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Arterial expression of 5-HT_{2B} and 5-HT_{1B} receptors during development of DOCA-salt hypertension

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Published: 15 September 2003

Received: 25 June 2003

BMC Pharmacology 2003, 3:12

Accepted: 15 September 2003

This article is available from: <http://www.biomedcentral.com/1471-2210/3/12>

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Abstract

Background: 5-hydroxytryptamine (5-HT)_{2B} and 5-HT_{1B} receptors are upregulated in arteries from hypertensive DOCA-salt rats and directly by mineralocorticoids. We hypothesized that increased 5-HT_{2B} and 5-HT_{1B} receptor density and contractile function would precede increased blood pressure in DOCA-high salt rats. We performed DOCA-salt time course (days 1, 3, 5 and 7) studies using treatment groups of: DOCA-high salt, DOCA-low salt, Sham and Sham-high salt rats.

Results: In isolated-tissue baths, DOCA-high salt aorta contracted to the 5-HT_{2B} receptor agonist BW723C86 on day 1; Sham aorta did not contract. The 5-HT_{1B} receptor agonist CP93129 had no effect in arteries from any group. On days 3, 5 and 7 CP93129 and BW723C86 contracted DOCA-high salt and Sham-high salt aorta; Sham and DOCA-low salt aorta did not respond. Western analysis of DOCA-high salt aortic homogenates revealed increased 5-HT_{2B} receptor levels by day 3; 5-HT_{1B} receptor density was unchanged. Aortic homogenates from the other groups showed unchanged 5-HT_{2B} and 5-HT_{1B} receptor levels.

Conclusion: These data suggest that functional changes of 5-HT_{2B} but not 5-HT_{1B} receptors may play a role in the development of DOCA-salt hypertension.

Background

5-HT is an autocooid with a myriad of actions in the cardiovascular system. One of the important vascular effects of 5-HT is its ability to act as a vasoconstrictor. The 5-HT_{2A}, 5-HT_{2B} and 5-HT_{1B} receptors have been implicated as mediators of 5-HT-induced contraction in vascular smooth muscle. 5-HT_{2A} receptors mediate contraction in many arteries including the rat thoracic aorta [1] and pulmonary arteries [2]. 5-HT_{2B} receptors mediate 5-HT-induced contraction in the rat stomach fundus, the aorta and mesenteric arteries from hypertensive deoxycorticosterone acetate (DOCA)-salt rats [3–5]. The 5-HT_{2B} receptor does not appear to mediate contraction in arteries

taken from normotensive Sham rats [4,5]. Involvement of 5-HT_{1B} receptors in mediating 5-HT-induced arterial contraction in normal vessels has been described in the rat tail artery [6], human temporal artery [7], human umbilical artery [8], human pulmonary artery [9] and human coronary artery [10]. Additionally, 5-HT_{1B} receptors mediate 5-HT-induced contraction in mesenteric arteries from hypertensive DOCA-salt rats [11] and pulmonary arteries from rats with pulmonary hypertension [12,2]. Furthermore, arteries from DOCA-salt hypertensive rats have approximately a 2-fold increase in the expression of the 5-HT_{2B} and 5-HT_{1B} receptor proteins [13]. These findings suggest that the change in the function of the 5-HT_{2B} and

5-HT_{1B} receptors may be due to the alterations in the level of the receptor proteins.

Recent *in vivo* studies with the selective 5-HT_{2B} receptor antagonist LY272015 have revealed that LY272015 lowered the blood pressure of DOCA-salt rats with established hypertension [4]. These findings suggest that the 5-HT_{2B} receptor is endogenously activated under conditions of hypertension to participate in the maintenance of the elevated blood pressure. No studies have examined whether 5-HT_{1B} receptors are endogenously activated in established DOCA-salt hypertension. However, activation of 5-HT_{1B} receptors have been implicated as a causative factor of pulmonary hypertension. [12,14,2]. Collectively, these studies suggest that 5-HT_{2B} and 5-HT_{1B} receptors can be endogenously activated and, by their ability to mediate 5-HT-induced vasoconstriction, may participate in the maintenance of the increased blood pressure.

We have recently found that aldosterone, *in vitro*, upregulates the 5-HT_{2B} and 5-HT_{1B} receptor proteins in endothelium-denuded aorta from rats with normal blood pressure [13]. This upregulation was inhibited in the presence of the mineralocorticoid receptor antagonist spironolactone. Furthermore, studies in the Wistar-Furth rat model, a rat model which is relatively resistant to the hypertensive effects of DOCA and salt treatment [15], demonstrated that in blood pressure matched rats, the presence of elevated levels of DOCA and salt resulted in enhanced contraction to the 5-HT_{2B} receptor agonist BW723C86 [16]. These findings suggest that mineralocorticoids, such as DOCA, may be important independent regulators of these 5-HT receptors. A separation of the effects of DOCA, an increase in blood pressure, increased levels of salt and the combined effects of an increase in salt and DOCA on 5-HT receptor function and protein levels has never been performed. Separating the effects of these factors is necessary to understand their contribution to regulation of 5-HT_{2B} and 5-HT_{1B} receptors. Therefore, we proposed to determine when in the course of the DOCA-salt hypertension an increase in receptor density occurs and if the increase in the level of receptor proteins and functional response preceded the increase in blood pressure. We hypothesized that the increase in the 5-HT_{2B} and 5-HT_{1B} receptor density and functional responses would precede the increase in blood pressure observed in the DOCA-salt rats. We used the rodent selective 5-HT_{1B} receptor agonist CP93129 and the 5-HT_{2B} receptor agonist BW723C86 to test this hypothesis.

Results

On days 1, 3, 5 and 7 after surgery the rats were weighed and the systolic blood pressure was measured. Only the Sham rats on day seven of the protocol showed a significant gain in body weight (data not shown). None of the

rats in the Sham-high salt, DOCA-high salt and DOCA-low salt groups gained significant body weight during the protocol. DOCA-high salt rats consumed significantly more fluid than the Sham, Sham-high salt and DOCA-low salt rats by day three and this continued through day seven (figure 1). Additionally, rats placed on high salt water alone increased intake significantly by day five and remained elevated through day seven (figure 1, bottom left and right, respectively). The Sham and DOCA-low salt rats did not vary significantly in their fluid consumption during the study.

DOCA-high salt rats showed a significant increase in blood pressure by day three [average systolic blood pressure (SBP) 124 ± 7.6 mm Hg, 110 ± 2.6 mm Hg and 112 ± 2.4 mm Hg, DOCA-high salt and Sham, respectively] (figure 2). The DOCA-salt rats reached hypertensive levels by day five (average SBP 147 ± 14.0). Sham-high salt rats had elevated blood pressures by day seven (average SBP 130 ± 9.3 mm Hg). Furthermore, by day seven the DOCA-low salt rats had significantly lower blood pressure than their Sham, DOCA-high salt and Sham-high salt counterparts (average SBP 114 ± 2.3 mm Hg, 142 ± 8.4 mm Hg, 130 ± 9.3 mm Hg and 101 ± 2.1 mm Hg, Sham, DOCA-high salt, Sham-high salt and DOCA-low salt, respectively). For further data please see Figure 9.

Contractile Studies on Day 1

Although there was no increase in systolic blood pressure on day one, arteries from DOCA-high salt rats contracted to the 5-HT_{2B} receptor agonist BW723C86 (maximal contraction 52.5 ± 10.5 % of PE contraction). Arteries from DOCA-low salt, Sham-high salt and Sham rats did not respond to either BW723C86 or to the 5-HT_{1B} receptor agonist CP93129 (figures 3A and 3B). There was no enhanced contraction to 5-HT observed in any of the arteries from the treatment groups (figure 3C). These data suggest that while 5-HT_{2B} receptors are functional on day one, as indicated by contraction to BW723C86, this is not sufficient to result in an enhanced contraction to 5-HT. Additionally, the 5-HT_{1B} receptor agonist CP93129 was unable to stimulate contraction, suggesting that the 5-HT_{1B} receptor was not functionally coupled to contraction on day one.

Contractile Studies on Day 3

On day three, BW723C86 contracted arteries from DOCA-high salt and Sham-high salt rats (figure 4A). There was also a significant contraction observed to CP93129 in arteries from DOCA-high salt rats (figure 4B). Arteries from DOCA-low salt and Sham rats did not contract to BW723C86 or CP93129 (figures 4A and 4B). Contraction to 5-HT was enhanced, as defined by an increased maximal contraction, decreased threshold for contraction and/or an increased potency, in arteries from Sham-high salt

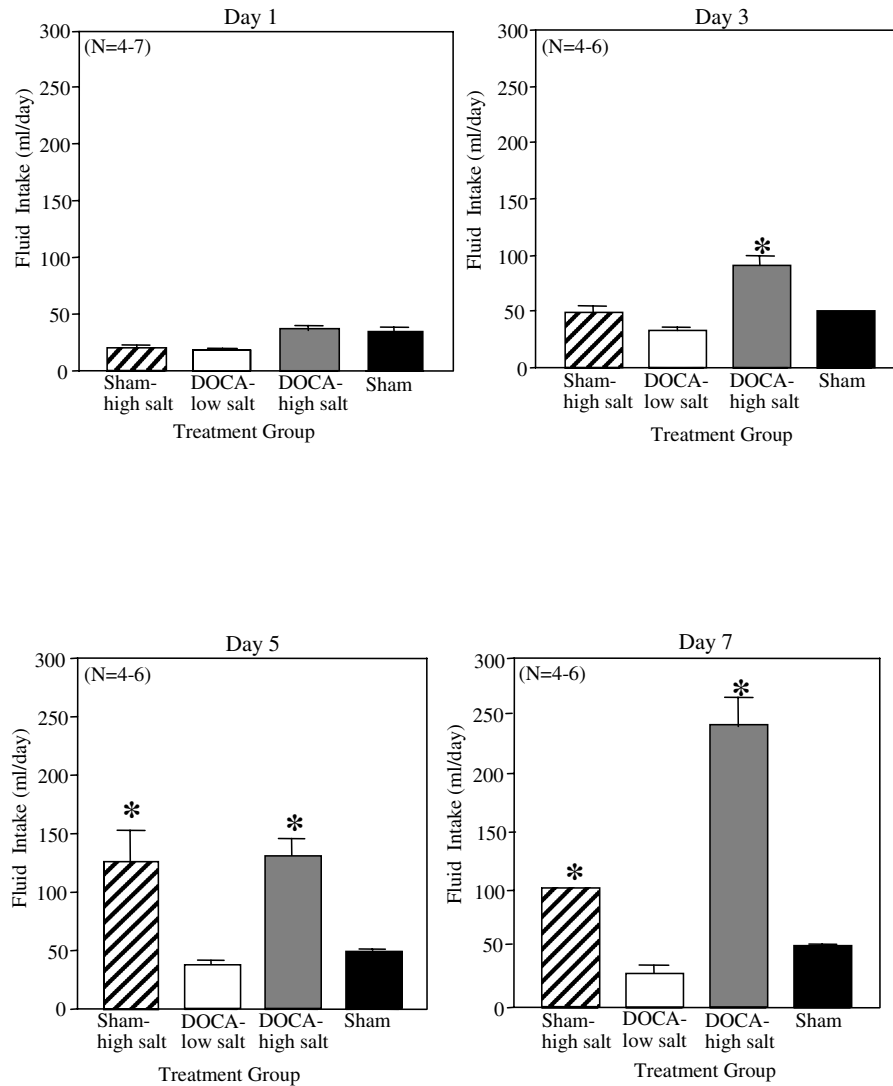


Figure 1

Measurement of fluid consumption by day and treatment group. Vertical bars represent the mean \pm sem for the number of animals indicated in parentheses. * represents statistically significant difference ($p < 0.05$) between the response obtained from day one of treatment.

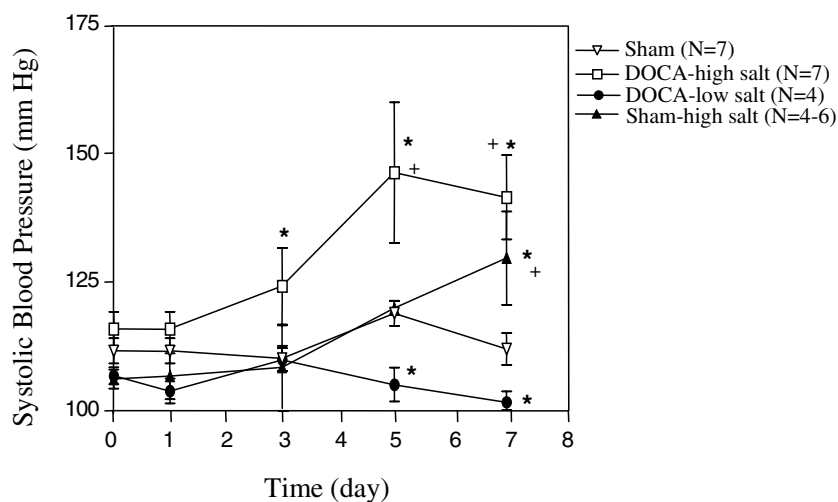


Figure 2

Measurement of systolic blood pressures. Data are reported in mm Hg. Points represent the mean \pm sem for the number of experiments indicated in parentheses. * represents statistically significant difference ($p < 0.05$) between the response obtained from the Sham response on each day. + represents statistically significant difference ($p < 0.05$) between the response obtained from the day zero measurement from each respective group.

rats (figure 4C). The arteries from the DOCA-high salt rats showed no change in the maximal contraction to 5-HT but demonstrated enhanced 5-HT potency ($-\log EC_{50}$ [M] values 6.02 ± 0.08 , 5.74 ± 0.05 DOCA-high salt day 3 and Sham, respectively). Unexpectedly, arteries from DOCA-low salt rats showed an increased maximal response to 5-HT (maximal contraction 141.2 ± 11.8 % PE contraction). Since there was no response to CP93129 and very little response to BW723C86, this enhanced contraction to 5-HT was surprising. However, this enhancement of 5-HT-induced contraction in arteries from DOCA-low salt rats only occurred on day three. These findings suggest that the 5-HT_{1B} receptor requires the presence of elevated levels of DOCA, salt and pressure to become functionally coupled to contraction. 5-HT_{2B} receptors were able to mediate contraction in arteries from both DOCA-high salt and Sham-high salt rats, suggesting that the elevated levels of salt are

required to observe functional changes in the 5-HT_{2B} receptor.

Contractile Studies on Day 5

Experiments performed on day five of the time course demonstrated a similar profile of results. Arteries from DOCA-high salt and Sham-high salt rats contracted to both BW723C86 (maximal contraction 54.3 ± 11.9 % and 46.3 ± 6.6 % of PE contraction, DOCA-high salt and Sham-high salt, respectively) and CP93129 (maximal contraction 35.0 ± 8.6 % and 27.8 ± 7.4 % of PE contraction, DOCA-high salt and Sham-high salt, respectively) (figures 5A and 5B). Arteries from Sham-high salt rats also displayed an increased maximal contraction to 5-HT (maximal contraction 148.5 ± 11.4 % and 106.2 ± 8.7 % of PE contraction, Sham-high salt and Sham, respectively), a decrease in the threshold of activation of contraction and an increase in potency, and as compared to

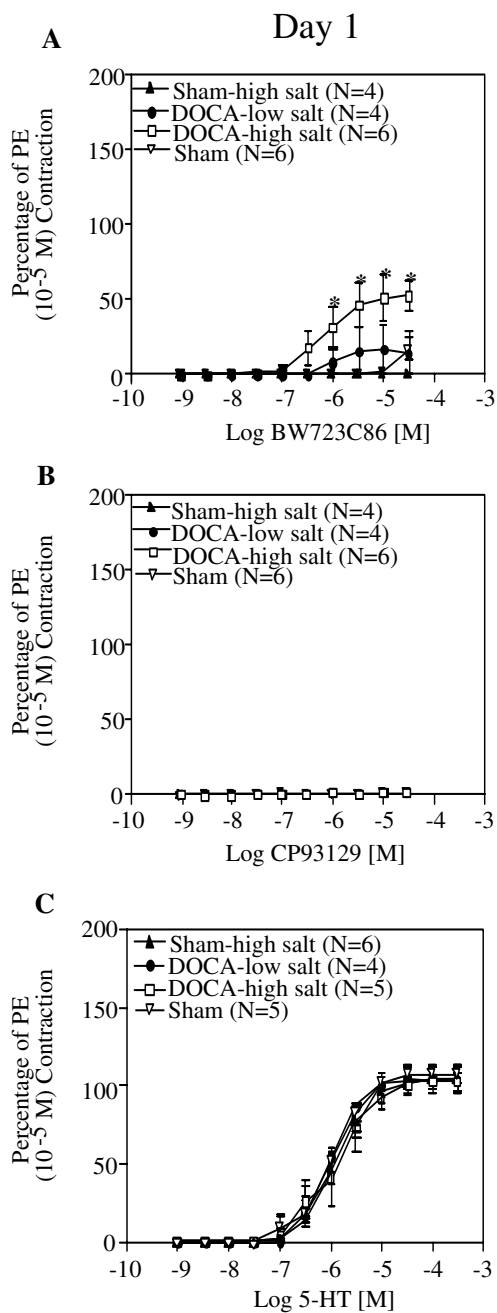


Figure 3

Day 1 A: Effect of the 5-HT_{2B} receptor agonist BW723C86 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **B:** Effect of the 5-HT_{1B} receptor agonist CP93129 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **C:** Effect of 5-HT in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. Data are reported as a percentage of the initial PE 10⁻⁵ M contraction. Points represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the Sham artery.

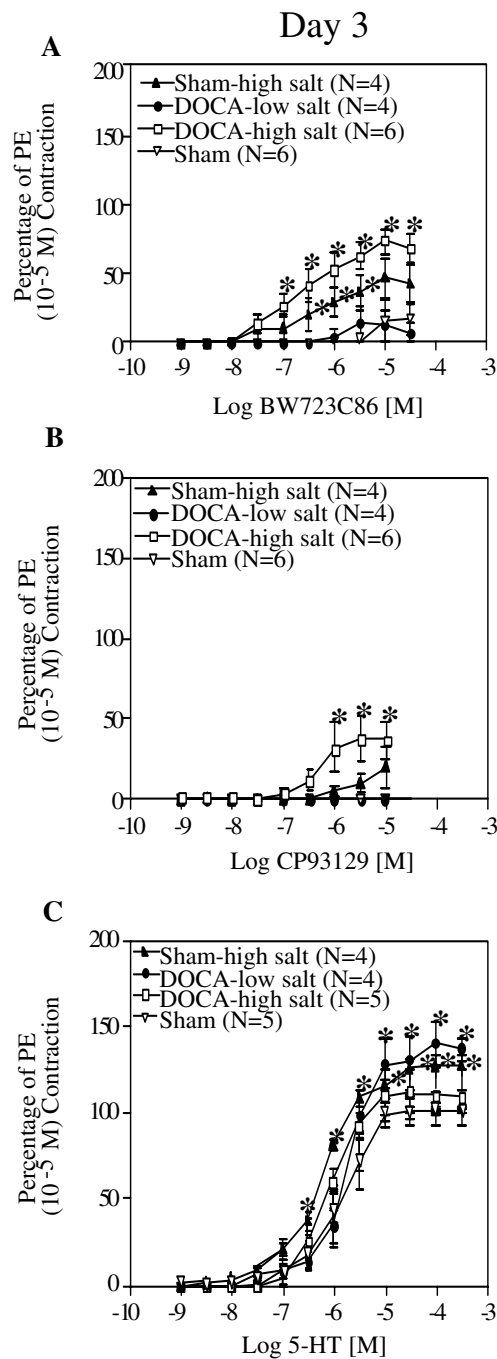


Figure 4

Day 3 A: Effect of the 5-HT_{2B} receptor agonist BW723C86 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **B:** Effect of the 5-HT_{1B} receptor agonist CP93129 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **C:** Effect of 5-HT in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. Data are reported as a percentage of the initial PE 10⁻⁵ M contraction. Points represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the Sham artery.

Sham arteries (-log EC₅₀ value [M] 6.06 ± 0.05 and 5.60 ± 0.07 Sham-high salt and Sham, respectively) (figure 5C). The arteries from DOCA-high salt rats also show a decrease in the threshold of activation of contraction (figure 5C). These results suggest that additional changes in the contractile mechanisms utilized by 5-HT, in addition to the 5-HT_{1B} and 5-HT_{2B} receptors being functionally coupled to contraction, are required in arteries from DOCA-high salt hypertensive rats to mediate hyperresponsiveness to 5-HT.

Contractile Studies on Day 7

On day seven, arteries from DOCA-high salt rats contracted to both BW723C86 (maximal contraction 57.4 ± 5.9 % of PE contraction) and CP93129 (maximal contraction 63.8 ± 7.8 % of PE contraction) (figures 6A and 6B). Arteries from Sham-high salt rats also contracted to both BW723C86 (maximal contraction 24.5 ± 9.4 % of PE contraction) and CP93129 (maximal contraction 19.6 ± 8.1 % of PE contraction) (figures 6A and 6B). Interestingly, although the arteries from Sham-high salt rats were exposed to both increased levels of salt and pressure, these arteries did not contract to the same magnitude in the presence of BW723C86 (maximal contraction 24.5 ± 9.4 % and 57.4 ± 5.9 % of PE contraction, Sham-high salt and DOCA-high salt, respectively) and CP93129 (maximal contraction 19.6 ± 8.1% and 63.8 ± 7.8 % of PE contraction, Sham-high salt and DOCA-high salt, respectively) as those that are taken from DOCA-high salt rats on day seven. Additionally, arteries from DOCA-high salt and Sham-high salt rats both show hyperresponsiveness to 5-HT (figure 6C). These arteries demonstrate a characteristic increased maximal contraction (maximal contraction 130.3 ± 19.5 % and 119.5 ± 5.1 % of PE contraction, DOCA-high salt and Sham-high salt, respectively), the decreased threshold for contraction and the increased potency (- log EC₅₀ value [M] 6.41 ± 0.09, 6.13 ± 0.03 and 5.70 ± 0.07, DOCA-high salt, Sham-high salt and Sham, respectively). These data suggest that the combined presence of elevated levels of salt, DOCA and pressure all contribute to the functional changes in 5-HT_{1B} and 5-HT_{2B} receptors observed in the arteries from the DOCA-high salt hypertensive rats.

Protein Analysis Studies

To determine if upregulation of 5-HT_{1B} and 5-HT_{2B} receptor proteins was responsible for the acquired contractility, we performed western analysis of total aortic homogenates. The authors acknowledge that the limitations of this technique do not speak to localization of the receptor proteins within the cell, only to the total amount of the receptor protein present. There was no increase in 5-HT_{1B} receptor protein levels in any of the treatment groups at any time point (figure 7), although there was a trend towards increasing levels of protein in the aortic homoge-

nates from the DOCA-high salt rats. This was an unexpected finding as the arteries from both the DOCA-high salt and Sham-high salt rats contract to the 5-HT_{1B} agonist CP93129 starting on day three of the time course. These data suggest that although the receptor protein is upregulated by 28 days of DOCA-salt treatment (13), upregulation is not required to enable this receptor to participate in contraction.

In contrast, the 5-HT_{2B} receptor was upregulated significantly by day 3 of DOCA-high salt treatment (figure 8). However, 5-HT_{2B} receptor protein levels were not increased in any of the other treatment groups at any time point. Interestingly, the level of receptor protein was not significantly increased on day 1, even though there was a significant contraction to the 5-HT_{2B} receptor agonist BW723C86 on this day. There was also no significant increase in 5-HT_{2B} receptor levels in the aortic homogenates from the Sham-high salt rats in which contraction to BW723C86 occurred on days 3, 5 and 7. These findings suggest that upregulation of the 5-HT_{2B} receptor proteins is not absolutely required for this receptor to mediate agonist-induced contraction.

Discussion

Since the isolation of 5-HT, a role for it in systemic hypertension has been suggested. While the specific role which 5-HT plays in hypertension remains a controversial topic, there is no argument that 5-HT is both a vasoconstrictor and a mitogen. Currently, the mRNA for the 5-HT_{2A}, 5-HT_{2B}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F} and 5-HT₇ receptors have been localized to vascular smooth muscle cells. [17]. However, only the 5-HT_{2A} receptor has been shown to activate the mitogen-activated protein kinase (MAPK) pathway and participate in arterial contraction under conditions of normal blood pressure in the rat thoracic aorta. [17]. Interestingly, the present studies suggest that 5-HT_{1B} and 5-HT_{2B} receptors are involved in mediating the arterial hyperresponsiveness to 5-HT observed in hypertension. This involvement appears to be due to changes independent of the level of receptor proteins as the functional response of contraction can be separated from an increase in the level of 5-HT_{2B} and 5-HT_{1B} receptor proteins.

Regulation of 5-HT Receptors

The studies presented here support the idea that mineralocorticoids combined with salt and increased blood pressure, but not mineralocorticoids alone *in vivo*, may be important mediators of the changes to both the level of 5-HT_{2B} and 5-HT_{1B} receptor protein levels and the signaling mechanisms utilized by these receptors. The aortic homogenates from DOCA-salt rats showed an increase in the level of the 5-HT_{2B} receptor protein on days 3, 5 and 7. Contrary to *in vitro* findings in which aldosterone alone

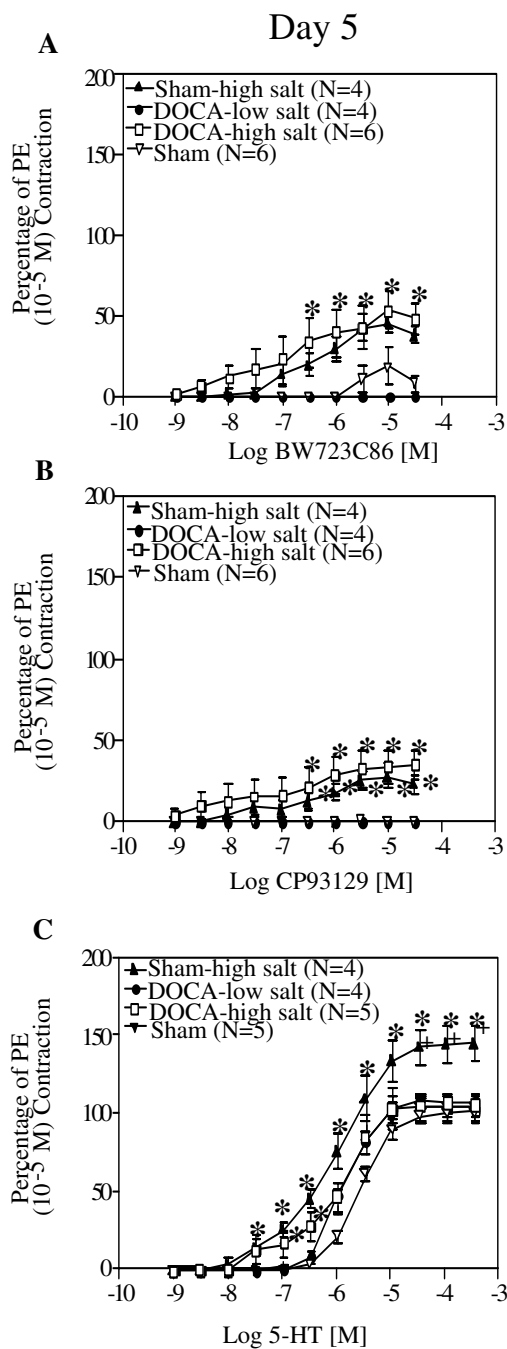


Figure 5

Day 5 A: Effect of the 5-HT_{2B} receptor agonist BW723C86 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **B:** Effect of the 5-HT_{1B} receptor agonist CP93129 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **C:** Effect of 5-HT in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. Data are reported as a percentage of the initial PE 10⁻⁵ M contraction. Points represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the Sham artery.

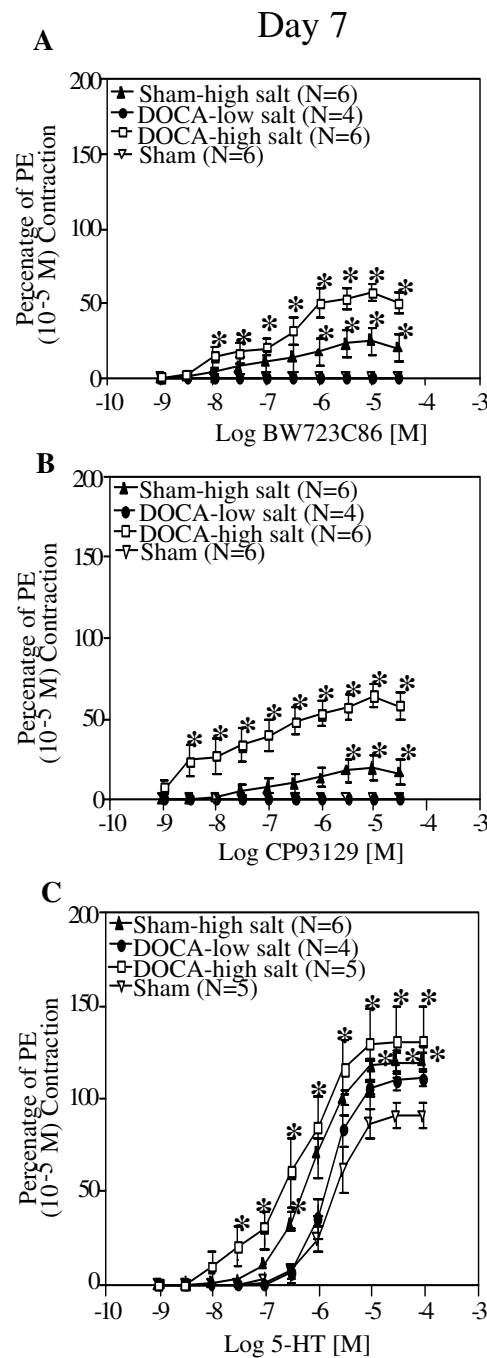


Figure 6

Day 7 A: Effect of the 5-HT_{2B} receptor agonist BW723C86 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **B:** Effect of the 5-HT_{1B} receptor agonist CP93129 in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. **C:** Effect of 5-HT in aorta from Sham-high salt, DOCA-high salt, DOCA-low salt and Sham rats. Data are reported as a percentage of the initial phenylephrine (PE) 10⁻⁵ M contraction. Points represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the Sham artery.

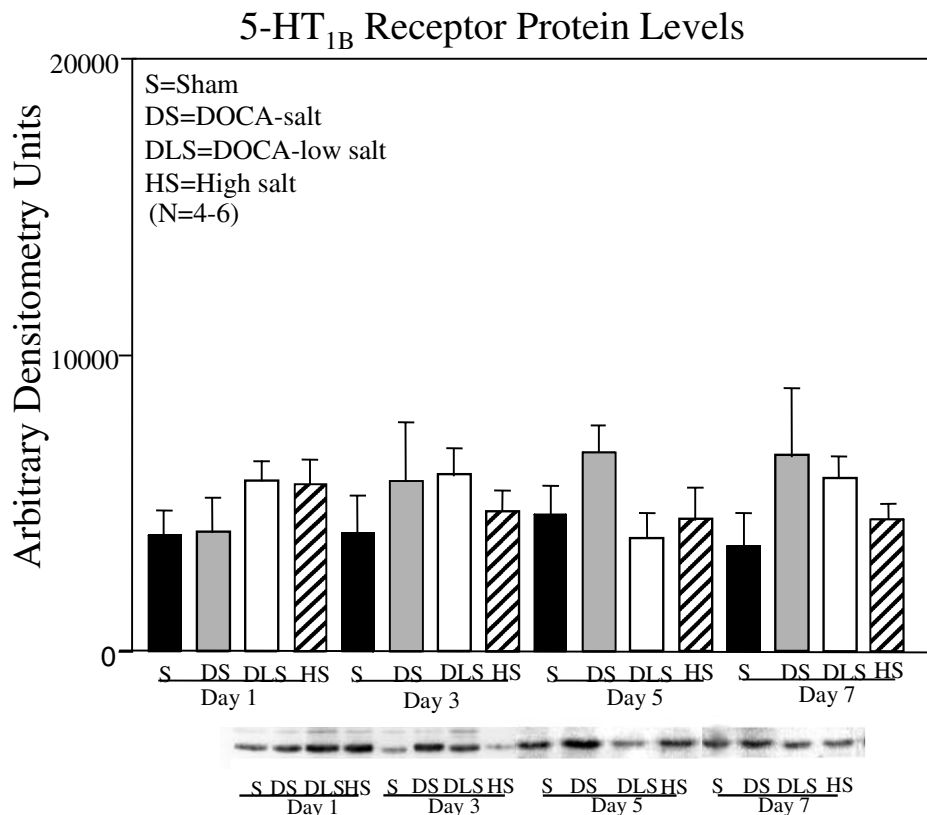


Figure 7

Top: Measurement of 5-HT_{1B} receptor protein density in aortic homogenates from Sham, DOCA-low salt, Sham-high salt and DOCA-high salt rats on days 1, 3, 5 and 7. Bottom: Representative western blot probed for the 5-HT_{1B} receptor protein. Units are reported as arbitrary densitometry units. Vertical bars represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the corresponding Sham. Abbreviations: S = Sham; DHS = DOCA-high salt; DLS = DOCA-low salt; SHS = Sham-high salt.

increased 5-HT_{2B} receptor [13], the aortic homogenates from the DOCA-low salt rats showed no increase in the level of 5-HT_{2B} receptor. These findings suggest that *in vivo* an increase in mineralocorticoids alone is not sufficient to upregulate the 5-HT_{2B} receptor. Upregulation of the 5-HT_{2B} receptor protein *in vivo* may require the elevated levels of salt, DOCA and pressure. On day one, arteries from the DOCA-salt rats with normal blood pressure contracted to BW723C86 but showed no increase in the level of the 5-HT_{2B} receptor protein.

Surprisingly, the *in vivo* time course experiments did not show any increase in the level of the 5-HT_{1B} receptor pro-

tein levels. These findings are also contrary to the *in vitro* data where aldosterone incubation produced an upregulation of these receptor protein levels [13]. In contrast to the data obtained on days 1, 3, 5 and 7, after 28 days of DOCA-high salt hypertension the 5-HT_{1B} and 5-HT_{2B} receptors are upregulated approximately 2-fold in the aorta from DOCA-high salt hypertensive rats [13]. These data suggest that *in vivo* regulation mechanisms of these receptors and their function maybe more complicated than the *in vitro* experiments suggested as elevated levels of mineralocorticoids alone *in vivo* was not sufficient to upregulate either the 5-HT_{1B} or the 5-HT_{2B} receptor proteins. However, as our studies did not address the issue of

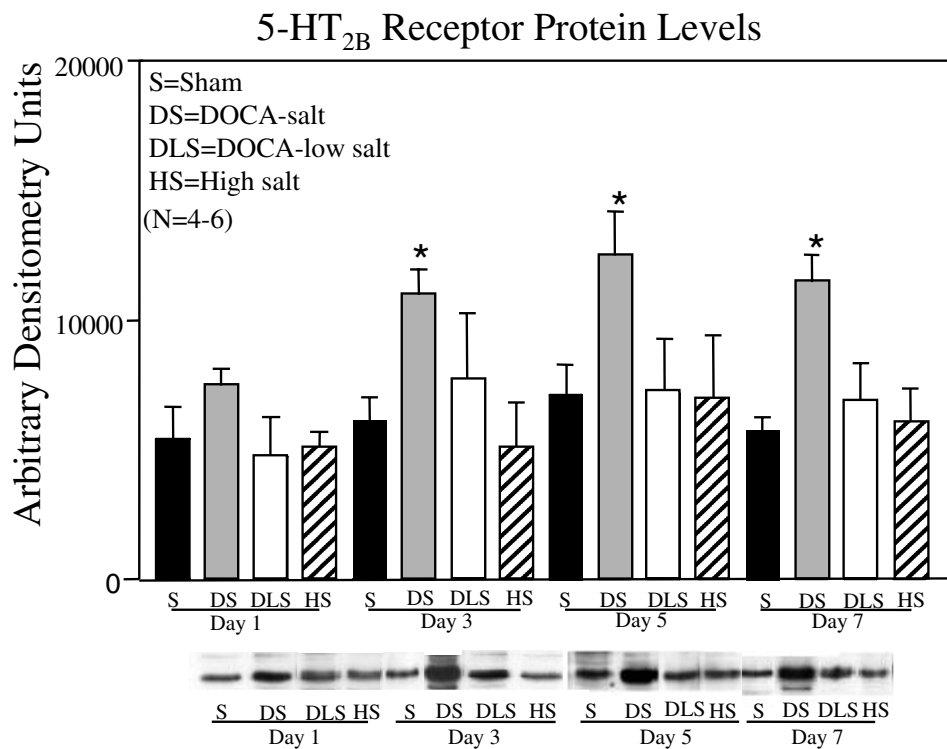


Figure 8

Top: Measurement of 5-HT_{2B} receptor protein density in aortic homogenates from Sham, DOCA-low salt, Sham-high salt and DOCA-high salt rats on days 1, 3, 5 and 7. Bottom: Representative western blot probed for the 5-HT_{1B} receptor protein. Units are reported as arbitrary densitometry units. Vertical bars represent the mean ± sem for the number of experiments indicated in parentheses. * represents statistically significant difference (p < 0.05) between the response obtained in the corresponding Sham. Abbreviations: S = Sham; DHS = DOCA-high salt; DLS = DOCA-low salt; SHS = Sham-high salt.

protein degradation, we can not rule out the possibility that there is an increase in the receptor proteins that is compensated for by an increase in protein degradation. Additionally, it is unknown at this point if the combination of elevated salt and mineralocorticoids affects the members of the signaling cascades used by the 5-HT_{1B} and 5-HT_{2B} receptors to enable enhanced contraction. This is an intriguing possibility since the ability of the 5-HT_{1B} and 5-HT_{2B} receptors to mediate contraction does not appear to be solely dependent on the level of the receptor protein.

Physiological Significance

The physiological importance of the 5-HT_{2B} receptor is emphasized by the finding that administration of the 5-HT_{2B} receptor antagonist LY272015, *in vivo*, decreased the blood pressure of hypertensive DOCA-salt rats and LNNA hypertensive rats [4,16]. Currently, studies using chronic *in vivo* administration of LY272015 to determine whether 5-HT_{2B} receptors are required for development of the increased blood pressure have not been done. Furthermore, the chronic administration of LY272015 would also address whether the 5-HT_{2B} receptor was involved in

Treatment Group	SBP mm Hg	- log EC ₅₀ value [M] for 5-HT Response	Maximal 5-HT Response	Maximal BW723C86 Response	Maximal CP93129 Response
Day 1					
Sham (7)	112 ± 2.4	5.95 ± 0.03	106.7 ± 5.5	7.7 ± 7.7	0
DOCA-high salt (7)	116 ± 3.4	5.87 ± 0.06	102.5 ± 8.4	52.5 ± 10.5	0
DOCA-low salt (4)	106 ± 2.5	6.00 ± 0.06	103.5 ± 0.5	16.8 ± 16.8	0
Sham-high salt (4)	107 ± 4.6	5.91 ± 0.03	104.3 ± 8.0	0	0
Day 3					
Sham (5)	110 ± 2.6	5.74 ± 0.05	93.2 ± 5.9	17.5 ± 11.0	0
DOCA-high salt (5)	124 ± 7.6	6.03 ± 0.08	110.1 ± 7.8	73.1 ± 9.1	37.1 ± 14.3
DOCA-low salt (4)	110 ± 2.3	5.71 ± 0.08	141.2 ± 11.8	0	0
Sham-high salt (4)	108 ± 8.3	6.20 ± 0.03	128.4 ± 6.3	46.7 ± 15.0	20.0 ± 12.2
Day 5					
Sham (5)	119 ± 2.5	5.60 ± 0.07	106.2 ± 8.7	18.5 ± 11.6	0
DOCA-high salt (5)	147 ± 14	5.98 ± 0.07	106.5 ± 7.7	54.3 ± 11.9	35.0 ± 8.6
DOCA-low salt (4)	105 ± 3.4	5.88 ± 0.06	110.9 ± 1.8	0	0
Sham-high salt (4)	120 ± 1.4	6.06 ± 0.05	148.5 ± 11.4	46.3 ± 6.6	27.8 ± 7.4
Day 7					
Sham (5)	114 ± 2.3	5.70 ± 0.07	90.4 ± 6.5	0	0
DOCA-high salt (5)	142 ± 8.4	6.41 ± 0.09	130.3 ± 19.5	57.4 ± 5.9	63.8 ± 7.8
DOCA-low salt (4)	101 ± 2.1	5.77 ± 0.08	110.1 ± 1.8	0	0
Sham-high salt (6)	130 ± 9.3	6.13 ± 0.03	119.5 ± 5.1	24.5 ± 9.4	19.6 ± 8.1

Figure 9

Systolic blood pressures (SBP), EC₅₀ values and the maximal contraction to 5-HT, BW723C86 and CP93129 represented as a percentage of phenylephrine (10 μM; PE) for Sham, Sham-high salt, DOCA-low salt and DOCA-high salt rats. Values represent the means ± standard error of the mean for each group. The number of animals, data from the data set used for the 5-HT graphs, in each group is indicated in parentheses. * represents statistical difference from response obtained in the Sham.

the smooth muscle cell hypertrophy and hyperplasia observed in hypertension. *In vivo* studies with the 5-HT_{1B} receptor antagonists in the model of DOCA-salt hypertension have not yet been performed. However, in the model of pulmonary hypertension, infusion of the 5-HT_{1B} receptor antagonist GR127935 (3 mg/kg/day) attenuated the increased right ventricular pressure, right ventricular hypertrophy and pulmonary vascular remodeling in the hypertensive rats [12]. These findings suggest that both the 5-HT_{2B} and 5-HT_{1B} receptors and their involvement in cardiovascular diseases, such as hypertension, merit further study.

Additional support for the importance of 5-HT_{2B} receptors in the cardiovascular system comes from 5-HT_{2B} receptor knockout mice. 5-HT_{2B} receptor knockout mice show severe cardiovascular abnormalities [18–20]. Since 5-HT_{2B} receptors are required for morphogenesis and cell migration, one can speculate that 5-HT_{2B} receptors may be important to the vascular changes observed in arteries during the development of hypertension. No studies have been done in the 5-HT_{1B} receptor knockout mice examining the progression of systemic cardiovascular diseases.

Speculations on Alternative Functional Changes

These studies were the first and an important step into understanding whether an upregulation of the 5-HT_{1B} and

5-HT_{2B} receptor proteins was sufficient to enable contraction to agonists of these receptors. While the lack of increase in the level of the 5-HT_{1B} and 5-HT_{2B} receptors to correspond with the functional changes may suggest nonspecific actions for CP93129 and BW723C86, there is evidence to refute this idea. BW723C86 possesses pK_i values of 6.2 at the 5-HT_{1B} receptor, 6.6 at the 5-HT_{2A} receptor and 7.9 at the 5-HT_{2B} receptor (21). There is currently no published information about the ability of CP93129 to interact with the 5-HT_{2B} receptor, one possible nonspecificity. However, it is known that contractions mediated by CP93129 can be inhibited with the 5-HT_{1B} receptor antagonist GR55562 (11). The ability of the 5-HT_{2B} and 5-HT_{1B} receptors to participate in contraction without a concomitant upregulation of the receptor protein levels suggest that changes in the signaling cascades and receptor/effector coupling are potentially involved in this enhanced contractility to 5-HT and agonists of the 5-HT_{2B} and 5-HT_{1B} receptors under conditions of DOCA-salt hypertension. Currently, the ability to track changes in 5-HT_{2A} receptor function is difficult due to limited tools available which includes a lack of 5-HT_{2A} receptor specific agonists and antibodies.

The mechanisms by which elevated levels of salt and pressure participate in the pathogenic changes in receptor function observed in hypertension are still unclear and were not the focus of this study. They are, however, an area of future investigation. Future avenues of research also include investigation of the Rho-Rho-kinase pathway as well as the acute *vs* chronic effects of increased pressure on 5-HT receptor subtypes.

Conclusion

We initially speculated that upregulation of 5-HT_{1B} and 5-HT_{2B} receptor proteins were, at least partially, responsible for the increased contractility to 5-HT seen in the arteries from hypertensive rats. However, the results of the DOCA-salt time course studies do not support this idea. Instead, these data support the argument that an upregulation of 5-HT_{1B} and 5-HT_{2B} receptors are not necessary to observe an increased contractile response to agonists of these receptors. Additionally, there was an increased contractile response to the 5-HT_{2B} receptor agonist BW723C86 prior to an increase in blood pressure in the DOCA-high salt and Sham-high salt treated rats. Interestingly, the hyperresponsiveness to 5-HT characteristic of hypertensive arteries was observed in Sham-high salt and DOCA-high salt arteries starting at day three. Collectively, the data presented herein suggest that 5-HT_{2B} receptors may be involved in the development of DOCA-salt hypertension. However, the precise role of these receptors in the development of hypertension and possibly vascular remodeling are yet to be elucidated.

Methods

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

Surgical Procedures and Systolic Blood Pressure Measurement

All rats used were adult male Sprague-Dawley rats (0.20–0.25 kg; Charles River Laboratories, Inc., Portage, MI). The rats were separated into four treatment groups: DOCA-high salt, DOCA-low salt, Sham and Sham-high salt. The DOCA-high salt and DOCA-low salt were given a subcutaneous silastic implant impregnated with DOCA (200 mg/kg) and were uninephrectomized (left side, flank incision) under isoflurane (IsoFlo, Abbott Laboratories, N. Chicago, IL) anesthesia. Sham and Sham-high salt rats did not receive an implant but were uninephrectomized. Postoperatively, rats in the DOCA-high salt and Sham-high salt groups received drinking water containing 1.0% NaCl and 0.2% KCl. Sham and DOCA-low salt rats received normal tap water. Animals in the DOCA-high salt, Sham-high salt and Sham groups were fed a diet of standard rat chow and received *ad libitum* access to both food and water. Rats in the DOCA-low salt group were fed a diet of chow containing .002 μEq NaCl/g. A baseline blood pressure and on days 1, 3, 5 and 7 the systolic blood pressures were measured using the standard tail cuff method. Daily fluid intake was measured by giving a standard amount of fluid and using a graduated cylinder to ascertain the amount required to return to the initial amount.

Isolated tissue bath procedure

The thoracic aorta 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 mM) NaCl, 103; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄•7H₂O, 1.17; CaCl₂•2H₂O, 1.6; 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 (1500 mg, determined previously). After a 1 hour equilibration, arteries were challenged with phenylephrine (10⁻⁵ M). Tissues were then washed and the lack of an intact endothelium was validated by observing the inability of acetylcholine (10⁻⁶ M) to relax arteries contracted with a half-maximal concentration of phenylephrine. Cumulative concentration response curves to agonists were performed. As there was no significant difference in the contractile response to PE (10⁻⁵ M) in any group at any day (data not shown) all the data is normalized and reported as a percentage of the PE (10⁻⁵ M) contraction.

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, 10% glycerol] with protease inhibitors (0.5 mM PMSF, 10 µg/ml aprotinin and 10 µg/ml leupeptin). Homogenates were centrifuged (11,000 g for 10 minutes, 4 °C) and supernatant total protein measured (BCA, Sigma, St. Louis, MO).

Immunoblotting protocol

Supernatant (50 µg total protein per lane, 4:1 in denaturing loading buffer, boiled 5 minutes) was loaded, separated on 10% denaturing SDS-polyacrylamide gels, and transferred to Immobilon-P membranes. Membranes were blocked for 3–4 hours in Tris buffer saline + Tween-20 (0.1%; TBS-T) containing 4% chick egg ovalbumin and 0.025% sodium azide. Mouse anti-5HT_{2B} receptor antibody (.5 µg/ml, Pharmingen, San Diego, CA) or guinea pig anti-5-HT_{1B} receptor antibody (1:1000, Chemicon, Temecula, CA) were incubated with blots overnight (4 °C). Following washes, secondary antibody linked to horseradish peroxidase [anti-mouse (1:10,000, Amersham Laboratories, Arlington Heights, IL) or anti-guinea pig (1:10,000 Chemicon, Temecula, CA)] was added for one hour and incubated with blots at 4 °C. Enhanced chemiluminescence was performed using standard reagents (Amersham Laboratories, Arlington Heights, IL). Each blot was washed and redeveloped using an α-smooth muscle actin antibody (1:400, Oncogene Research Products, Boston, MA; anti-mouse secondary antibody, 1:5,000, Amersham Laboratories, Arlington Heights, IL). Equal lane loading of protein was ensured by comparing α-smooth muscle actin densitometry.

Data analysis and statistics

Data are presented as means ± standard error of the mean for the number of animals in parentheses. When comparing more than two groups, either a one-way ANOVA was performed followed by a Student-Newman-Keuls post hoc test or a two-way ANOVA was used, as was appropriate. The systolic blood pressure data was analyzed with repeated measures. In all cases, a p value less than or equal to 0.05 was considered statistically significant.

Materials

Acetylcholine chloride, phenylephrine hydrochloride, DOCA and 5-hydroxytryptamine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). BW723C86 was purchased from Tocris (Ballwin, MO). CP93129 was a gift from Pfizer (Groton, CT, USA).

Authors' contributions

Studies were performed and analyzed primarily by AKLB. SWW assisted with study design and analysis.

Acknowledgements

This work was supported by the NIH grant HL58489 (SWW), an Atorvastatin Award (SWW) and a grant from the American Heart Association Award 0010194z (AKLB).

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