5-HT$_{2B}$-receptor antagonist LY-272015 is antihypertensive in DOCA-salt-hypertensive rats

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Watts, Stephanie W., and Gregory D. Fink. 5-HT$_{2B}$-receptor antagonist LY-272015 is antihypertensive in DOCA-salt-hypertensive rats. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H944–H952, 1999.—We previously demonstrated a change in the receptors mediating 5-hydroxytryptamine (5-HT)-induced contraction in arteries of deoxycorticosterone acetate (DOCA)-salt-hypertensive rats. Specifically, contraction to 5-HT is mediated primarily by 5-HT$_{2A}$ receptors in arteries from normotensive sham rats and by both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors in arteries from hypertensive rats. We hypothesized that the 5-HT$_{2B}$ receptor may play a role in maintaining the high blood pressure of DOCA-salt-hypertensive rats, and herein we provide data connecting in vitro and in vivo findings. The endothelium-denuded isolated superior mesenteric artery of DOCA-salt rats displayed a marked increase in maximum contraction to the newly available 5-HT$_{2B}$-receptor agonist BW-723C86 compared with that of arteries from sham rats, confirming that the 5-HT$_{2B}$ receptor plays a greater role in 5-HT-induced contraction in arteries from DOCA-salt rats. In chronically instrumented rats, the 5-HT$_{2B}$-receptor antagonist LY-272015 (0.3, 1.0, and 3.0 mg/kg iv at 30-min intervals) was given cumulatively 1 timewk during 4 wk of continued DOCA-salt treatment. LY-272015 did not reduce blood pressure of the sham-treated rats at any time or dose. However, LY-272015 (1.0 and 3.0 mg/kg) significantly reduced mean blood pressure in a subgroup of week 3 (20 mmHg) and week 4 DOCA-salt (40 mmHg) rats that had extremely high blood pressure (mean arterial blood pressure 200 mmHg). Blockade of 5-HT$_{2B}$ receptors by in vivo administration of LY-272015 (3.0 mg/kg) was verified by observing reduced 5-HT-induced contraction in rat stomach fundus, the tissue from which the 5-HT$_{2B}$ receptor was originally cloned. These data support the novel hypothesis that 5-HT$_{2B}$-receptor expression is induced during the development of DOCA-salt hypertension and contributes to the maintenance of severe blood pressure elevations.

5-hydroxytryptamine; deoxycorticosterone acetate; experimental hypertension; vasoconstriction; serotonin receptors

MULTIPLE STUDIES have investigated serotonin [5-hydroxytryptamine (5-HT)]-receptor antagonists, and in particular the 5-HT$_{2A}$-receptor antagonist ketanserin, for their effects on high blood pressure (4, 22, 31, 32). One of the reasons this has been done is that 5-HT is markedly more potent in stimulating contraction in vasculature isolated from several different animal models of experimental and/or genetic hypertension compared with vasculature isolated from animals with normal blood pressure (6, 14, 20, 23, 25, 29). These studies raise the question as to whether the dramatic increase in sensitivity to 5-HT could play a role in hypertension. Results of whole animal studies that used ketanserin have largely been negative in that ketanserin has not been effective in lowering blood pressure, or the antihypertensive effects of ketanserin could be attributed to its ability to block $\alpha_1$-adrenergic receptors (4, 22, 31, 32).

We have studied 5-HT in hypertension by focusing on one model, the deoxycorticosterone acetate (DOCA)-salt-hypertensive rat. Arterial smooth muscle from the DOCA-salt-hypertensive rat displays pronounced increases in sensitivity to 5-HT, although arteries from most models of hypertension show some increase in responsiveness to 5-HT (6, 14, 20, 23, 25, 29). Our laboratory previously reported that part of this increase in arterial sensitivity to 5-HT is due to a change in the receptor population that mediates contraction to 5-HT under conditions of DOCA-salt hypertension. Specifically, Watts and colleagues (35–38) presented pharmacological and molecular evidence that a 5-HT$_{2A}$-receptor population (ketanserin sensitive) primarily mediates contraction in arteries from normotensive rats, and a 5-HT$_{2B}$-receptor population (relatively ketanserin insensitive) primarily mediates arterial contraction in DOCA-salt hypertension. This “switch” is important because 5-HT possesses 300–1,000 times higher affinity for the 5-HT$_{2B}$ receptor compared with the 5-HT$_{2A}$ receptor (33). Thus lower concentrations of 5-HT are necessary to activate the 5-HT$_{2B}$ receptor. Moreover, this finding makes important the reexamination of hypertension with new pharmacological tools that block the 5-HT$_{2B}$ receptor, because the serotonergic antagonist most frequently tested has been ketanserin, and ketanserin possesses 1,000 times lower affinity for the 5-HT$_{2B}$ receptor compared with the 5-HT$_{2A}$ receptor (33). Thus we presently test the hypothesis that an increase in 5-HT$_{2B}$-receptor activation plays a role in maintaining high blood pressure in DOCA-salt hypertension. If correct, then a 5-HT$_{2B}$-receptor antagonist such as the recently developed LY-272015 (1, 5) should reduce elevated blood pressure. 5-HT may then be included among the other substances already known to participate in the pathophysiology of this model of hypertension (endothelin-1, vasopressin, and norepinephrine). By means of connecting findings from in vivo experiments to our past in vitro experiments, we provide in vitro and ex vivo evidence that a 5-HT$_{2B}$ receptor mediates 5-HT-induced arterial contraction in DOCA-salt hypertension.

METHODS
All animal procedures followed were in accordance with institutional guidelines of Michigan State University.
Surgery: DOCA-salt rats. Male Sprague-Dawley rats (300–350 g; Charles River, Portage, IN) underwent uninephrectomy (flank incision, left side) under Metofane anesthesia. In the same surgery, a Silastic implant impregnated with DOCA (200 mg/kg) was placed subcutaneously between the shoulder blades. Sham rats were also uninephrectomized but received no implant. All rats receiving DOCA were given water containing 1.0% NaCl and 0.2% KCl (salt water); sham rats received tap water. All animals were fed standard rat chow and had ad libitum access to both food and water. Some animals were allowed to continue on DOCA-salt therapy for 4 wk before tissue isolation, while others were taken into the catheterization protocol (described in Surgery-catheterization).

Isolated tissue bath protocol. Rats were euthanized (80 mg/kg pentobarbital sodium ip), and the superior mesenteric artery, thoracic aorta, or stomach fundus was removed. Arteries were dissected into helical strips (mesenteric artery, 0.7 × 10 mm; aorta, 1 × 10 mm), and the endothelial cell layer was removed by rubbing the luminal side of the vessel with a moistened cotton swab [the endothelial cell layer does not influence arterial responsiveness to the 5-HT-receptor agonists being examined (data not shown)]. Two longitudinal strips from the stomach fundus were dissected, and fundus from only sham rats was used. Tissues were placed in physiological buffer for measurement of isometric contractile force with standard bath procedures. Physiological salt solution contained (in mM) 130 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4·7H2O, 1.6 CaCl2·H2O, 14.9 NaHCO3, 5.5 dextrose, and 0.03 CaNa2-EDTA, pH 7.2. Tissues from DOCA-salt and sham rats were placed in the same glass tissue bath. One end of the preparation was attached to a stainless steel rod, and the other was attached to a force transducer (FT03, Grass Instruments, Quincy, MA) and placed under optimum resting tension (previously determined: mesenteric artery, 600 mg; aorta, 1,500 mg; and stomach fundus, 4,000 mg). Muscle baths were filled with warmed (37°C), aerated (95% O2-5% CO2) physiological salt solution. Changes in isometric force were recorded on a Grass polygraph (Grass Instruments).

After 1 h of equilibration with washes every 15 min, arteries were challenged with phenylephrine (10−10 M), and fundi were challenged with KCl (100 mM). All tissues were washed until responses returned to baseline. In arteries, we examined the status of the endothelium by observing arterial relaxation to the muscarinic agonist ACh (1 × 10−6 M) in tissues contracted with a concentration necessary for a half-maximal response (EC50) to the α1-adrenergic-receptor agonist phenylephrine (−1 × 10−6 M). All tissues were washed and taken through one of the following protocols.

Mesenteric artery experiments. These tissues were taken from animals that were not catheterized and went through a normal 4 wk of DOCA-salt treatment. Cumulative concentration-response curves to agonists (5-HT and BW-723C86) were performed. Tissues were exposed to both agonists with a 30-min washout period between agonists.

Surgery-catheterization. This surgery took place 3 days after DOCA-salt treatment was begun. Catheters were constructed of polyvinyl chloride with silicone rubber tips and advanced to the abdominal aorta and vena cava via the left femoral artery and vein in rats anesthetized with pentobarbital sodium (50 mg/kg ip). The ends of the catheters were tunneled subcutaneously to the head, where the catheters were stabilized to the skull with the use of jeweler’s screws and dental acrylic. Catheter ends were passed through a stainless steel spring attached to a plastic swivel, through which infusions were given. On regaining consciousness, rats were housed singly in stainless steel cages in a climate-controlled room with a 12:12-h light-dark cycle.

In vivo experiments. In experiments determining whether LY-272015 can reverse DOCA-salt hypertension, LY-272015 (0.3, 1, and 3 mg/kg iv) was given to conscious rats in a cumulative fashion over 90 min. This was done 1 timelw during 4 wk of developing DOCA-salt hypertension; thus responsiveness to LY-272015 was measured in the same rats during weeks 1–4. Mean arterial blood pressure and heart rate were monitored before, during, and for 30 min after each injection with a computerized DigiMed system. In week 4, some animals were given the 5-HT2A-receptor agonist α-methyl-5-HT before and after the 5-HT2A-receptor antagonist ketanserin (1 mg/kg iv).

Ex vivo experiments. These experiments were performed with the purpose of demonstrating that the dosing protocol of the rats with LY-272015 was sufficient for the drug to exert the appropriate pharmacological blockade (e.g., blockade of 5-HT2A receptors). In the conscious state, animals (both sham and DOCA-salt) were given the highest dose of LY-272015 (3.0 mg/kg) or vehicle. After 30 min, animals were euthanized with pentobarbital sodium, and the aorta or stomach fundus was removed and taken through the isolated tissue bath protocol as described but with a few modifications. The 30-min time period was chosen because this was the maximal time each dose of LY-272015 was allowed to equilibrate in the in vivo experiments. The modifications of the tissue bath experiments included the following: the equilibration of the tissue was only 30 min, and the tissues were washed only once during this time to minimize the washing out of LY-272015 from the tissues. Arteries, the luminal sides of which were rubbed with a cotton swab, were initially challenged with phenylephrine, and the fundus was challenged with KCl, but the arterial endothelial status was not examined to minimize time between removal of the tissues from the animal and exposure to 5-HT. All tissues were washed three times after initial challenges to remove the stimulus and allowed to resume baseline tension. Cumulative concentration-response curves to 5-HT were performed to examine the status of receptor blockade.

Data analysis. Contractile data are presented as means ± SE for n animals and are reported as milligrams or as a percentage of the initial phenylephrine (10−5 M) contraction. Blood pressure is reported as mean arterial blood pressure (mmHg) or a change in mean arterial blood pressure (ΔmmHg). Unpaired or paired Student’s t-tests were used when appropriate in comparing two group responses, and repeated-measure ANOVA followed by Tukey’s post hoc test was used when responses of three or more measurements were compared in the same group of animals (P < 0.05 was considered significant). The agonist EC50 values were calculated with a nonlinear regression analysis using the algorithm [effect = [maximal response − (EC50/agonist concentration)]].

Materials. Compounds were prepared for use in deionized water unless indicated otherwise. ACh chloride, DOCA, 5-HT hydrochloride, and α-methyl-5-HT hydrochloride were from Sigma Chemical (St. Louis, MO); ketanserin tartrate and ketanserin were from Research Biochemicals (Natick, MA). BW-723C86 was from Dr. Doug Bonhaus (Roche BioScience, Palo Alto, CA), and LY-272015 was graciously provided by Dr. Jim Audia (Eli Lilly; Indianapolis, IN).

RESULTS

The first set of experiments examined contraction caused by 5-HT and the 5-HT2A-receptor agonist BW-723C86 in arteries from DOCA-salt-hypertensive and...
sham normotensive rats. Noninstrumented sham and DOCA-salt rats that had been given DOCA and salt for 4 wk were used in these experiments. The mean systolic blood pressures for the eight DOCA rats and for the sham rats were 204 ± 15 and 123 ± 4 mmHg, respectively (P < 0.05). In these experiments, we used the endothelium-denuded superior mesenteric artery. Figure 1A demonstrates the dramatic decrease in threshold to 5-HT, increase in potency, and increase in maximal response to 5-HT in arteries from DOCA-salt rats compared with arteries from sham rats. These data are reported as a percentage of an initial phenylephrine (10⁻⁵ M) contraction (sham 442 ± 67 mg and DOCA-salt 336 ± 33 mg) that was not significantly different (P > 0.05, 1-way Student’s t-test) between the two groups of animals. The \(\text{logEC}_{50}\) values (in M) for 5-HT were 5.82 ± 0.16 in sham arteries and 6.98 ± 0.24 in the DOCA-salt group (P < 0.05). Arteries from DOCA-salt rats were also significantly hyperresponsive to the 5-HT₂B-receptor agonist BW-723C86 (Fig. 1B). \(\text{EC}_{50}\) values were difficult to measure in the sham arteries because the response was so poor. However, a \(\text{logEC}_{50}\) value (in M) for BW-723C86 in DOCA-salt rats could be measured and was 7.21 ± 0.11. Moreover, maximal contraction to BW-723C86 was dramatically increased in the DOCA-salt rat arteries (in percentage of phenylephrine contraction, sham 24.9 ± 9.1% and DOCA-salt 79.5 ± 12.7%). The 5-HT₂B-receptor antagonist LY-272015 (50 nM) shifted BW-723C86-induced contraction in arteries from DOCA-salt rats 30-fold to the right (data not shown). These data confirm and extend our previous findings that it is likely that the vascular 5-HT₂B-receptor population is increased in DOCA-salt hypertension and, in part, mediates contraction to 5-HT.

We next moved to experiments designed to test the hypothesis at hand. Is the increase in functional vascular 5-HT₂B-receptor expression physiologically important and does this change play a role in the hypertension experienced by DOCA-salt rats? Rats were chronically instrumented for measurement of blood pressure and heart rate throughout 4 wk of DOCA-salt therapy. Each animal’s response to the 5-HT₂B-receptor antagonist LY-272015 was followed week to week. Figure 2A displays weekly mean arterial blood pressure and heart rate (Fig. 2B) for the animals in this study. Although blood pressure and heart rate did not increase over the 4 wk of testing in sham rats, it became clear that there were subpopulations of DOCA-salt rats. Specifically there was a group that developed moderate hypertension (DOCA-M) and then a group that developed hypertension at a more rapid or severe rate (DOCA-S). These two groups of DOCA-salt animals were distinguished by differences in heart rate levels throughout the experiment and segregated as such in terms of their responsiveness to LY-272015. The heart rate of DOCA-S animals was significantly elevated in weeks 3 and 4 compared with that of the DOCA-M group (Fig. 2B). We do not know the reason for this difference.

The weekly responses of these three groups (sham, DOCA-M, and DOCA-S) to increasing doses of the 5-HT₂B-receptor antagonist LY-272015 are shown in Figs. 3 and 4. During weeks 1 and 2, LY-272015 did not cause a reduction in blood pressure in any of the three groups (Fig. 3). However, by week 3, the DOCA-S group tended to show a reduced blood pressure on administration of 1.0 mg/kg LY-272015 and clearly responded to the highest dose of LY-272015 with a blood pressure drop of \(\pm 20\) mmHg (Fig. 4A). This is in significant contrast to the DOCA-M group, which displayed a mild pressor response to LY-272015. This trend of increasing sensitivity to LY-272015 in the DOCA-S group became more marked in week 4: 1.0 mg/kg LY-272015 reduced blood pressure, and 3 mg/kg caused a significant fall in blood pressure (>40 mmHg) after 30 min (Fig. 4B).
Thus these data suggest that it is in the later stages of hypertension that the 5-HT$_{2B}$ receptor plays some role in supporting severely elevated blood pressure.

Further support for an increased activity of vascular 5-HT$_{2B}$ receptors in the DOCA-s rat is that in DOCA-s rats, there was a residual pressor response to the 5-HT$_{2A}$-receptor agonist $\alpha$-methyl-5-HT after 5-HT$_{2A}$-receptor blockade with ketanserin (Fig. 5). This residual response could be abolished by LY-272015. Moreover, this LY-272015-inhibitable pressor response to $\alpha$-methyl-5-HT was not present in sham or DOCA-M rats.

To test whether the effects of LY-272015 were due to $\alpha_1$-adrenergic-receptor blockade, the highest dose (3.0 mg/kg) of LY-272015 was examined against a pressor response to the $\alpha_1$-adrenergic-receptor agonist phenylephrine in sham rats. Figure 6 demonstrates that LY-272015 at this dose does not interact with $\alpha_1$-adrenergic receptors because LY-272015 did not alter the pressor response to phenylephrine.

We performed ex vivo experiments on some of the animals that went through the catheterization protocol to demonstrate that 5-HT$_{2B}$-receptor blockade occurred in animals receiving LY-272015. Animals (sham or DOCA-salt) were given vehicle or the highest dose of LY-272015 (3.0 mg/kg iv). Thirty minutes later, animals were euthanized, and their aorta or stomach fundus was removed. The aorta from a sham rat given LY-272015 was used to demonstrate a lack of blockade on 5-HT$_{2A}$ receptors (3), whereas the stomach fundus was used as a preparation in which 5-HT-induced contraction is mediated by 5-HT$_{2B}$ receptors. Finally, aorta from DOCA-salt rats that displayed a decrease in blood pressure to LY-272015 was taken to examine whether vascular contraction to 5-HT was reduced. Concentration-dependent contraction to 5-HT in the stomach fundus of animals not given LY-272015 could be observed but was not observed in the stomach fundus of rats given LY-272015 (Fig. 7). Fundal strips had similar responses to KCl at the beginning of the experiment, indicating that the strip, the response of which is depicted in Fig. 7B, was viable. These experiments

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**Fig. 2.** Mean arterial blood pressure (MAP; A) and heart rate (B) of sham (control), moderately hypertensive DOCA rats (DOCA-M), and severely hypertensive DOCA rats (DOCA-S). Data are means ± SE for no. of animals indicated in parentheses. *Significant difference (P < 0.05) from sham data. † Significant difference (P < 0.05) between DOCA-S and DOCA-M groups.

**Fig. 3.** Week 1 (A) and week 2 (B) responses of control, DOCA-M, and DOCA-S rats to dose-dependent increases in 5-HT$_{2B}$-receptor antagonist LY-272015 (LY; 0.3–3.0 mg/kg). Data are means ± SE for no. of animals indicated in parentheses.
support a significant blockade of 5-HT<sub>2B</sub> receptors in our experiments. This dose of LY-272015 did not block 5-HT<sub>2A</sub> receptors because 5-HT-induced contraction in aorta from sham rats was not altered by in vivo treatment with LY-272015 (0.3–3.0 mg/kg). Data are means ± SE for no. of animals indicated in parentheses. *Significant difference (P < 0.05) from control MAP.

**DISCUSSION**

The 5-HT<sub>2</sub>-receptor family comprises three subtypes, the 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> subtypes (13). The 5-HT<sub>2B</sub> receptor, first called an atypical 5-HT receptor (30), was originally described by Vane (30) as a highly sensitive 5-HT receptor in the longitudinal smooth muscle of the rat stomach fundus. Once cloned and sequenced, it was recognized that the receptor belonged in the 5-HT<sub>2</sub>-receptor family (11, 17, 26, 33, 34). Ketanserin, a classical 5-HT<sub>2</sub>-receptor antagonist, has a low affinity for the 5-HT<sub>2B</sub> receptor and a high affinity for the 5-HT<sub>2A</sub> receptor. Because of the significantly higher affinity of 5-HT for the 5-HT<sub>2B</sub> receptor compared with the 5-HT<sub>2A</sub> receptor, we suggested the involvement of this relatively new receptor in the increase in vascular reactivity to 5-HT observed in hypertension (35–38). We have previously demonstrated support for this novel hypothesis at the level of the vasculature in vitro and now connect these data with in vitro, in vivo, and

**Fig. 4.** Week 3 (A) and week 4 (B) responses of control, DOCA-M, and DOCA-S rats to dose-dependent increases in 5-HT<sub>2B</sub>-receptor antagonist LY-272015 (0.3–3.0 mg/kg). Data are means ± SE for no. of animals indicated in parentheses. *Significant difference (P < 0.05) from control MAP.

**DISCUSSION**

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**Fig. 5.** Pressor response of control (●) and DOCA rats (□, DOCA-M; △, DOCA-S) to 5-HT<sub>2</sub>-receptor agonist α-methyl-5-HT in absence (A) and presence (B) of ketanserin (1 mg/kg iv). ▲, Response to 100 µg/kg α-methyl-5-HT after ketanserin and LY-272015 (3.0 mg/kg iv); ΔMAP, change in MAP. Data are means ± SE. *Significant difference from responses in control animals. + Significant difference from 100 µg/kg response of DOCA-S rats.

**Fig. 6.** Change in MAP of normotensive sham rats to α<sub>1</sub>-adrenergic agonist PE in absence (control) and presence of 5-HT<sub>2B</sub>-receptor antagonist LY-272015 (3.0 mg/kg). Data are means ± SE for no. of animals indicated in parentheses.
The results of the contractility experiments presented within this study confirm and extend our previous findings: 5-HT$_{2B}$-receptor signaling is upregulated in DOCA-salt hypertension (35–38). We have not measured actual 5-HT$_{2B}$-receptor protein levels in arteries from DOCA-salt and sham rats but have done a qualitative comparison of 5-HT$_{2B}$-receptor mRNA in mesenteric arteries from DOCA-salt and sham rats (37). We observed a two- to threefold increase in the 5-HT$_{2B}$-receptor mRNA in the arteries from DOCA-salt rats compared with those in the sham rats. The message was measurable in the sham arteries so the receptor may be present, but the 5-HT$_{2B}$ receptor clearly does not serve 5-HT-induced contraction in endothelium-denuded arteries from a normotensive rat. All of our work has been performed with the use of arteries in which the endothelium has been removed. The reasons for doing this were to simplify interpretation of the data and address primarily vascular smooth muscle cell signaling. There are reports describing 5-HT-induced vascular relaxation via an endothelial 5-HT$_{2B}$ receptor (10, 12). It has been postulated that an endothelial 5-HT$_{2B}$ receptor in the meninges may play a role in migraine (27). One can speculate that the slight pressor response to LY-272015 in the sham and moderately hypertensive DOCA-salt rats in week 4 (Fig. 4) may reflect blockade of such an endothelial receptor. Change in this endothelial receptor population and/or activation under conditions of hypertension would indeed be interesting to investigate but was not the focus of the present study.

The intent of this project was to test the hypothesis that an increase in the vascular expression of a 5-HT receptor more sensitive to 5-HT plays a role in the hypertension caused by treatment with DOCA-salt. We
found two different levels of hypertension produced by DOCA-salt treatment in rats. Both groups clearly had an increased blood pressure compared with that of the sham rats, but the absolute level of blood pressure attained by the rats in the two groups differed. One group developed hypertension in a more moderate fashion, and, by week 4, mean arterial blood pressure was ~150 mmHg. By contrast, the other group developed hypertension more rapidly and attained a much higher mean blood pressure in week 4 (close to 200 mmHg). We have had a difficult time elucidating the cause of this different response to DOCA and salt. The animals were the same age and weight at the beginning of the experiment, were all from the same supplier, and were treated as identically as possible. One physiological parameter that was different between these two DOCA groups was heart rate, and we have used this parameter as a means by which to segregate animals that did or did not respond to LY-272015. In week 2, heart rate was elevated in the severely hypertensive rats and stayed elevated throughout the 4 wk of the experiment. The cause of this difference is unknown, but it was this more rapid development of high blood pressure and high heart rate in week 2 that was the criterion for grouping the animals in the DOCA-M and DOCA-S subsets post hoc. The systolic blood pressures for the DOCA-salt rats from which data in Fig. 1 were produced ranged from virtually normotensive values (136 mmHg) to 152 mmHg to >250 mmHg, thus including animals from both the DOCA-S and DOCA-M group. The data from the contractile experiments are tight for a group of rats in which blood pressures covered quite a wide range. Thus there is no obvious graded scale between enhanced vascular reactivity to either 5-HT or the 5-HT2B-receptor agonist BW-723C86 and systolic blood pressure. In other words, using in vitro techniques, we cannot pick out those rats that would have responded in vivo to LY-272015. This raises concerns as to whether the change in vascular contraction can thus be truly associated with hypertension, and we address this concern in Speculations.

LY-272015 is a tetrahydro-β-carboline recently developed as a 5-HT2B-receptor antagonist (1, 5). This serotonergic receptor antagonist was an antihypertensive agent in those animals that had severely elevated blood pressure. The effects of LY-272015 were not observed until week 3, indicating that the magnitude of the increase in 5-HT2B-receptor signaling only then became sufficient to affect overall blood pressure regulation in vivo. Watts (35) previously published a study investigating the time course over which 5-HT2B-receptor expression was increased in arterial tissue from DOCA-salt rats. Those pharmacological data showed that an increase in sensitivity to 5-HT is measurable by day 5, and decreased antagonism by ketanserin, a 5-HT2A-receptor antagonist with low affinity for the 5-HT2B receptor, is measurable by day 7. Thus the vascular changes as measured in vitro occur during the first 2 wk of DOCA-salt treatment. The present data suggest that in the severely hypertensive rats, this change becomes physiologically important starting in week 3 because this was the first time at which LY-272015 (3.0 mg/kg) reduced DOCA-salt blood pressure. The fall in blood pressure in week 4 is dramatic, and blood pressure of the DOCA-S rats remained low for over 6 h after the highest dose of LY-272015 was given (unpublished observations). How does LY-272015 cause this fall in blood pressure?

We postulate that it does so through blocking vascular 5-HT2B receptors. Ex vivo experiments support that the 5-HT2B receptor is functionally blocked in these experiments and that LY-272015 can reduce 5-HT-induced arterial contraction in DOCA-salt-hypertensive but not sham rats. Furthermore, in DOCA-S rats, acute pressor responses to α1-methyl-5-HT persisted after blockade of 5-HT2A receptors, and these responses could be antagonized by LY-272015, suggesting a non-5-HT2A-receptor component. Rapid pressor effects of this kind are almost surely mediated by direct vascular contraction. It is unlikely that the antihypertensive effects of LY-272015 can be attributed to α1-adrenergic-receptor blockade because the highest dose of LY-272015 did not shift or reduce dose-dependent pressor responses to the α1-adrenergic-receptor agonist phenylephrine (Fig. 6). A nonselective effect on α1-adrenergic receptors has been the greatest criticism of 5-HT-receptor antagonists as antihypertensives, and the criticism is partially warranted for drugs such as ketanserin (4, 22). However, this does not appear to be the case for LY-272015. At this point, our hypothesis is that the effects of LY-272015 are vascular in nature, but we cannot rule out the possibility that LY-272015 may have central effects. There is a controversy as to whether 5-HT2B receptors are expressed in rat brain. Some groups have been unable to find mRNA for the 5-HT2B receptor (2), whereas others have shown that modulators of the 5-HT2B receptor can affect centrally mediated responses such as anxiety (9).

Speculations. Induction of receptors in disease states is a concept recently receiving greater attention (8). An increased expression of a vascular smooth muscle 5-HT2B receptor is important for several reasons. First, 5-HT has a higher affinity for this receptor compared with the normally expressed 5-HT2A receptor and thus lower concentrations of 5-HT are necessary to activate the receptor. Second, circulating levels of 5-HT may be increased in hypertension. Measures of either plasma or serum levels of 5-HT have not been obtained in DOCA-salt hypertension, but brain 5-HT levels were increased in this model in the first 2 wk of treatment (7). How does an increase in receptor signaling, seen functionally in both DOCA-M and DOCA-S rats early on in hypertension, translate into mediating the high blood pressure in severely but not moderately hypertensive rats later in hypertension?

One possible explanation for this is that, at later but still not malignant phases of hypertension, another change in the DOCA-S rat has occurred that makes the unmasking of the antihypertensive effects of LY-272015 possible. Specifically, we speculate that circulating 5-HT levels are increased in these but not the DOCA-M rats. Several points from the literature suggest this to be a reasonable explanation. First, there is a decreased...
uptake of 5-HT in platelets from hypertensive patients (15). Because platelets are the main storage source for circulating 5-HT, this could result in an increase in free 5-HT. Second, any defect in endothelial cell function, especially in pulmonary endothelial cells, would result in a decreased clearance of 5-HT from the blood, and endothelial dysfunction is well documented in hypertension. Third, platelets are more fragile in hypertension and appear to be more easily able to aggregate in hypertension. Again, as platelets are the main storage source of circulating 5-HT, this would lead to increased amounts of free 5-HT in the blood. We have not measured the plasma levels of 5-HT, and in a hypertensive rat it may be difficult to do so. Platelets are fragile and easily ruptured, and distinguishing between the levels of free and stored 5-HT is paramount to evaluating this theory. This increase in free 5-HT would have an impact on those receptors for which it has the highest affinity, and 5-HT has a 300-fold higher affinity for the 5-HT2B receptor compared with the 5-HT2A receptor. Thus it would be the combination of changes in receptor expression and an increase in circulating levels of 5-HT that is important. This combination is necessary to understand data from experiments using arteries from DOCA-M animals, animals that do not display antihypertensive effects to LY-272015 but do display the switch in pharmacological profile from a 5-HT2A to a 5-HT2B receptor.

With respect to why in vitro changes (e.g., increased contraction to 5-HT and decreased blocking ability of ketanserin) were seen earlier in development of DOCA-salt hypertension than in vivo responses to LY-272015, it is possible that there is a steady increase in 5-HT2B expression in blood vessels that is simply insufficient to affect overall blood pressure regulation (which is multifactorial) until a critical threshold is reached (3 wk). In vitro studies are much more sensitive for detecting changes in receptor function as they provide information separate from many of the compensatory responses that may occur in vivo.

Another possible explanation is that, because 5-HT is a more potent vasoconstrictor, the ability of 5-HT to potentiate vascular contraction to other agonists may be enhanced (21, 24). These agonists include important cardiovascular hormones such as norepinephrine (28) and endothelin-1, two substances involved in DOCA-salt hypertension. An additional impact of increased 5-HT2B receptor expression and activation is the following: activation of the 5-HT2B receptor, naturally expressed in a carcinoma cell line or stably expressed in mouse fibroblast LMTK- cell lines, causes a rapid and transient activation of p21ras and can activate the extracellular signal-regulated kinases, members of the mitogen-activated protein kinase family (18, 19). Because 5-HT is a vascular mitogen (16), such an interaction holds obvious implications for the involvement of 5-HT2B receptor in the vascular growth often observed in hypertension.

Collectively, these data suggest that the 5-HT2B receptor signaling is significantly enhanced in DOCA-salt hypertension. This enhancement is revealed as a vascular hyperreactivity to 5-HT, the 5-HT2B-receptor agonist BW-723C86, and, in a subpopulation of DOCA-rats, as a drop in blood pressure due to the 5-HT2B-receptor antagonist LY-272015. Moreover, these data support the novel hypothesis that 5-HT2B-receptor expression is induced during the development of DOCA-salt hypertension and contributes to the maintenance of severe blood pressure elevations and suggest that the involvement of 5-HT in hypertension should be reexamined.

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