubscript{1B} receptor mediates contraction.\textsuperscript{5,6} In some models of hypertension, arterial smooth muscle cells exhibit a slightly depolarized resting membrane potential.\textsuperscript{17} This may be due to defects in calcium handling\textsuperscript{18} and/or changes in potassium channel function.\textsuperscript{19} We speculated that a depolarizing stimulus, such as KCl, in normotensive tissues would enable the 5-HT\textsubscript{1B} receptor to mimic the depolarized state of smooth muscle in hypertensive tissues. If these assumptions are correct, then (1) a 5-HT\textsubscript{1B} receptor–mediated contraction should be observed in depolarized arteries from normotensive rats and (2) arteries from hypertensive rats should also display an enhanced response to 5-HT\textsubscript{1B} receptor activation. We tested the hypothesis that the 5-HT\textsubscript{1B} receptor mediates contraction in the superior mesenteric artery from hypertensive deoxycorticosterone acetate
(DOCA–salt) rats. We also tested the hypothesis that depolarization alone would enable an artery from a normotensive sham rat to respond with the same potency and to the same maximum as an artery from a DOCA–salt hypertensive rat.

Methods

All procedures that involved animals were performed in accordance with the institutional guidelines of Michigan State University.

Surgical Procedures and Systolic Blood Pressure Measurement

Adult male Sprague-Dawley rats (weight, 0.20 to 0.25 kg; Charles River Laboratories, Portage, Mich) were given a subcutaneous silastic implant impregnated with DOCA (200 mg · kg⁻¹) and were uninephrectomized (left side, flank incision) under isoflurane (IsoFlo, Abbott Laboratories) anesthesia. Control rats did not receive an implant but were uninephrectomized. Postoperatively, rats given DOCA received drinking water containing 1.0% NaCl and 0.2% KCl. Control rats received normal tap water. All animals were fed a diet of standard rat chow and received ad libitum access to food and water. After 4 weeks, the systolic blood pressures were measured by the standard tail-cuff method.

Isolated Tissue Preparation

Rats were anesthetized with pentobarbital (50 mg · kg⁻¹ IP) and killed via a pneumothorax. The superior mesenteric artery was removed, cleaned of debris, and cut into helical strips. These strips were denuded of endothelial cells and placed in isolated tissues baths containing physiological salt solution consisting of (in mmol/L) NaCl 103, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.2, NaHCO₃ 14.9, dextrose 5.5, and CaNa₂EDTA 0.03. Tissues were mounted on stainless steel holders in tissue baths (50 mL) for isometric tension recordings and placed under optimum resting tension (600 mg, determined previously). Strips from normotensive and hypertensive rats were placed in the same bath, thereby controlling for experimental variations. After a 1-hour equilibration period, arteries were challenged with phenylephrine (10⁻² mol/L). Tissues were then washed, and the lack of an intact endothelium was validated by observing the inability of acetylcholine (10⁻⁴ mol/L) to relax arteries contracted with a half-maximal concentration of phenylephrine. Cumulative concentration-response curves to agonists were performed. Antagonists or vehicle was incubated with vessels for 1 hour before experimentation.

Depolarization Protocol

Tissues were incubated for 1 hour in the presence or absence of the 5-HT₂A receptor antagonist ketanserin (10 nmol/L) to block the effects mediated by the 5-HT₂A receptor. Tissues were depolarized with 15 mmol/L KCl, and this contraction was allowed to plateau (15% to 20% of maximal phenylephrine [10⁻² mol/L] contraction). Cumulative concentration-response curves to agonists were then performed. The initial KCl-induced contraction was subtracted from the final agonist-induced contraction values used to construct the concentration-response curves.

Data Analysis and Statistics

Data are presented as mean±SEM for the number of animals (in parentheses). Contractions are reported as a percentage of response to phenylephrine (10⁻² mol/L). The appropriate Student’s t test was used to compare 2 groups. In all cases, P≤0.05 was considered statistically significant.

Materials

Acetylcholine chloride, phenylephrine hydrochloride, DOCA, and 5-HT hydrochloride were purchased from Sigma Chemical Co. Ketanserin tartrate was purchased from Research Biochemical International. GR55562 was purchased from Tocris. 5H-Pyrrolol[3,2-b]pyridin-5-one,1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)

Figure 1. Top, Effect of the 5-HT₁B receptor antagonist GR55562 (100 nmol/L) on 5-HT–induced contraction in mesenteric arteries from normotensive (left) and DOCA-salt hypertensive (right) rats. Bottom, Effect of GR55562 (100 nmol/L) on the rodent selective 5-HT₁B agonist CP93129-induced contraction in mesenteric arteries from normotensive (left) and DOCA-salt hypertensive (right) rats. Data are reported as a percentage of the phenylephrine (PE) (10⁻² mol/L) response. Points represent mean±SEM of the number of animals indicated in parentheses. *Statistically significant (P<0.05) difference vs vehicle response. (CP93129) was a gift from Pfizer (Groton, Conn), and sumatriptan was a gift from Glaxo (Stevenage, Hertfordshire, UK).

Results

The average systolic blood pressure of the normotensive sham rats used was 123±2 mm Hg, whereas the average systolic blood pressure of the hypertensive DOCA-salt rats was 194±10 mm Hg (P<0.05). Confirming previous findings, we observed that in the superior mesenteric artery, 5-HT was significantly more potent in arteries from hypertensive DOCA-salt rats than in arteries from normotensive sham rats (log EC₅₀ values [mol/L], 6.68±0.11 and 5.91±0.03 in DOCA-salt and sham, respectively; Figure 1). The 5-HT₁B antagonist GR55562 (100 nmol/L) shifted 5-HT–induced contraction in the arteries from hypertensive DOCA-salt rats (Figure 1, top) (log EC₅₀ values [mol/L], 6.90±0.10 and 6.29±0.13 for vehicle and GR55562, respectively). This is a small but significant shift, and it demonstrates that in arteries from hypertensive rats, 5-HT–induced contraction is mediated in part by the 5-HT₁B receptor. By contrast, 5-HT–induced contraction in the arteries from the normotensive sham rats was not sensitive to inhibition by GR55562. These data suggest that the 5-HT₁B receptor is involved in mediating 5-HT–induced contraction under conditions of hypertension but not under conditions of normal blood pressure. The 5-HT₈(III)/D₁F receptor agonist sumatriptan (data not shown) and the rodent selective 5-HT₁B receptor agonist
CP93129 did not cause contraction in the arteries from normotensive rats (Figure 1, bottom). However, sumatriptan and CP93129 (shown in Figure 1, bottom right) both caused contraction in the mesenteric artery from hypertensive DOCA-salt rats (log EC50 values [mol/L], 5.52±0.04 and 6.92±0.07, respectively). In arteries from hypertensive DOCA-salt rats, CP93129 caused a maximal contraction of 51±15% of phenylephrine (10⁻⁵ mol/L) contraction, and sumatriptan caused a maximal contraction of 54.4±9.9% of phenylephrine (10⁻⁵ mol/L) contraction. The 5-HT₁B receptor antagonist GR55562 (100 nmol/L) inhibited CP93129-induced contraction in arteries from hypertensive DOCA-salt rats (Figure 1, bottom). Collectively, these data strongly support the conclusion that in the mesenteric artery, the 5-HT₁B receptor is involved in mediating 5-HT- and CP93129-induced contraction under conditions of hypertension.

We next performed isolated tissue bath experiments on mesenteric arteries from normotensive Sprague-Dawley rats in the presence of depolarizing KCl (15 mmol/L). In some experiments, ketanserin (10 nmol/L) was used to inhibit the contribution of the 5-HT₂A receptor to 5-HT–induced contraction and did not inhibit the 5-HT₁B receptor at this concentration. The concentration-response curves for 5-HT–induced contraction in the arteries from normotensive sham and DOCA-salt rats without KCl treatment are shown for comparison. In the absence of ketanserin, acute depolarization alone, as stimulated by KCl (15 mmol/L), was not sufficient to result in an increase in the maximal 5-HT–induced contraction elicited in the superior mesenteric artery from the normal Sprague-Dawley rat (Figure 2, top). The observed leftward shift (log EC₅₀ values [mol/L], 5.91±0.03 and 6.55±0.08 without and with KCl, respectively) may be due to the intervention of the 5-HT₁B receptor. In the presence of ketanserin (10 nmol/L) and KCl (15 mmol/L), however, there was a rightward shift in 5-HT–induced contraction (log EC₅₀ values [mol/L], 6.55±0.08 and 5.24±0.03 with KCl/without ketanserin and with ketanserin/with KCl treatment, respectively; Figure 2, bottom right). This suggests that under depolarized conditions, the ketanserin-sensitive 5-HT₂A receptor remains the primary 5-HT contractile receptor. Unlike the response to 5-HT in the depolarized arteries, an even higher concentration of ketanserin (30 nmol/L) was unable to inhibit 5-HT–induced contraction in the arteries from hypertensive DOCA-salt rats (Figure 2, bottom left). These results suggest that under conditions of DOCA-salt hypertension, 5-HT is clearly activating a different complement of receptors to mediate contraction.

To determine whether the leftward shift to 5-HT in the presence of KCl was specific to 5-HT or an artifact of KCl treatment, we repeated the depolarization protocol with the α₁-adrenergic agonist phenylephrine (Figure 3). Treatment with KCl also did not increase the responsiveness to phenylephrine, as evidenced by the lack of change in the potency of the phenylephrine response curves (Figure 3). These data suggest that acute depolarization alone does not produce global increases in agonist sensitivity. Moreover, ketanserin did not affect the phenylephrine-induced contraction (Figure 3), demonstrating that this concentration of ketanserin (10 nmol/L) is selectively inhibiting the 5-HT₂A receptor and not α₁-adrenergic receptors.

We lastly performed acute depolarization studies using the rodent selective 5-HT₁B agonist CP93129. Even in the presence of a depolarizing stimulus, the 5-HT₁B receptor agonist CP93129 did not elicit a contraction in the mesenteric artery from normotensive rats (Figure 2, top). CP93129-induced contraction was also not observed in depolarized arteries incubated with ketanserin (data not shown). These additional data do not support the role of silent 5-HT₁B receptors in the normotensive rat mesenteric artery.

**Discussion**

We hypothesized that the 5-HT₁B receptor mediates contraction under conditions of DOCA-salt hypertension. Our experiments using 5-HT, the 5-HT₁B antagonist GR55562, and the rodent selective 5-HT₁B receptor agonist CP93129 support the conclusion that the 5-HT₁B receptor may mediate at least a portion of the 5-HT– and CP93129-induced contraction under conditions of DOCA-salt hypertension. Additionally, we
conclude that the 5-HT\textsubscript{1B} receptor does not mediate arterial contraction under normotensive conditions. We also hypothesized that membrane depolarization alone would enable an artery from a normotensive sham rat to respond in the same manner as an artery from a hypertensive DOCA-salt rat. The present studies support the conclusion that membrane depolarization alone may be a factor, but not the sole factor, involved in the hyperresponsiveness to 5-HT observed in arteries from hypertensive rats. Because we were unable to observe a contraction even in the presence of KCl (15 mmol/L) to the rodent selective 5-HT\textsubscript{1B} receptor agonist CP93129 in arteries from normotensive rats, we conclude that these data do not support a role for silent 5-HT\textsubscript{1B} receptors in normotensive rat mesenteric arteries.

Collectively, these studies present the novel finding of an enhanced contractile responsive of the 5-HT\textsubscript{1B} receptor under the conditions of DOCA-salt hypertension. This functional change has potential significant physiological ramifications. Although the role of serotonin in many cardiovascular diseases remains controversial, its involvement has been suggested in hypertension, migraine, vasospastic angina, and coronary artery disease. In many of these diseases, a common finding is that arteries display an increased responsiveness to 5-HT. One side effect of migraine therapy with sumatriptan is chest pain. Sumatriptan is an agonist with high affinity for the human 5-HT\textsubscript{1B} receptor as well as affinity for other 5-HT\textsubscript{1} receptors and is used clinically to cause vasoconstriction in cerebral blood vessels, thus alleviating the vasoconstriction associated with the pain of migraine. We have reported that arterial responsiveness to sumatriptan is significantly enhanced in hypertension. Understanding the 5-HT receptors that mediate vascular contraction under conditions of normal blood pressure and hypertension will allow for targeting of drug therapy for migraine and depression with fewer systemic side effects, as well as potential treatments for the cardiovascular diseases.

Currently, the mechanisms of receptor unmasking are not clearly understood. However, the work by Smith and colleagues in the rabbit ear artery suggests that both the 5-HT\textsubscript{1B} and 5-HT\textsubscript{2A} receptors may function as “silent receptors.” This may be due to a lack of coupling to second messenger systems under nondepolarized conditions. They suggest that the depolarization caused by elevated K\textsuperscript{+} may induce an influx of extracellular calcium, which in turn enhances the coupling to the second messenger pathways that mediate vasoconstriction. It is not known whether the depolarization-dependent unmasking is a phenomenon selective for serotonergic receptors or whether other receptors might also be unmasked by this mechanism. However, data presented herein demonstrate that acute depolarization alone is not sufficient to enhance arterial responses to agonists of the 5-HT\textsubscript{1B} receptor.

Other mechanisms, such as changes in receptor density, may be responsible for the observed enhanced contraction to CP93129 and 5-HT under conditions of hypertension. Thus, although regulation of the vascular 5-HT\textsubscript{1B} receptor in disease states is not clearly defined, we speculate that mineralocorticoids may be involved. Analysis of the human 5-HT\textsubscript{1B} promoter revealed that there are multiple mineralocorticoid response elements located in the promoter. This is relevant to the regulation of this receptor in a disease state such as in the DOCA-salt model, in which hypertension is induced by the use of mineralocorticoids, as well as other models of hypertension in which elevated levels of aldosterone may influence expression of this receptor. Further study will be required to demonstrate the ability of mineralocorticoids to modulate expression of the 5-HT\textsubscript{1B} receptor, as well as to determine whether the expression of the 5-HT\textsubscript{1B} receptor is altered in other models of hypertension.

Previous data from our laboratory have also implicated the 5-HT\textsubscript{2B} receptor as a player in the hypersensitivity to 5-HT displayed in arteries from DOCA-salt hypertensive rats. Both the 5-HT\textsubscript{1B} and the 5-HT\textsubscript{2B} receptors may be involved in the hyperresponsiveness to 5-HT. With the recent availability of antibodies to both the 5-HT\textsubscript{1B} and 5-HT\textsubscript{2B} receptors, analysis of protein expression will now be possible. Because acute depolarization was not sufficient to unmask a response to agonists of the 5-HT\textsubscript{1B} receptor (Figure 1, top) and 5-HT\textsubscript{2B} receptors (data not shown), we speculate that there will be an increase in the expression of these receptors in vascular smooth muscle, which would provide an explanation for the physiological and pharmacological data that indicate a change in the complement of contractile serotonergic receptors in vascular smooth muscle under conditions of hypertension.

The data presented support the conclusions that the 5-HT\textsubscript{1B} receptor does not mediate contraction under normotensive conditions but does mediate CP93129- and 5-HT-induced contraction under conditions of DOCA-salt hypertension. The data also support the conclusion that a slightly depolarized membrane potential of smooth muscle cells is not the sole factor that causes an increased responsiveness to 5-HT observed under conditions of hypertension, nor can the depolarization of tissues from animals with normal blood pressure unmask a response mediated by the 5-HT\textsubscript{1B} receptor. These findings are the first to implicate the 5-HT\textsubscript{1B} receptor in mediating arterial contraction in cardiovascular disease.
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