Vascular Responses to Serotonin in Steroid Hypertensive Rats

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SUMMARY This study investigates the mechanism responsible for increased vascular sensitivity to serotonin in deoxycorticosterone acetate (DOCA)-salt hypertension. Femoral arteries from normotensive and hypertensive rats were excised and cut into helical strips for isometric force recording. Dose-response curves to serotonin were shifted significantly to the left in arteries from DOCA-salt hypertensive rats compared to those from normotensive rats (ED₅₀: DOCA = 7.1 x 10⁻⁸ M; control = 27 x 10⁻⁸ M). The partial agonistic properties of methysergide were increased in femoral arteries from DOCA-salt hypertensive rats. The competitive antagonism of serotonin by methysergide or ketanserin was similar in arteries from control and DOCA-salt hypertensive rats (pA₂: methysergide, control = 10.4, DOCA = 10.5; and ketanserin, control = 10.4, DOCA = 10.4). After cellular calcium (Ca) depletion with EGTA, dose-response curves to Ca were obtained in the presence of serotonin (5.7 x 10⁻⁵ M). The Ca sensitivity of vessels from hypertensive rats was not statistically different from that in arteries from normotensive rats. Contractile responses to serotonin in calcium-free solution following loading of a cellular store with Ca were 50% greater in arteries from DOCA hypertensive rats. These results suggest that the enhanced sensitivity to serotonin in DOCA-salt hypertensive rats is not related to a change in receptor affinity nor to an alteration in transmembrane movement of Ca following receptor activation. The increased serotonin sensitivity is related to an altered mobilization of Ca from a cellular store. (Hypertension 6: 887-892,1984)

KEY WORDS • vascular sensitivity • isolated arterial strips • ketanserin • methysergide • calcium

In various forms of hypertension, there is a generalized increase in vascular reactivity to constrictor stimuli such as norepinephrine, barium, and angiotensin II.¹⁻³ In addition, a specifically enhanced reactivity to serotonin compared to other vasoconstrictor agents has been reported.¹⁻⁵ Such a specific hypersensitivity to one agonist cannot be explained on the basis of a structural change in the vessel wall alone, but suggests that there is, in hypertension, a functional alteration that is specific for serotonin. The difference in response to serotonin in normotensive and hypertensive animals may be due to a change in membrane receptors for serotonin⁶ or to an alteration in the sequence of events that follow receptor activation.

This study investigates three cellular mechanisms that may contribute to increased sensitivity to serotonin in femoral arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive rats: 1) serotonin receptor affinity (pharmacological determination of pA₂ value); 2) transmembrane movement of calcium (Ca) following receptor activation by serotonin; and 3) mobilization of Ca from a cellular store by serotonin.

Methods

Animals and Preparation

This study was performed on male, Sprague-Dawley rats weighing 300 to 350 g. Approximately one-half of the rats were anesthetized with ether, their left kidney was removed through a flank incision, and DOCA (200 mg/kg) impregnated in Silastic was implanted subcutaneously. These rats were maintained on standard Purina laboratory rat chow and salt water (1% NaCl, 0.2% KCl) ad libitum. In most experiments, the control rats did not undergo sham treatment and received tap water to drink. In eight experiments, the rats were sham-treated (unilateral nephrectomy; 1% NaCl, 0.2% KCl drinking water; Silastic implants without DOCA). Systolic blood pressures were determined by tail cuff measurements in the conscious rat. Experiments were performed after 4 to 6 weeks of DOCA-salt treatment.
All rats were killed by a blow to the head, and the femoral arteries were excised and stored overnight in physiological salt solution (PSS) at a cold temperature (4°C). This storage did not alter the contractile properties of arteries from hypertensive or normotensive rats. The millimolar composition of the PSS was: NaCl 130, KC1 14.7, K2HPO4 1.18, MgSO4-7H2O 1.17, NaHCO3 14.9, CaNa2EGTA 0.026, and dextrose 5.5. Unless stated otherwise, the concentration of CaCl2-H2O in the buffer system was 1.0 mM. The next morning the arteries were cut helically into strips (0.8-1.0 x 8-10 mm) under a dissecting microscope and mounted vertically on a glass holder in a muscle bath containing oxygenated PSS (95% O2, 5% CO2) at 37°C. The upper ends of the strips were connected to force transducers (Grass FT 0.03, Grass Instrument Company, Quincy, Massachusetts), and a passive force (500 mg) that allowed maximum contraction to norepinephrine (NE) was placed on each strip. Strips were allowed to equilibrate under these conditions for 90 to 120 minutes before experimentation.

Methysergide-Ketanserin Antagonism

The first series of experiments was conducted to determine the pA2 values for methysergide vs serotonin and ketanserin vs serotonin. Cumulative concentration-response curves to serotonin were obtained in the absence and presence of three concentrations of methysergide (3.4 x 10^-10, 1.3 x 10^-9, 5.3 x 10^-9 M) or ketanserin (4.0 x 10^-10, 1.6 x 10^-9, 6.3 x 10^-9 M). Responses were normalized to the maximum serotonin response of each curve to allow interpretation of vascular sensitivity. The strips were washed several times between each concentration-response curve to remove any remaining drug. Four dose-response curves to serotonin (control, and in the presence of three concentrations of antagonist) were performed on a given strip. Time control experiments were performed to take into consideration any shift in the dose-response curves to serotonin during the course of the experiment. Contractile responses induced by the partial agonistic properties of methysergide were excluded from the analysis (i.e., the change in baseline caused by methysergide was operationally defined as the zero before addition of serotonin to the muscle bath).

Response to Calcium

To test the possibility that altered sensitivity to serotonin results from changes in the Ca handling, two types of experiments were performed: 1) dose-response to CaCl2; and 2) release of CaCl2 from a cellular store by serotonin. Dose-response curves to CaCl2 were obtained in the presence of a maximal concentration of serotonin (5.7 x 10^-9 M). Arterial strips were depleted of Ca by placing them for 15 minutes in a Ca-free PSS solution containing 1 mM EGTA and challenged with serotonin (5.7 x 10^-9 M). No response was obtained. The strips were then placed in Ca-free PSS, serotonin was added, and cumulative dose-response curves to CaCl2 were obtained. A similar experiment was performed with use of a 45-minute exposure to Ca-free PSS with no EGTA, which was substituted for the 15-minute exposure in Ca-free PSS containing EGTA.

Contractile responses to serotonin in Ca-free solution after loading of a cellular store for Ca were investigated according to the method of Karaki et al. The cellular stores of Ca were depleted and then loaded with Ca by placing the tissue in a known concentration of Ca (0.025-7.5 mM) for 5 minutes. Following this loading procedure, the strips were placed in Ca-free EGTA (1.0 mM) PSS for 1 minute, and a contraction to a maximum dose of serotonin (5.7 x 10^-9 M) was obtained. This contraction was compared to the maximum contraction to serotonin in normal (1.6 mM CaCl2) PSS.

Contractile Responses to Norepinephrine

Cumulative concentration response curves to norepinephrine (NE) were also obtained on femoral arteries from six pairs of control and DOCA-salt rats. In addition, contractile responses to NE in Ca-free solution were obtained following loading to a cellular Ca store according to the method of Karaki et al. The loading concentrations of Ca used were 0.5, 1.0, and 2.0 mM.

Drugs

Drugs used in this study were: serotonin creatinine sulfate (Sigma Chemical Company, St. Louis, Missouri), ketanserin (compliments of Janssen Pharmaceutical Company), methysergide maleate (compliments of Sandoz Pharmaceutical Company), and NE (Sigma Chemical Company).

Statistical Analysis

The ED50 values (dose of agonist producing a half-maximal response) were determined following logit transformation of dose-response curves. The pA values were determined according to the method of Schild and corrected for shifts over time. Data are reported as the means ± standard error of the mean (SEM), and comparisons between hypertensive and normotensive animals were made with unpaired Student's t test. The 0.05 level of probability was regarded as significant.

Results

Serotonin and Methysergide Dose-Response Curves

The systolic blood pressure of the DOCA-salt rats was significantly (p < 0.05, Student's t test) higher than that of the untreated controls and of the sham-treated controls (DOCA = 180 ± 6 mm Hg; untreated control = 122 ± 4 mm Hg; sham control = 126 ± 4 mm Hg). Dose-response curves to serotonin were shifted to the left in arteries from DOCA-salt hypertensive rats compared to the normotensive controls (ED50; DOCA hypertensive = 7.1 [± 0.93] x 10^-8 M; control = 27.0 [± 4.0] x 10^-8 M; Figure 1). Dose-response curves to serotonin in femoral arteries from sham-treated control rats (ED50 = 26.8 [± 9.3] x
10^{-8} \text{ M} [n = 8]) were not significantly different from those in arteries from untreated control rats. Maximum contractions to serotonin were not different between arterial strips from untreated control (731 ± 89 mg), sham control (872 ± 117 mg), and DOCA-salt hypertensive (662 ± 76 mg) rats. Methysergide caused a dose-related contraction in the arteries from all rats, which was much greater in the femoral arteries from DOCA-salt hypertensive rats than in those from control rats. The maximum contraction to methysergide (2.1 \times 10^{-6} \text{ M}) was 40\% of the maximum serotonin contraction in hypertensive rats, but only 10\% in the control rats (Figure 1). Ketanserin (1.6 \times 10^{-6} \text{ M}) caused a 50\% reduction of the methysergide (2 \times 10^{-5} \text{ M}) contraction. Ketanserin, in concentrations up to 10^{-5} \text{ M}, failed to produce contractions in any of the vessels.

**Calcium Dose-Response Curves**

The ED₅₀ values for Ca in the presence of serotonin (5.7 \times 10^{-5} \text{ M}) following cellular depletion of Ca with EGTA-containing solution were not significantly different in arteries from DOCA-salt hypertensive rats compared to controls (Figure 3; ED₅₀: control = 2.48 [± 0.78] \times 10^{-4} \text{ M}; DOCA = 3.81 [± 0.70] \times 10^{-4} \text{ M}). Arterial strips from DOCA-salt hypertensive rats reached their maximum contraction at a higher Ca concentration than the control strips. Maximum contractions were not different in strips from control and DOCA-salt hypertensive rats (447 ± 53 mg and 390 ± 44 mg, respectively). Similar results were obtained in seven pairs of strips following depletion of Ca by prolonged exposure to Ca-free solution without EGTA (ED₅₀: control = 2.14[± 0.41] \times 10^{-4} \text{ M}; DOCA = 2.84 [± 0.58] \times 10^{-4} \text{ M}).

Figure 4 left illustrates the protocol used to investigate the involvement of a cellular store of Ca that can be mobilized by serotonin to produce contraction. Arterial strips were made to contract to a maximal dose of serotonin (5.7 \times 10^{-5} \text{ M}) in normal PSS that contained 1.6 mM CaCl₂. After the contractile response had reached a plateau (10 minutes), the strips were placed for 10 minutes in Ca-free PSS that contained 1.0 mM EGTA, to deplete the cellular Ca stores. This procedure produced complete depletion of Ca in vessels from both control and DOCA-salt hypertensive rats, since the addition of serotonin (5.7 \times 10^{-5} \text{ M}) failed to produce a contraction. Following this depletion of Ca, the vessels were loaded by placing them in

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**FIGURE 1.** Effect of serotonin and methysergide on isometric force of femoral artery strips from control and DOCA-salt hypertensive rats. Serotonin and methysergide dose-response curves are normalized to the maximum serotonin response. Data are shown as means ± SEM of 21 and 20 observations for serotonin and five and six for methysergide in controls and DOCA-salt hypertensive vessels, respectively. Asterisks indicate a significant difference from the control strip response at p < 0.05.

**FIGURE 2.** Schild plots of antagonism of serotonin response (ED₅₀) by methysergide (MS) and ketanserin (KET). There were no significant differences found among any of the pA₂ values, and slopes of the lines were not different from — 1.0. Negative log of the antagonist (MS or KET) concentration is plotted on the X axis, and the log of the ED₅₀ for serotonin in the presence/absence of the antagonist minus 1 is plotted on the Y axis. Each point represents the mean ± SEM of six observations.

**Methysergide and Ketanserin Antagonism of Serotonin-Induced Contractions**

The competitive antagonistic properties of methysergide and ketanserin vs serotonin were similar in arteries from control and DOCA-salt hypertensive rats (Figure 2). The pA₂ values for methysergide vs serotonin were 10.42 and 10.47 in control and hypertensive rats, respectively, and 10.41 and 10.37 for ketanserin vs serotonin in controls and hypertensives. The slopes of these lines were not significantly different from −1.00, which indicated competitive antagonism of serotonin by both ketanserin and methysergide in these preparations.
PSS with a known concentration of Ca (0.025-7.5 mM) for 5 minutes. At the end of this loading period, the strips were placed in Ca-free EGTA PSS for 1 minute, and then serotonin (5.7 x 10^-5 M) was added to the muscle bath. The contraction produced was compared to the initial contraction in 1.6 mM CaCl₂ PSS. Although contractions tended to be larger at most concentrations of CaCl₂, only significantly larger contractions were produced in vessels from DOCA-salt hypertensive rats compared to control vessels at concentrations of CaCl₂ between 1.0 and 7.5 mM (Figure 4, right). On a percentage basis, the contractions for femoral arteries from hypertensive rats were approximately 50% greater than those for arteries from normotensive rats at these loading concentrations of Ca.

**Contractile Responses to Norepinephrine**

Cumulative addition of NE to the bath caused a dose-related contraction of the femoral artery strips. In this study, there was no difference in sensitivity to NE between strips from untreated control rats and from DOCA-salt hypertensive rats (ED₅₀: control = 6.1 [± 1.2] x 10⁻⁸ M; DOCA = 5.3 [± 1.0] x 10⁻⁸ M). There was no significant difference in the maximum contractile responses to NE (5.9 x 10⁻⁶ M) between femoral arteries from control (530 ± 52 mg) or DOCA-salt (457 ± 68 mg) rats. Furthermore, contractile responses to NE (5.9 x 10⁻⁶ M) in Ca-free solution following loading of a cellular Ca store (0.5, 1.0, and 2.0 mM CaCl₂ load) were approximately 25% of the maximum contractions to NE and did not differ between strips from control and DOCA-salt rats (DOCA responses: 0.5 mM CaCl₂ = 20% ± 4%; 1.0 mM CaCl₂ = 26% ± 4%; 2.0 mM CaCl₂ = 23% ± 3%; control responses: 0.5 mM CaCl₂ = 22% ± 4%; 1.0 mM CaCl₂ = 26% ± 6%; 2.0 mM CaCl₂ = 27% ± 3%; n = 9 for each concentration; p > 0.05).

**FIGURE 3.** Dose-response curves to CaCl₂ in the presence of serotonin (5.7 x 10⁻⁵ M) of femoral arteries from control and DOCA-salt hypertensive rats. Each curve was normalized to its own maximum. Each point is expressed as the mean ± SEM of seven observations. Asterisks indicate a significant difference from the control strip response at p < 0.05.

**FIGURE 4.** Membrane store of calcium (dose-response relationship). Left: Femoral arteries were made to contract to serotonin (5.7 x 10⁻⁵ M) in 1.6 Ca PSS (normal PSS). The strips were then depleted of Ca by placement in EGTA-Ca-free PSS for 10 minutes. Following this Ca depletion, the strips were loaded for 5 minutes in PSS with a known concentration of Ca, then placed in EGTA-Ca-free PSS, and then challenged with serotonin (5.7 x 10⁻⁵ M). Right: Data are expressed as a percentage of the contraction in 1.6 mM Ca PSS and represent the mean ± SEM of responses from six strips. Contractile responses to serotonin in femoral arteries from DOCA-salt hypertensive rats were significantly greater (p < 0.05) than responses from control rats at loading concentrations of Ca above 0.5 mM as indicated by an asterisk.
Discussion

This study demonstrates that isolated femoral arteries from DOCA-salt hypertensive rats are more sensitive (lower EDJQ) to the contractile effects of serotonin than those from normotensive rats. Furthermore, femoral arteries from hypertensive rats were more responsive to the partial agonistic properties of the serotonin antagonist methysergide than femoral arteries from normotensive rats. The change in sensitivity to serotonin in arteries from DOCA-salt hypertensive rats does not appear to be related to a change in receptor affinity nor to an alteration in the transmembrane movement of Ca following receptor activation. The increased serotonin sensitivity is accompanied by the mobilization of an increased amount of sequestered Ca from a cellular store by the agonist. The following paragraphs document these conclusions.

Serotonin Receptor Affinity and Number

Enhanced sensitivity to a contractile agonist may be caused by an increased affinity of membrane receptors or by an increased number of receptors. The pA2 value is a measure of competitive antagonism of a given agonist-antagonist pair as well as the affinity of the receptors for the agonist. Based on the pA2 values determined in this study, the affinity of receptors for serotonin in femoral arteries from DOCA-salt hypertensive rats is not different from that in femoral arteries from normotensive rats. Both ketanserin and methysergide displayed competitive antagonism (slopes of the Schild analysis were not different from - 1.0) for serotonin, and the pA2 values for both antagonists were identical in the femoral arteries from the two groups of rats. Methysergide, at the concentrations employed to determine pA2 values, has minimal agonistic activity and did not interfere with the Schild analysis.

The pA2 values obtained for ketanserin and methysergide in this study are higher than values previously reported in the literature.13 However, species and vessel differences may be responsible, in part, for the difference. These are the first reported pA2 values for methysergide and ketanserin in the rat femoral artery. In our preliminary experiments on the rat caudal artery, the pA2 values for ketanserin vs serotonin were in the range supported by Van Nueten et al.14 in that vessel.

Methysergide and ketanserin were used in this study as antagonists to evaluate the affinity of the serotonin receptor. The identical pA2 values and parallel slopes obtained from Schild analysis suggest that methysergide and ketanserin produce competitive inhibition of the same type of serotonin receptor in this vessel. Similar findings have been reported in the canine coronary artery, where ketanserin and methysergide have similar potency in antagonizing serotonin.15 In the dog coronary artery, methysergide was a competitive antagonist at all concentrations tested, and ketanserin appeared to act competitively only at lower concentrations similar to those employed in our study. We conclude that the type of serotonin receptor in femoral arteries of DOCA-salt hypertensive rats and normotensive rats is similar in these pharmacological characteristics.

Femoral arteries from DOCA-salt hypertensive rats contract to a greater level in response to methysergide than those from normotensive rats. Since maximal contractile responses to serotonin were similar in arteries from DOCA-salt hypertensive and normotensive rats, this change in responsiveness to methysergide suggests that there may be a greater number of receptors that mediate the response to methysergide. Recently, Greenberg and Curro16 have demonstrated an increased maximum-binding capacity for radioactive methysergide in vascular smooth muscle from renal hypertensive dogs. The agonist effect of methysergide may be due to its action on any of the serotonin receptor types or on another class of receptors. Van Nueten et al.14 have demonstrated that contractions produced in the rabbit femoral artery by methysergide are blocked by ketanserin, an S2 antagonist in that vessel. On the other hand, Apperley and associates17 have reported that methysergide is an agonist of serotonin contractions and, in addition, possesses agonistic properties in the rabbit aorta that are abolished by phentolamine. In the present study, ketanserin had only weak antagonistic properties against contractions induced by methysergide (dose of ketanserin to cause a 50% inhibition = 1.6 X 10^-6 M).

Calcium and Contractile Responses to Serotonin

Alterations in the delivery of Ca to the contractile machinery following receptor activation may cause increased sensitivity to a contractile agent. It appears that increased sensitivity to serotonin is not due to a change in the influx of extracellular Ca following receptor activation. Following cellular Ca depletion with EGTA, the EDJQ values for Ca in the presence of serotonin were the same in femoral arteries from DOCA-salt hypertensive rats as those in arteries from normotensive rats. Although it is possible that Ca-free conditions may alter membrane properties, 1 mM EGTA apparently has no effect, since similar results were obtained when vessels were first depleted with Ca-free PSS without EGTA.

It seems likely that the difference in responsiveness to serotonin between DOCA-salt hypertensive and normotensive rats is related to a greater release of Ca from a cellular pool. The technique used to determine the role of a cellular store of Ca was developed by Karaki et al.? This technique examines the contribution of a cellular pool of Ca in an agonist-induced contraction. The phasic contractile response produced by the agonist in Ca-free solution containing EGTA is not blocked by agents (verapamil, lanthanum) that decrease the transmembrane movement of Ca. Our results with this technique suggest that this cellular pool of Ca plays a larger role in serotonin-mediated contractions in arteries from DOCA-salt hypertensive rats than in those from normotensive rats. In contrast, sensitivity to NE was not increased in the femoral arteries from DOCA-salt hypertensive rats compared to controls, and the contribution of a cellular pool of Ca by
NE was not different between vessels from controls and DOCA-salt hypertensive rats. There are a number of possible explanations for these observations: 1) the cellular pool for Ca may be larger in arteries from DOCA-salt hypertensive rats; 2) the number of serotonin receptors that act to cause release of a cellular pool of Ca may be increased in arteries from DOCA-salt hypertensive rats; and 3) receptor activation by serotonin may cause a greater release of Ca from this pool in the arteries from hypertensive rats.

In conclusion, the results from these experiments suggest that enhanced sensitivity to serotonin in the femoral artery of DOCA-salt hypertensive rats is not mediated by changes in receptor affinity nor by an alteration in the transmembrane movement of Ca following receptor activation. The change in sensitivity to serotonin is related to an increased mobilization of Ca ion from cellular stores. There is an increase in the response of or number of receptors that mediate contraction induced by methysergide in the arteries from DOCA-salt hypertensive rats.

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