Upregulation of Arterial Serotonin 1B and 2B Receptors in Deoxycorticosterone Acetate-Salt Hypertension

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Abstract—Previous studies have established a role for 5-hydroxytryptamine (5-HT) and 5-HT receptors in mediating enhanced contraction to serotonin (5-HT) in arteries from hypertensive deoxycorticosterone acetate (DOCA)-salt rats. To determine whether the observed increase in responsiveness was due to upregulation of 5-HT receptors, we used Western analysis to measure 5-HT and 5-HT receptor protein density. In endothelium-denuded aortas from hypertensive DOCA-salt rats (mean systolic blood pressure 192±6 mm Hg), 5-HT and 5-HT receptor proteins were upregulated 2-fold compared with the response in the aortas of sham-operated control rats (mean systolic blood pressure 119±2 mm Hg). Contraction to 5-HT receptor agonists was also enhanced in arteries from Wistar-Furth rats given DOCA and salt. This strain is relatively resistant to the hypertensive effects of DOCA and salt treatment. A common factor between the model of DOCA-salt hypertension and the DOCA-salt–treated Wistar-Furth rats is the presence of mineralocorticoids. Therefore, we tested the hypothesis that mineralocorticoids can upregulate 5-HT and 5-HT receptors. Aortas from normal Sprague-Dawley rats were incubated with aldosterone (100 nmol/L) for 8, 12, 24, and 48 hours. The expression of 5-HT and 5-HT receptor proteins was significantly increased (2-fold over vehicle treatment) by 8 hours. 5-HT and 5-HT receptor proteins were upregulated by aldosterone in a concentration-dependent manner, and incubation with spironolactone (10 μmol/L) blocked this upregulation. These data support the conclusion that the increased expression of 5-HT and 5-HT receptors observed in arteries from DOCA-salt rats may be partially due to mineralocorticoids acting via the mineralocorticoid receptor to modulate gene expression. (Hypertension. 2002; 39[part 2]:394-398.)

Key Words: serotonin ▪ aldosterone ▪ arteries ▪ hypertension, sodium-dependent ▪ receptors, serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a molecule with a controversial role in hypertension. Previous studies have established that the 5-HT receptor mediates 5-HT–induced contraction in many arteries from normotensive rats.1–2 Recently, in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, 5-HT and 5-HT receptors have been implicated as mediators of 5-HT contractile hyperresponsiveness.3–6 5-HT and 5-HT receptors do not appear to be involved in mediating contraction to 5-HT under conditions of normal blood pressure. The 5-HT receptor has also been implicated as a mediator of 5-HT–induced contraction in the mesenteric arteries of Wistar and Wistar-Furth rats, which undergo the DOCA-salt protocol.7 Interestingly, the Wistar-Furth rat is relatively resistant to the hypertensive effects of mineralocorticoids, 8–10. This is important to note because although the Wistar-Furth DOCA-salt–treated rats do not obtain the same magnitude of change in blood pressure as do the Wistar DOCA-salt–treated rats, arteries from both of these rats show an increase in response to the 5-HT receptor agonist BW723C6.7 These previous studies, from the Wistar-Furth as well as the Sprague-Dawley DOCA-salt–treated rats, have demonstrated a functional upregulation of the 5-HT receptors. However, none of these studies has addressed the issue of changes in the levels of 5-HT receptor proteins. We speculated that the change in contractility to 5-HT, 5-HT receptor agonists, and 5-HT receptor agonists is due to an upregulation of 5-HT receptor proteins.

Because of the commonality of mineralocorticoids in the model of DOCA-salt hypertension and Wistar-Furth rats treated with DOCA and salt, we speculated that mineralocorticoids would have the ability to upregulate 5-HT receptors. Previous studies have demonstrated the ability of aldosterone to increase the expression of Na+,K+-ATPase in vascular smooth muscle cells.11 Investigation of the promoters of the rat genes for the 5-HT receptor and the 5-HT receptor revealed that they contain mineralocorticoid response elements (MREs).12,13 Therefore, we tested the hypothesis that aldosterone incubation would cause an upregulation of 5-HT receptor and 5-HT receptor proteins, independent of an increase in blood pressure.

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 (0.20 to 0.25 kg; Charles River Laboratories, Inc, 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. After surgery, the 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 both food and water. After 4 weeks, the systolic blood pressures were measured by using the standard tail-cuff method.

Incubation Experiments

The aorta was removed, cleaned, denuded of endothelial cells, and cut into helical strips. The tissue was then cut into 4 segments and placed into DMEM (GIBCO-BRL) supplemented with 10% FBS (HyClone), and either vehicle or aldosterone was added to each culture plate. For experiments involving spironolactone, either vehicle or spironolactone was added 30 minutes before the addition of aldosterone. Culture plates were maintained at 37°C in a humidified incubator with 5% CO₂. After incubation, the tissues were removed from the media, and protein was isolated as described below.

Western Analysis

Protein Isolation

The aorta was removed, cleaned, denuded of endothelial cells, and cut into helical strips. The tissue was frozen in liquid nitrogen, pulverized in a liquid nitrogen-cooled mortar and pestle, and solubilized in a lysis buffer (0.5 mol/L Tris HCl [pH 6.8], 10% SDS, and 10% glycerol) with protease inhibitors (0.5 mmol/L phenylmethanesulfonyl fluoride, 10 μg/mL aprotinin, and 10 μg/mL leupeptin). Homogenates were centrifuged (11 000 g for 10 minutes, 4°C), and supernatant total protein was measured (BCA, Sigma).

Immunoblotting Protocol

Supernatant (4:1 in denaturing loading buffer, boiled for 5 minutes) was loaded, separated on 10% denaturing SDS-polyacrylamide gels, and transferred to Immobilon-P membranes (Millipore). Membranes were blocked for 3 to 4 hours in Tris-buffered saline plus Tween 20 (0.1%) containing 4% chick egg ovalbumin and 0.025% sodium azide. Mouse anti-5-HT₁B receptor antibody (0.5 μg/mL, Pharmingen) and guinea pig anti-5-HT₂B receptor antibody (1:1000, Chemicon) were incubated with blots overnight (4°C). After washes, secondary antibody linked to horseradish peroxidase (anti-mouse, 1:10,000, Amersham Laboratories, or anti–guinea pig, 1:10 000, Chemicon) was added for 1 hour and incubated with blots overnight (4°C). After washes, secondary antibody linked to horseradish peroxidase (anti-mouse, 1:10,000, Amersham Laboratories, or anti–guinea pig, 1:10 000, Chemicon) was added for 1 hour and incubated with blots overnight (4°C). After washes, secondary antibody linked to horseradish peroxidase (anti-mouse, 1:10,000, Amersham Laboratories, or anti–guinea pig, 1:10 000, Chemicon) was added for 1 hour and incubated with blots overnight (4°C). Enhanced chemiluminescence was performed by using standard reagents (Amersham Laboratories). Each blot was washed and redeveloped by using an α-smooth muscle actin antibody (1:400, Oncogene Research Products; anti-mouse secondary antibody, 1:3000, Amersham Laboratories). Equal lane loading of protein was ensured by comparing α-smooth muscle actin densitometry.

Data Analysis and Statistics

Data are presented as mean±SEM for the number of animals in parentheses. When 2 groups were compared, the appropriate Student t test was used. When >2 groups were compared, a 1-way ANOVA was performed, followed by a Student-Newman-Keuls post hoc test. In all cases, a value of P≤0.05 was considered statistically significant.

Materials

Acetylcholine chloride, phenylephrine hydrochloride, DOCA, 5-HT hydrochloride, spironolactone, and d-aldosterone were purchased from Sigma Chemical Co.

Results

The average systolic blood pressure of the hypertensive DOCA-salt rats was 192±6 mm Hg; the average systolic blood pressure of the normotensive sham-operated rats was 119±2 mm Hg (P<0.05). The role of 5-HT₂B and 5-HT₁B receptors in mediating 5-HT–induced contraction and hyper-reactivity in arteries from Sprague-Dawley DOCA-salt hypertensive rats has been previously established.²⁻⁶ We used Western analysis and antibodies to 5-HT₁B and 5-HT₂B receptors to determine whether the change in contractility to 5-HT₁B receptor and 5-HT₂B receptor agonists was due to an increase in the levels of the receptor protein. 5-HT₁B and 5-HT₂B receptor proteins were upregulated ∼2-fold in aortas from Sprague-Dawley DOCA-salt hypertensive rats compared with aortas from control rats (Figure 1). These data suggest that an increase in the level of receptor protein may be at least partially responsible for the changes observed to 5-HT₁B receptor and 5-HT₂B receptor agonists.

There are data to support a functional upregulation of the 5-HT₂B receptor in Wistar and Wistar-Furth rats treated with DOCA and salt.⁷ A common factor between the Wistar and Wistar-Furth rats treated with DOCA and salt and the Sprague-Dawley DOCA-salt rat model of hypertension is the presence of mineralocorticoids. Therefore, we hypothesized...
that mineralocorticoids may regulate the expression of 5-HT1B and 5-HT2B receptors. We incubated with aldosterone the endothelium-denuded thoracic aortas from normotensive Sprague-Dawley rats under tissue culture conditions. Incubation with aldosterone (100 nmol/L) for 8 and 12 hours resulted in a significant increase (2-fold above vehicle treatment) in both 5-HT1B receptor (top) and 5-HT2B receptor (bottom) protein density in aldosterone-incubated aortas is shown. Blots are representative of 7 experiments. Bars represent mean±SEM of the animals indicated in parentheses. *P<0.05 compared with vehicle response.

Figure 2. Effect of aldosterone (Aldo, 100 nmol/L) incubation for variable lengths of time (8, 12, 24, and 48 hours) in thoracic aortas from normal Sprague-Dawley rats. Measurement of 5-HT1B receptor (top) and 5-HT2B receptor (bottom) protein density in aldosterone-incubated aortas is shown. Blots are representative of 7 experiments. Bars represent mean±SEM of the animals indicated in parentheses. *P<0.05 compared with vehicle response.

Figure 3. Top, Effect of Aldo (1 nmol/L to 100 nmol/L) incubation in thoracic aortas from normal Sprague-Dawley rats. Measurement of 5-HT1B receptor (top, left) and 5-HT2B receptor (top, right) protein density in Aldo-incubated aortas is shown. V indicates vehicle. Blots are representative of 4 to 10 experiments. Bottom, Effect of spironolactone (SP, 10 μmol/L) incubation on Aldo (100 nmol/L)-induced upregulation of the 5-HT1B (bottom, left) and 5-HT2B receptor (bottom, right) protein density in thoracic aortas from normal Sprague-Dawley rats. DMSO indicates dimethyl sulfoxide; ETOH, ethanol. Bars represent mean±SEM of the animals indicated in parentheses. Blots are representative of 7 experiments *P<0.05 compared with vehicle response; †P<0.05 compared with Aldo and SP treatment.

Discussion
These data demonstrate an increase in the levels of 5-HT1B and 5-HT2B receptor proteins under conditions of DOCA-salt hypertension. We speculate that this upregulation of 5-HT1B and 5-HT2B receptor proteins is, at least partially, responsible for the increased contractility to 5-HT seen in the arteries of hypertensive rats.

The mesenteric arteries of the Wistar and Wistar-Furth rats treated with DOCA and salt demonstrated increased responsiveness to 5-HT and also to the 5-HT2B agonist BW723C86.7 The increase in maximal response to BW723C86 was smaller in the arteries from the Wistar-Furth DOCA-salt rats than in
the arteries from the Wistar DOCA-salt rats. These Wistar-Furth DOCA-salt rats had an increase in systolic blood pressure of \(\approx 17\) mm Hg, but the Wistar DOCA-salt rats had an increase of \(\approx 66\) mm Hg. These data suggest that this increase in receptor density could be blood pressure dependent. However, when the animals were matched for systolic blood pressure, arteries from the Wistar-Furth DOCA-salt rats had an increased maximal response to BW723C86 compared with the response in arteries from the Wistar-Furth sham-operated rats. Thus, such data do suggest that mineralocorticoids, independent of an increase in blood pressure, can modulate 5-HT\(_{2B}\) receptor function. Enhanced contraction to the 5-HT\(_{2B}\) receptor agonist BW723C86 has also been seen in arteries from the \(\text{N}^\text{nitro\text{-l-arginine}}\) hypertensive rat model\(^{11,12}\) as well as in the spontaneously hypertensive rat model of hypertension.\(^{15}\) Interestingly, in the spontaneously hypertensive rat model\(^{16}\) and the \(\text{N}^\text{nitro\text{-l-arginine methyl ester}}\) model\(^{17}\) of hypertension, treatment with the mineralocorticoid receptor antagonist spironolactone reduces blood pressure. Although these studies do involve an increase in blood pressure, they also suggest that there is a dependence on mineralocorticoids in these models. Because of the general finding of an increase in contraction in hypertensive animals, the purpose of the Wistar-Furth experiments was to understand the effects of an increase in blood pressure on the regulation of the 5-HT\(_{2B}\) receptor. Surprisingly, the studies in the Wistar-Furth and Sprague-Dawley rats treated with DOCA and salt suggest that in the presence of increased levels of mineralocorticoids or salt, there is a functional upregulation of the 5-HT\(_{2B}\) receptor, independent of an increase in blood pressure. Currently, there are no studies in the Wistar and Wistar-Furth rats treated with DOCA and salt that have examined the role of the 5-HT\(_{1B}\) receptor. There are also no studies in the Wistar and Wistar-Furth rats treated with DOCA and salt that have examined 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptor protein levels. However, the data that are available suggest that the functional contractile response to stimulation of the 5-HT\(_{2B}\) receptor, induced by the presence of increased levels of mineralocorticoids and salt in vivo, is increased. This information, coupled with the findings in experiments using Sprague-Dawley DOCA-salt rats, led us to speculate that mineralocorticoids and increases in blood pressure may independently regulate the expression of the 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptors.

In an attempt to separate the effects of an increase in blood pressure from the direct effects of mineralocorticoids, we incubated with aldosterone the thoracic aortas of Sprague-Dawley rats with normal blood pressure. Aldosterone has been implicated in increasing the expression of human Na\(^+\),K\(^+\)-ATPase \(\beta 1\)\(^{16}\) and K-ras\(^{19}\) and in decreasing the 5-HT\(_{1A}\) receptor expression in the rat dentate gyrus.\(^{20}\) Aldosterone decreases the mRNA expression of c-Myc, c-Jun, and c-Fos by posttranscriptional mechanisms while increasing Fra-2 mRNA by a transcriptional mechanism in epithelial cells.\(^{21}\) This is interesting to note because it suggests that aldosterone may act directly at a promoter via the MRE as well as indirectly through its actions on transcription factors. Additionally, aldosterone induces methylation of ras in renal epithelial cells.\(^{22}\) This suggests that aldosterone changes not

only the level of protein expression but also the state of activation of proteins involved in signaling cascades. Whether aldosterone is acting directly at the MREs or through its actions on other transcription factors to cause the upregulation of the 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptors is unknown.

These results, which demonstrate an increase in vascular smooth muscle 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptors under conditions of DOCA-salt hypertension and increased mineralocorticoids, have potential significant physiological ramifications. The 5-HT\(_{1B}\) receptor has been described as a mediator of 5-HT–induced contraction in the human coronary,\(^{23}\) pulmonary,\(^{24}\) and cerebral\(^{25}\) arteries. Clinically, 5-HT\(_{1B}\) receptor agonists, such as Sumatriptan, are used in the treatment of migraines. Understanding the regulation of this receptor in disease states may prevent the administration of 5-HT\(_{1B}\) agonists to patients with risk factors and thereby prevent complications.

Recently, the 5-HT\(_{2B}\) receptor has been implicated as an important developmentally expressed receptor in the heart.\(^{26}\) In 5-HT\(_{2B}\) receptor knockout mice, the heart shows abnormal structural development as well as abnormal function.\(^{27,28}\) Interestingly, aldosterone has been implicated as a mediator of cardiac fibrosis and myocardial necrosis.\(^{29,30}\) Currently, there are no studies published that have addressed the interaction of the 5-HT\(_{2B}\) receptor and aldosterone in the heart under the pathological condition of hypertension. Therefore, one can speculate that under conditions of hypertension, particularly in the forms of hypertension that are aldosterone dependent, the 5-HT\(_{2B}\) receptor expression may be changed in arteries and also in the heart. These changes may play an important role in the development and/or maintenance of the high blood pressure and organ damage observed in this pathophysiological condition.

The data presented support the conclusion that 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptor proteins are upregulated in arteries from DOCA-salt hypertensive rats. Furthermore, the incubation of aortas from normotensive rats with aldosterone, in the absence of pressure, resulted in an upregulation of 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptor proteins. This increase was blocked by the mineralocorticoid receptor antagonist spironolactone. These data support the conclusion that mineralocorticoids, independent of a change in blood pressure, can upregulate 5-HT\(_{1B}\) and 5-HT\(_{2B}\) receptors. The authors acknowledge that the present study does not directly address the role of high salt in the regulation of these receptors. High salt may play an important regulatory role in vivo, but whether the effects are due to direct actions in the tissue or are secondarily due to the increase in blood pressure is, as yet, undetermined. The authors also acknowledge that there may be other growth factors and hormones that may contribute and/or have the ability to modulate the expression of these receptors. To date, very little is currently known about the regulation of these receptors. The present study demonstrates the importance of mineralocorticoids, specifically aldosterone, as an important modulator of gene expression. The ability to change receptor expression, kinase expression, and kinase activity levels suggests that aldosterone has a plethora of effects on vascular function. These effects may act alone or in combination to contribute to the pathogenesis of vascular dysfunction associated with hypertension.
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References
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