SUMMARY This study characterizes vascular responsiveness to serotonin and its metabolites and to several monoamines that are structurally related to serotonin in deoxycorticosterone acetate (DOCA)-salt hypertension. Mesenteric arteries from normotensive and hypertensive rats were excised and cut into helical strips for isometric force recording. Dose-response curves to serotonin in arteries from hypertensive rats were shifted significantly to the left compared with those in arteries from normotensive rats (ED50; DOCA-treated = 2.4 x 10^-8 M; control = 17.1 x 10^-8 M). Contractile responses to 5-hydroxyindole acetic acid and 5-hydroxytryptophol were greater in mesenteric arteries from hypertensive rats, whereas reactivity to 5-methoxytryptamine and melatonin in arteries from hypertensive rats did not differ from that in arteries from normotensive rats. Mesenteric arteries from both rat groups were unresponsive to the serotonin metabolite N-acetylserotonin. Contractile responses to 5,6-dihydroxytryptamine and 6-hydroxytryptamine were greater in mesenteric arteries from hypertensive rats, whereas responsiveness to 3-hydroxytryptamine in hypertensive arteries did not differ from normotensive values. Contractile responses to serotonin and its metabolites and to the structurally related monoamines were inhibited by the serotonergic antagonist ketanserin. These results demonstrate that vascular sensitivity to serotonin is increased in DOCA-hypertensive rats. Based on the experiments with serotonin metabolites and with other monoamines, the increased responsiveness to these compounds appears to be related to the structural location of hydroxyl and amine moieties.

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KEY WORDS • mesenteric artery • serotonin • deoxycorticosterone acetate • mineralocorticoid hypertensive rats • ketanserin • vascular hyperreactivity

SEVERAL investigators have proposed that the peripheral vasoconstrictor effects of serotonin may contribute to increased total peripheral vascular resistance in hypertension.1-7 Sensitivity to the direct vasoconstrictor properties of serotonin is increased in the hypertensive state,8,9 and its indirect sympathomimetic effect has been observed to be exaggerated in young spontaneously hypertensive rats.10 Tachyphylaxis to serotonin develops at a slower rate in blood vessels from hypertensive animals, and the potentiating effect of serotonin on other vasoconstrictor agents is increased in hypertension.6 The partial agonistic properties of serotonin antagonists are increased in hypertensive blood vessels.8 Despite these observations of altered vascular effects of serotonin in hypertension, it has been difficult to assess the physiological importance of serotonin in hypertension for three reasons: 1) circulating levels of free serotonin in the blood are low;11 2) the vascular actions of serotonin are complex and include both vasodilatation and vasoconstriction; and 3) serotonin antagonists, used to lower blood pressure, contain agonistic as well as antagonistic properties and vary in potency and specificity.13-19

This study features two aspects of vascular responsiveness to serotonin that have not previously been characterized in hypertension: 1) contractile properties of metabolites of serotonin and 2) vascular reactivity to several monoamines that are structurally related to serotonin. The overall objective was to examine contractile actions of compounds that may provide mechanistic information about increased vascular sensitivity to serotonin in hypertension.
Materials and Methods

Animal and Tissue Preparation

Sprague-Dawley rats (Harlan; weight, 200-250 g) were made hypertensive by Silastic implants containing deoxycorticosterone acetate (DOCA), 200 mg/kg. Animals were anesthetized with sodium pentobarbital (50 mg/kg) and uninephrectomized, and the implants were placed subcutaneously in a flank skin incision. Following surgical recovery, rats were given 1% NaCl and 0.2% KCl drinking water, and after 4 to 5 weeks systolic blood pressures (tail cuff technique; pneumatic transducer) in rats receiving DOCA were elevated above those of control animals. Control rats received 1% NaCl and 0.2% KCl in the drinking water and were sham operated, but they did not receive Silastic implants. Normotensive and hypertensive rats (4-5 weeks after implant) were killed using a lethal dose of sodium pentobarbital. Superior mesenteric arteries were removed and placed in cold physiological salt solution of the following composition (mM): NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄, 7H₂O, 1.17; CaCl₂, 2H₂O, 1.6; NaHCO₃, 14.9; dextrose, 5.5; and CaNa₂EDTA, 0.03. Mesenteric arteries (outside diameter, ~1.5 mm) were cut into helical strips (1 mm x 10 mm) and suspended vertically in tissue chambers containing physiological salt solution (37°C), aerated with 95% O₂, 5% CO₂. Strips were attached to a metal base and force transducer to measure isometric tension development to various agents. The resting tension of each strip was adjusted so that it developed maximum active force in response to a standard dose of norepinephrine (5.9 x 10⁻⁷ M; see Reference 21 for details). Strips were allowed to equilibrate for 1 hour before the start of each experiment.

Cumulative concentration-response curves for serotonin (5-hydroxytryptamine) were determined using arteries from normotensive and hypertensive rats. Concentration-response curves to serotonin-like monoamines (3-hydroxytryptamine, 5-hydroxytryptamine, and 5,6-dihydroxytryptamine) and serotonin metabolites (5-methoxytryptamine, 5-hydroxyindole-3-acetic acid, 5-hydroxytryptophol, and melatonin) were determined in a similar manner for both rat groups. Contractile responses to various agonists were measured following 2 minutes of steady state responses. Contractile responses to serotonin-related monoamines and serotonin metabolites were expressed as a percentage of the maximal response to serotonin. Concentration-response curves were determined for approximately three compounds per strip. Vascular sensitivity to serotonin-like compounds was defined as the dose that produced a contractile response that was 25% of the maximal response to serotonin (EDS₅₀ max value). These values were estimated from graphic representation of the dose-response curves. This procedure was necessary since many of these compounds did not produce a maximal response over the dose range tested.

Statistical Analyses

Data are reported as means ± SEM. Vascular sensitivity to serotonin was determined by comparison of ED₅₀ values (effective dose that caused a 25% maximal response). As with ED₅₀ max values, ED₅₀ values for serotonin were estimated from graphic representations of the dose-response curves. An unpaired analysis (Student's t test) was used to compare systolic blood pressures, absolute maximal contractile responses, and ED₅₀ and ED₅₀ max values (expressed as -log values). A p value less than 0.05 was considered statistically significant.

Drugs

The following drugs were used: serotonin (5-hydroxytryptamine creatinine sulfate), 3-hydroxytryptamine hydrochloride, 6-hydroxytryptamine, 5,6-dihydroxytryptamine, 5-hydroxyindole-3-acetic acid, 5-hydroxytryptophol, 5-methoxytryptamine, melatonin (N-acetyl-5-methoxytryptamine), and N-acetylserotonin (N-acetyl-5-hydroxytryptamine). These agents were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ketanserin was provided by Janssen Pharmaceutical Company (Piscataway, NJ, USA).

Results

Four to 5 weeks after implantation, systolic blood pressures in DOCA-treated rats (168 ± 5 mm Hg; n = 24, p < 0.05) were elevated above those in control animals (122 ± 3 mm Hg, n = 22).

Vascular Responses to Serotonin and Related Analogs

Cumulative addition of serotonin (5-hydroxytryptamine), 3-hydroxytryptamine, 5,6-dihydroxytryptamine, and 6-hydroxytryptamine to the muscle bath caused dose-dependent contractions in all mesenteric artery strips (Figure 1). Dose-response curves to serotonin in mesenteric arteries from DOCA-hypertensive rats were shifted to the left of those obtained in mesenteric arteries from control rats (see Figure 1, upper left panel). The ED₅₀ values for serotonin in arterial strips from hypertensive rats (2.4 x 10⁻⁸ M; -log ED₅₀ = 8.23 ± 0.36; n = 8) were significantly lower (p < 0.05) than those in arterial strips from normotensive rats (17.1 x 10⁻⁸ M; -log ED₅₀ = 7.13 ± 0.28; n = 8), indicating increased sensitivity to the amine. Maximal force developed in response to serotonin in mesenteric arteries from hypertensive rats (506 ± 58 mg; n = 8) did not differ from that in arterial strips from normotensive rats (465 ± 68 mg; n = 8).

Contractile sensitivity to 5,6-dihydroxytryptamine (EDS₅₀ max = 1.4 x 10⁻⁷ M; -log EDS₅₀ max = 6.90 ± 0.19; n = 4; see Figure 1 lower left panel) and 6-hydroxytryptamine (EDS₅₀ max = 1.2 x 10⁻⁶ M; -log EDS₅₀ max = 5.96 ± 0.18; n = 6; see Figure 1 lower right panel) was significantly lower (p < 0.05) in mesenteric arteries from DOCA-hypertensive rats than that in mesenteric arteries from normotensive rats (EDS₅₀ max = 6.8 x 10⁻⁷ M; -log EDS₅₀ max = 6.24 ± 0.15; n = 4; and EDS₅₀ max = 9.5 x 10⁻⁴ M; -log EDS₅₀ max = 5.24 ± 0.20; n = 6, respectively). Contractile sensitivity to 3-hydroxytryptamine in hypertensive arteries (EDS₅₀ max = 1.5 x 10⁻⁷ M; -log EDS₅₀ max = 6.91 ± 0.32; n = 5) did not differ from normotensive values (EDS₅₀ max = 1.5 x 10⁻⁷ M; -log EDS₅₀ max = 6.91 ± 0.32; n = 5).
Vascular Responses to Serotonin Metabolites

Contrast responsiveness to serotonin metabolites (5-methoxytryptamine, 5-hydroxyindole acetic acid, melatonin, 5-hydroxytryptophol, and N-acetylsertotonin) was measured in a manner similar to that for serotonin. Mesenteric arteries from hypertensive (n = 5) and normotensive rats (n = 5) did not contract in response to the cumulative addition of N-acetylsertotonin (5 × 10^{-9} to 5 × 10^{-4} M) to the muscle bath (data not shown). Vascular sensitivity to 5-methoxytryptamine in mesenteric arteries from hypertensive rats (EDS_{max} = 6.9 × 10^{-4} M; -log EDS_{max} = 7.47 ± 0.20; n = 9) did not differ from that in arteries from normotensive rats (EDS_{max} = 29 × 10^{-8} M; -log EDS_{max} = 6.82 ± 0.28; n = 5; Figure 2 upper left panel).

Contrast responsiveness to 5-hydroxyindole acetic acid, melatonin, and 5-hydroxytryptophol varied (see Figure 2). In response to 5-hydroxyindole acetic acid (see Figure 2 upper right panel), four out of six arteries from normotensive rats (maximal response expressed as a percentage of serotonin maximum] = 6 ± 5%; n = 6) and two out of 10 arteries from hypertensive rats (maximal response = 28 ± 9%; n = 10) did not contract. Following cumulative addition of melatonin to the muscle bath (see Figure 2 lower left panel), four out of six arteries from both rat groups (maximal response = 9 ± 5%; n = 6) and 15 ± 10%; n = 6; normotensive and hypertensive, respectively) did not contract. All mesenteric arteries from hypertensive rats (n = 6) contracted in response to 5-hydroxytryptophol (see Figure 2, lower right panel; maximal response = 21 ± 9%), whereas only one of the eight normotensive arteries (maximal response = 3 ± 3%) contracted. Vascular sensitivity to 5-hydroxyindole acetic acid, melatonin, and 5-hydroxytryptophol was not evaluated, since the contractile responses to these agents did not reach the determined level of response to serotonin (EDS_{max} values).

Antagonistic Properties of Ketanserin

Following addition of a maximally effective dose of each compound, ketanserin (3 × 10^{-7} M) was added to determine an inhibitory action of this serotoninergic antagonist. Inhibition was measured as the steady state level of relaxation following addition of ketanserin to the muscle bath. Ketanserin inhibited contractile responses to all agents (17-97% inhibition of agonist-induced contraction; n = 4-6). Percentage of inhibition by ketanserin varied with the magnitude of contraction (i.e., the larger the contractile response, the smaller the inhibition).

Discussion

This study demonstrates that the contractile properties of serotonin and several related compounds are altered in mesenteric arteries isolated from mineralocorticoid hypertensive rats. The altered vascular response in hypertension relates to specific chemical properties of the drugs. Additionally, the experiments provide general information about contractile properties of serotonin as it relates to the amine and hydroxyl moieties. Contractile responses to all compounds were inhibited by ketanserin, indicating activation of serotoninergic receptors. It remains unknown whether part of the contractile response to serotonin and related compounds is also mediated by α-adrenergic receptor activation. In addition, other cellular mechanisms responsible for altered vascular sensitivity in hypertensive rats include altered metabolism or uptake (or both) of serotonin by endothelium and nerve endings. The following discussion covers aspects of altered vascular responsiveness to serotonin in hypertension with special reference to serotonin metabolites and analogues.

Magnitude of Force Generation

Absolute maximal force generation (in milligrams) in response to serotonin in mesenteric arteries from hypertensive rats did not differ significantly from that in arteries from normotensive rats. All drugs that contained an amine moiety (e.g., 3-hydroxytryptamine,

![Figure 1. Responsiveness to serotonin and related analogues in mesenteric arteries. The chemical structures of serotonin (5-hydroxytryptamine) and its structurally related monoamines: 3-hydroxytryptamine, 6-hydroxytryptamine, and 5,6-dihydroxytryptamine are indicated. Differences in these compounds occur only in the location of the hydroxyl groups on the six-carbon ring. Contractile forces are expressed as a percentage of the maximal force generated in response to serotonin. Values are means ± SEM. The number of rats in each experimental group is indicated parenthetically.](https://hyper.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.116.180287)
5,6-dihydroxytryptamine, 6-hydroxytryptamine, and 5-methoxytryptamine) as a terminal group on the side chain of the five-membered ring caused contractions that were near maximal in relation to that caused by serotonin. These results suggest that the efficacy of serotonin in producing a maximal contraction is importantly related to the terminal amine group. The structure-activity relationship of serotonin and its related analogues is similar to other amine-containing compounds (i.e., catecholamines), in that the magnitude of the contractile response is related to the chemical configuration of the functional groups attached to the ring structure.22

Vascular Sensitivity to Serotonin

Sensitivity to serotonin is increased in mesenteric arteries from DOCA-hypertensive rats, as demonstrated by the lower ED$_{25}$ values. Based on the dose-response relationships to serotonin-related analogues and metabolites, the change in vascular sensitivity appears to be related to the presence of a hydroxyl group on position 5 or 6 of the six-carbon ring. Vascular responsiveness to 5,6-dihydroxytryptamine, 6-hydroxytryptamine, 5-hydroxyindole acetic acid, and 5-hydroxytryptophol in arteries from DOCA-hypertensive rats was increased (lower ED$_{25}$max values) compared with that in normotensive arteries. When the hydroxyl group was substituted with a methoxyl group (5-methoxytryptamine and melatonin) vascular sensitivity in arteries from DOCA-hypertensive rats did not differ significantly from that in arteries from normotensive rats. Enhanced contractile responses to 5-hydroxyindole acetic acid and 5-hydroxytryptophol in hypertensive but not normotensive arteries indicate the importance of the hydroxyl group in determining increased vascular sensitivity.

Although the observations of this study strongly support the concept that increased vascular sensitivity to serotonin is associated with an alteration in a membrane receptor in hypertension, the precise abnormality is not apparent. There may be two classes of serotoninergic receptors that could be analogous to $\alpha_1$-adrenergic and $\alpha_2$-adrenergic receptors.22 Serotonin and ketanserin could be nonselective at these receptors in a manner similar to the way that norepinephrine and phenolamine act nonselectively at the two subtypes of the $\alpha$-adrenergic receptor. Thus, a specific change (affinity or number) in one type of a serotoninergic receptor may account for the augmented responsiveness to compounds that contain the hydroxyl group in position 5 or 6. This anomalous receptor is not present in arteries from normotensive rats, as indicated by the lack of responsiveness to 5-hydroxyindole acetic acid and 5-hydroxytryptophol. This anomalous receptor would not interact with compounds that have a methoxyl group substitution at position 5 or 6 (melatonin and N-acetylserotonin).

In conclusion, this study demonstrates that vascular sensitivity to serotonin is increased in mineralocorticoid-hypertensive rats. The placement of the hydroxyl group relates to this change in vascular sensitivity. Maximal force generation appears to relate to the presence of a terminal amine group on the five-membered ring, and this characteristic does not differ in hypertensive and normotensive rat groups. The experiments on serotonin metabolites may have particular importance to studies demonstrating that the metabolism of serotonin is altered in hypertension.23 The degradative products of serotonin at the local level may contribute to increased vascular responsiveness; however, the physiological role of serotonin metabolites in hypertension remains unknown.

References

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