The Fenfluramine Metabolite (+)-Norfenfluramine Is Vasoactive

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ABSTRACT

The anorexigen (+)-fenfluramine was used for treatment of obesity until the association of use with valvular heart disease and primary pulmonary hypertension. (+)-Fenfluramine has been found in Chinese and Korean slimming pills. The hepatic metabolite of (+)-fenfluramine, (+)-norfenfluramine, has affinity for 5-hydroxytryptamine (5-HT)2A and 5-HT2B receptors. We tested the hypothesis that (+)-norfenfluramine contracts arterial smooth muscle in a 5-HT receptor-dependent manner and acts as a pressor in the conscious rat. Isometric contraction experiments showed that (+)-norfenfluramine (10 nM, 100 μM) but not (+)-fenfluramine nor the isomer (-)-norfenfluramine caused concentration-dependent contraction in arteries [−log EC50 (moles per liter), thoracic aorta = 5.77 ± 0.09; renal artery = 6.29 ± 0.02; mesenteric resistance artery = 5.70 ± 0.06]. Contraction was dependent on the 5-HT2A receptor because ketanserin (10 nM) rightward shifted (+)-norfenfluramine response curves (aorta = 16-fold, renal artery = 26-fold, and resistance artery = >100-fold). Dependence on activation of 5-HT2A receptors and independence of (+)-norfenfluramine-induced contraction from stimulation of α-adrenergic receptors and the sympathetic nervous system was validated by demonstrating 1) unchanged contraction to (+)-norfenfluramine in arteries from chemically denervated rats; 2) a minimal effect of the αi-adrenergic receptor antagonist prazosin (100 nM) on contraction; and 3) antagonism by [6-methyl-l-(1-methyllethy)ergoline-8β-carboxylic acid 2-hydroxy-1 methylpropyl ester maleate] LY53857 [6-methyl-1-(1-methyllethy)ergoline-8β-carboxylic acid 2-hydroxy-1 methylpropyl ester maleate], a 5-HT2 receptor antagonist without α-receptor affinity. (+)-Norfenfluramine (10–300 μg/kg i.v.) caused a dose-dependent increase in mean arterial blood pressure in conscious rats, the maximum of which could be virtually abolished by ketanserin (3 mg/kg i.v.) but not prazosin (0.2 mg/kg i.v.). Our findings demonstrate for the first time that (+)-norfenfluramine is vasoactive and has the potential to increase blood pressure.
receptor was cloned in rat stomach fundus in 1992 (Foguet et al., 1992; Kursar et al., 1992) and plays an important role in mediating arterial contraction in deoxytocosterone acetate-salt hypertensive rats (Watts, 1998). Thus, we hypothesized that (+)-norfenfluramine may activate 5-HT receptors and be a vasoactive substance.

Few studies have tested whether (+)-fenfluramine can alter smooth muscle contractility (Desta et al., 1998), and it has never been determined whether (+)-norfenfluramine contracts smooth muscle. We investigated whether (+)-norfenfluramine causes a contraction-dependent contraction in three isolated arteries and whether the contraction depends on 5-HT receptors, sympathetic nerves [a site of potential uptake of (+)-norfenfluramine], or α-adrenergic receptors. We then investigated whether and the mechanism by which (+)-norfenfluramine could alter mean arterial blood pressure in conscious normal rats, with the intent of connecting our data derived from in vitro experiments to the whole animal.

Materials and Methods

Animal Use

The normal male Sprague-Dawley rats (0.225–0.300 kg; Charles River Laboratories, Inc., Portage, MI) were used in these experiments.

Isolated Tissue Bath Protocol

Thoracic aorta and renal artery were removed and placed in normal physiological salt solution (PSS) containing 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄, 1.6 mM CaCl₂, 14.9 mM NaHCO₃, 5.5 mM dextrose, and 0.03 mM CaNa₂EDTA (pH 7.2). Vessels were trimmed of fat and cut into helical strips (aorta, 1 × 10 mm; renal artery, 1 × 5 mm). We used endothelium-intact arteries in our experiments.

Tissues were attached to a fixed, stainless steel rod at one end and to a force transducer (FT03; Grass Instruments, Quincy, MA) at the other. Baths were filled with PSS, warmed to 37°C, and aerated with 95% oxygen and 5% carbon dioxide. Each strip was placed under optimum resting tension (previously determined; rat aorta, 1500 mg; renal artery, 500 mg) and allowed to equilibrate for 1 h with frequent buffer changes. Tissues were then challenged with a maximal concentration of PE (10⁻⁵ M) to initiate a maximal contraction and wash repeatedly until tone returned to baseline. To examine the state of the arterial endothelium, tissues were contracted with a half-maximal concentration of PE (10⁻⁶–10⁻⁷ M), and once the contraction plateaued, the muscarinic agonist acetylcholine (10⁻⁶ M) was administered. We observed a relaxation to acetylcholine greater than 60% of the PE (10⁻⁶–10⁻⁷ M)-induced contraction as endothelium intact. Tissues were then again washed until baseline was reached, and then one of the following protocols was followed.


Concentration-response curves to fluramines were performed in a cumulative manner. Each concentration incubated a minimum of 3 min; when contraction reached a maximum, the next higher concentration of agonist was added. Contraction to agonist was normalized by maximal contraction to PE.

Protocol 2: Testing of Effect of Antagonists on (+)-Norfenfluramine-Induced Contraction. Vehicle or antagonist was added to the bath. Antagonists incubated with the tissues for 1 h at which time the cumulative response to (+)-norfenfluramine in the presence of vehicle or antagonist was examined. In some experiments using LYS₃₈₅, vehicle (0.1% dimethyl sulfoxide) or LYS₃₈₅ (10 mM) was added once the maximum contraction to (+)-norfenfluramine was established, and reduction in contraction after 30 min was recorded.

Myograph Protocol

The whole intestine was placed in a silastic-filled petri dish in cold PSS and pinned down. Second and third order arteries (200–300 μm, inner diameter) were carefully dissected from the vein and placed in cold PSS. Two tungsten wires (California Wire, Grover Beach, CA) were threaded through the lumen of the artery. One wire was mounted to a micrometer and the other to a microtransducer. All arteries were examined with an intact endothelium. Baths, filled with PSS, were warmed by a water-circulated jacket around the bath and are aerated with 95% O₂, 5% CO₂. Tissues equilibrated for 30 min before an optimal passive tension of 400 mg (determined previously; Watts, 2002) was applied using the micrometer. Tissues equilibrated another 30 min with frequent buffer changes before challenge with a maximal concentration of PE (10 μM). Experimental protocols are as described above.

Denervation Experiment Protocol

Sympathetic neuronal denervation was induced by 6-hydroxydopamine (6-OHDA) injections (McCafferty et al., 1997). Rats were treated with four doses of 6-OHDA over 7 days (50 mg/kg on days 1 and 2 and 100 mg/kg on days 6 and 7; 0.1% ascorbic acid in physiological saline as vehicle, i.p.). Rats were euthanized (pentobarbital, 60 mg/kg, i.p.), and arteries were removed on day 8. Contractility experiments were performed on the aorta, renal artery, and mesenteric resistance arteries from vehicle-treated and denervated rats.

Glyoxylic Acid Protocol

Aorta and mesenteric resistance arteries were removed and placed in normal PSS. After fat was trimmed off, arteries were immersed into glyoxylic acid (2%) for 5 min. Blood vessels were mounted on a microscope slide and placed in an oven (100°C) for 5 min. The slides were removed and blood vessels mounted in mineral oil and coverslipped. Vessels were viewed using a fluorescence microscope (Nikon LABOPHOT) and UV illumination (G-1A; excitation, 546/10 nm; barrier filter, BA580). The absence of a fluorescent network of staining of catecholamines in arteries confirms the effectiveness of 6-OHDA in causing sympathetic denervation. Method is according to Ruijtenbeek et al. (2000). Only pictures of small resistance arteries are shown as validation of this protocol, because no sympathetic network was readily discernible in the aorta.

In Vivo Experiments

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 (50 mg/kg, i.p.). The ends of the catheters were tunneled subcutaneously to the head where the catheters were stabilized to the skull by using 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 (venous end) and arterial end was connected to a pressure transducer (TRN050; Kent Scientific Corp., Litchfield, CT). Upon regaining consciousness, rats were housed singly in stainless steel cages in a climate-controlled room with a 12-h light/dark cycle. At least 1-week recovery was allowed before experiments were begun.

(+)-Norfenfluramine (10–300 μg/kg i.v.) was given in a cumulative manner at 6-min intervals. Mean arterial blood pressure and heart rate were monitored before, during, and for 6 min after each injection with a computerized DigiMed system. Antagonist or vehicle was given 30 min before (+)-norfenfluramine.

Statistics

Contractile data are expressed as ±S.E.M. and reported as a percentage of the maximal contraction to PE (10⁻⁵ M). Unpaired t tests were performed and a p value ≤0.05 was considered statistically significant. Agonist EC₅₀ values were calculated using a non-linear regression analysis using the algorithm [effect = maximum
response/1 + (EC\textsubscript{50}/agonist concentration)) in the program GraphPad Prism. Apparent antagonist dissociation constants (K\textsubscript{B} values) were calculating using the following equation: \( \log(dr) = \log[B] - \log K_B \), where \( dr \) is the EC\textsubscript{50} value of agonist in the presence of the antagonist divided by the EC\textsubscript{50} value of agonist in the absence of the antagonist, and \( [B] \) is the concentration of the antagonist tested. Blood pressure is reported as a change in mean arterial blood pressure and a repeated measures analysis of variance was used to ascertain statistical differences between groups.

### Chemicals

Acetylcholine chloride, (+)-fenfluramine, 5-hydroxytryptamine hydrochloride, ketanserin tartrate, LY53857, phenylephrine hydrochloride, prazosin hydrochloride, RX821002, UK14304, 6-OHDA, and glyoxylic acid were purchased from Sigma-Aldrich (St. Louis, MO). LY272015 was a generous gift from Eli Lilly and Company (Indianapolis, IN) and (+)-norfenfluramine was graciously provided by SRI International (Menlo Park, CA).

### Results

**Response of Normal Rat Arteries to the Fluramines.**

Figure 1A compares response of endothelium-intact aorta from normal rats to (-)-norfenfluramine, (+)-norfenfluramine, and (+)-fenfluramine. Only (+)-norfenfluramine contracted the aorta in a concentration-dependent manner with a \(-\log EC_{50} \) value of 5.77 \pm 0.09 mM, whereas (+)-fenfluramine and (-)-norfenfluramine were virtually inactive. Similarly, (+)-norfenfluramine (Fig. 1B) but not (+)-fenfluramine (Fig. 1C) contracted endothelium-intact renal arteries (\(-\log EC_{50} = 6.29 \pm 0.02 \) mM) and mesenteric resistance arteries (\(-\log EC_{50} = 5.70 \pm 0.06 \) mM). The potency of (+)-norfenfluramine-induced contraction in these three arteries was relatively similar, but there were notable differences in the maximal contraction. In preliminary experiments in the rat aorta, removal of the endothelial cell layer did not affect (+)-norfenfluramine-induced contraction (\(-\log EC_{50} = 5.62 \pm 0.08 \) mM, max = 75.89 \pm 4.29). Thus, in all experiments we have kept the endothelial cell layer intact to most closely parallel the in vivo situation.

**Inhibition of (+)-Norfenfluramine-Induced Contraction in Aorta and Pressor Response in Normal Rats by 5-HT\textsubscript{2A/B} Receptor Antagonist Ketanserin.** To determine the receptor type mediating (+)-norfenfluramine-induced contraction, contraction in aorta was examined in the presence of 5-HT and \( \alpha \)-adrenergic receptor antagonists. Ketanserin (Fig. 2A; 10 nM) competitively shifted the (+)-norfenfluramine concentration-response curve rightward 16-fold in aorta from normal rats. The apparent dissociation constant calculated from this shift (\( pK_B = 9.27 \pm 0.8 \)) is consistent with antagonism of the 5-HT\textsubscript{2A} receptor. Antagonists for other relevant heptahelical receptors, e.g., the 5-HT\textsubscript{2B} receptor antagonist LY272015 (10 nM), \( \alpha \)-adrenergic receptor antagonist prazosin (100 nM), and \( \alpha \)-adrenergic receptor antagonist RX821002 (1 \( \mu \)M), did not significantly alter the potency or maximal response elicited by (+)-norfenfluramine (Table 1). These antagonists were tested in concentrations that are largely unable to interact with the 5-HT\textsubscript{2A} receptor and were effective at their specific receptor as suggested by reported \( K_B \) values in the Table 1. We validated that these antagonists were used in appropriate concentrations in control experiments. Specifically, LY272015 (10 nM) rightward-shifted 5-HT-induced contraction of the isolated rat stomach fundus (52.5-fold), prazosin (100 nM) abolished a PE-induced contraction in rat aorta (percentage of 10 \textsuperscript{-5} M of PE-induced contraction, control maximal = 111.8 \pm 4.9, prazosin-incubated maximal = 3.05 \pm 1.7; \( p < 0.05 \)) and RX821002 (1 \( \mu \)M) inhibited a UK14304-induced aortic contraction (percentage of 10 \textsuperscript{-5} M PE-induced contraction, control maximal = 50.4 \pm 4.5, RX821002-incubate maximal = 7.56 \pm 1.9; \( p < 0.05 \)).

Ketanserin (10 nM) inhibited (+)-norfenfluramine-induced contraction in renal artery (Fig. 2B) and mesenteric resistance artery (Fig. 2C). Notably, ketanserin produced an inhibition that was quantitatively greater in resistance arteries (>100-fold shift) as compared with that observed in the renal artery or aorta. These results suggest that the predom-
inant receptor mediating (+)-norfenfluramine contraction in arteries from normal rats is a 5-HT2A receptor but that there is either a difference in the coupling of 5-HT2A receptors or ketanserin may cause mild α-adrenergic antagonism in resistance arteries.

Before discriminating between these possibilities, we examined the effect of (+)-norfenfluramine on blood pressure in the conscious rat. Given intravenously, (+)-norfenfluramine increased blood pressure in a dose-dependent manner in conscious normal rats without a concomitant change in heart rate (data not shown). This pressor response was markedly reduced by ketanserin (3 mg/kg; Fig. 2D) and again raised the possibility that (+)-norfenfluramine was interacting with the α-adrenergic system/sympathetic nervous system.

Effect of Chemical Sympathectomy on (+)-Norfenfluramine-Induced Contraction in Aorta. To determine whether (+)-norfenfluramine-induced contraction and pressor response were dependent on the sympathetic nervous system, we performed experiments on sympathectomized rats. Figure 3A shows the ablation of the sympathetic nervous system (SNS) in mesenteric resistance arteries from rats that received 6-OHDA. The fluorescence network is composed of sympathetic nerve fibers as induced by the glyoxylic acid reaction product formed with biogenic amines in small arteries from vehicle-treated rats (vehicle); we do not show pictures for aorta here because no discernible network was observed. Arteries from 6-OHDA-treated rats lost most of the nerve innervation, as seen by the loss of a fine white network of fibers (Fig. 3A). Importantly, we did not observe a significant difference in contraction induced by (+)-norfenfluramine in aorta from normal rats and sympathectomized rats (Fig. 3B, and (+)-norfenfluramine-induced contraction in arteries from denervated rats was similarly antagonized by ketanserin compared with controls (Fig. 3B).

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target (Kᵢ)</th>
<th>log EC₅₀</th>
<th>Maximum Contraction</th>
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<tr>
<td>Vehicle</td>
<td></td>
<td>5.77 ± 0.09</td>
<td>82.54 ± 2.53</td>
</tr>
<tr>
<td>LY272015 (10 nM)</td>
<td>5-HT₁A Receptor (0.75)</td>
<td>5.75 ± 0.01</td>
<td>91.61 ± 2.96</td>
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<tr>
<td>Prazosin (100 nM)</td>
<td>α₁-Adrenergic receptor (0.01)</td>
<td>5.64 ± 0.01</td>
<td>82.23 ± 3.79</td>
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<tr>
<td>RX821002 (1 μM)</td>
<td>α₂-Adrenergic receptor (0.95)</td>
<td>5.55 ± 0.02</td>
<td>85.54 ± 3.03</td>
</tr>
</tbody>
</table>

* Cohen et al. (1996).
* Jones et al. (1987).
* Naselsky et al. (2001).
ramine-induced contraction in the renal artery and mesenteric resistance arteries of denervated rats was not altered compared with control (Table 2), and maximal contraction to (+)-norfenfluramine was significantly reduced by the 5-HT2 receptor antagonist LY53857 (10 nM; Fig 3, C and D). Thus, it is unlikely that (+)-norfenfluramine-induced contraction depends on the peripheral SNS.

**Effect of LY53857 and Prazosin on (+)-Norfenfluramine-Induced Vasoreactivity.** To further rule out the ability of (+)-norfenfluramine to stimulate contraction by directly interacting with α-adrenergic receptors in small vessels, and to confirm the involvement of the 5-HT2A receptor, we examined the effects of LY53857 (30 nM) on (+)-norfenfluramine-induced contraction. This antagonist does not discriminate between 5-HT2A and 5-HT2B receptors, but it has little α-adrenergic receptor affinity (Cohen et al., 1988). Figure 4 depicts that in aorta and in mesenteric resistance arteries, LY53857 significantly reduced (+)-norfenfluramine-induced contraction.

The ability of (+)-norfenfluramine to interact directly with α-adrenergic receptors was investigated in mesenteric resistance arteries. At a concentration that abolishes contraction to a maximal concentration of the α1-adrenergic receptor agonist PE (10^{-5} M), prazosin (100 nM) reduced contraction to (+)-norfenfluramine in arteries from control rats only at the lower concentrations of (+)-norfenfluramine (Fig. 5A). The potency of (+)-norfenfluramine was not significantly reduced by prazosin (−log EC_{50} control = 5.40 ± 0.13 M; prazosin = 5.05 ± 0.17 M; p = 0.08). Importantly, prazosin exerted only a minimal inhibition on (+)-norfenfluramine-induced contraction in the renal artery and mesenteric resistance arteries of denervated rats was not altered compared with control (Table 2), and maximal contraction to (+)-norfenfluramine was significantly reduced by the 5-HT2 receptor antagonist LY53857 (10 nM; Fig 3, C and D). Thus, it is unlikely that (+)-norfenfluramine-induced contraction depends on the peripheral SNS.

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**Table 2**

Potency (−log EC_{50}) and maximum contraction elicited by (+)-norfenfluramine in arteries from vehicle- and 6-OHDA-treated rats n = 3 to 7.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>6-OHDA</th>
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<tbody>
<tr>
<td></td>
<td>−log EC_{50}</td>
<td>% PE Contraction</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>5.79 ± 0.31</td>
<td>83.9 ± 4.7</td>
</tr>
<tr>
<td>Renal artery</td>
<td>5.51 ± 0.10</td>
<td>120.3 ± 5.6</td>
</tr>
<tr>
<td>Mesenteric resistance artery</td>
<td>5.40 ± 0.13</td>
<td>52.3 ± 8.6</td>
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Fig. 3. A, glyoxylic acid fluorescence in small mesenteric resistance arteries from vehicle or 6-OHDA-treated rats. The white network is sympathetic nerve fibers. B, effect of the 5-HT2A receptor antagonist ketanserin (10 nM) on (+)-norfenfluramine-induced contraction in aorta from 6-OHDA-treated rats. Dashed lines are data representing (+)-norfenfluramine-induced contraction in normal rats. C and D, effect the 5-HT2 receptor antagonist LY53857 (10 nM) had on maximum contraction to (+)-norfenfluramine in renal artery (C) and mesenteric resistance arteries (D). Points/bars represent means and vertical lines the S.E.M. for the number of animals indicated in parentheses. Asterisk (*) indicates statistically significant differences (p < 0.05) between control/vehicle and artery incubated with antagonist.
induced contraction in mesenteric resistance arteries that were from 6-OHDA denervated rats (Fig. 5B), a tissue in which direct interaction with α-adrenergic receptors could most simply be examined. Again, the potency of (+)-norfenfluramine was not significantly reduced by prazosin (control = 5.62 ± 0.08 M, prazosin = 5.41 ± 0.12 M; p = 0.10). Finally, prazosin (0.2 mg/kg i.v.; Oates, 1979) only modestly reduced the pressor response to (+)-norfenfluramine in conscious rats (Fig. 5C).

Discussion

The correlation between body weight and blood pressure is significant and real in the human (Chiang et al., 1969; Stamler et al., 1978; for review, see Montani et al., 2002). Flechtner-Mors et al. (1988) reported that short-term treatment with (+)-fenfluramine in obese postmenopausal women decreased blood pressure and heart rate. By contrast, long-term use of anorexigen agent (+)-fenfluramine to control body
weight has resulted in primary pulmonary hypertension (Abenhaim et al., 1996), valvular disease (Connolly et al., 1997), and systemic hypertension in some populations (Mabadeje, 1974). We demonstrate here the ability of (+)-nortenfluramine, a metabolite of (+)-fenfluramine, to be vasoactive primarily through activation of a 5-HT receptor.

**Contraction to (+)-Nortenfluramine.** Our results show that it is (+)-nortenfluramine (Fig. 1, A and B), and not (+)-fenfluramine (Fig. 1C), that causes concentration-dependent contraction in various arteries. Aorta and renal artery were used as conduit arteries and were examined in addition to a true resistance artery from the mesentry. Use of these small resistance vessels is important because they contribute to determination of total peripheral resistance. Our data demonstrate that (+)-nortenfluramine contracts both conduit and resistance arteries (Fig. 1, A and B), so contraction is not vessel-specific. Moreover, the contractility experiments were performed in arteries with intact endothelium, thereby most closely approaching the in vivo situation.

Our data suggest that (+)-nortenfluramine contracts arterial smooth muscle in a 5-HT2A receptor-dependent manner. Using a series of amine receptor antagonists, we found that contraction to (+)-nortenfluramine was significantly inhibited by the 5-HT2A/2C receptor antagonist ketanserin (10 nM) in aorta from normal rats (Fig. 2A). The same concentration of ketanserin also inhibited (+)-nortenfluramine-induced contraction in renal artery and mesenteric resistance artery (Fig. 2, B and C). In theory, this concentration of ketanserin has little effect on α1-adrenergic receptor activation (ketanserin at 5-HT2A receptor, KI = 0.39 nmol, Leysen et al., 1982; and ketanserin at α1-adrenergic receptor, KI = 72.4 nmol; Korstanje et al., 1986). The 5-HT2C receptor, another receptor for which ketanserin has significant affinity, has never definitively been found in periphery (Barnes and Sharp, 1999). Importantly, the nonselective 5-HT2 receptor antagonist LY53857, which has significantly lower affinity for an adrenergic receptor compared with ketanserin (Cohen et al., 1988), also antagonized (+)-nortenfluramine-induced contraction in aorta. Collectively, these data suggest that the 5-HT2A receptor is likely involved in (+)-nortenfluramine-induced contraction.

However, it was a concern that the antagonism exerted by ketanserin in the mesenteric resistance arteries was quantitatively greater than that observed in either aorta or renal artery. We interpreted these data to suggest that either the 5-HT2A receptors in the small resistance arteries were exquisite sensitive to antagonism of 5-HT receptors and/or that ketanserin might exert α-adrenergic antagonism in these small arteries. Because of these data and the fact that (+)-nortenfluramine has been described as a norepinephrine transporter substrate (Rothman et al., 2003), it was crucial to determine whether (+)-nortenfluramine interacted directly with α-adrenergic receptors or indirectly through release of NE.

**Independence from SNS and Modest Dependence on α-Adrenergic Receptor.** Our previous experiments raise the question of whether (+)-nortenfluramine activates 5-HT2A and/or α-adrenergic receptor directly to cause contraction or stimulates local neuronal 5-HT or NE release, which in turn contracts the arteries. Although few peripheral arteries are innervated with serotonergic nerves, 5-HT and (+)-nortenfluramine have the potential to be taken up by norepinephrine transporter. In large arteries, we do not believe (+)-nortenfluramine interacts directly with α-adrenergic receptors or releases NE to cause contraction because 1) the α1-adrenergic receptor antagonist prazosin and α2-adrenergic receptor antagonist RX821002 were unable to block (+)-nortenfluramine-induced contraction in normal aorta; and 2) we did not observe a significant difference between the contraction to (+)-nortenfluramine in aorta from denervated rats and normal rats. Thus, it is unlikely that NE is released from effrent sympathetic nerve endings and involved in (+)-nortenfluramine-induced contraction in aorta.

We next determined whether a similar independence existed in arteries smaller than the aorta. (+)-Nortenfluramine induced contraction in both renal arteries and mesenteric resistance arteries from 6-OHDA denervated rats, indicating that the sympathetic nervous system was not necessary. Importantly, contraction to (+)-nortenfluramine in these arteries could be antagonized by LY53857, supporting the involvement of a 5-HT2A receptor in contraction. Our findings suggest a weak direct interaction of (+)-nortenfluramine with the adrenergic receptor. This is on the basis of the weak antagonism exerted by prazosin in mesenteric resistance arteries from control and 6-OHDA-treated rats and the fact that prazosin only modestly affected (+)-nortenfluramine-induced increases in blood pressure. To our knowledge, pharmacological interaction of (+)-nortenfluramine with α-adrenergic receptors has not been reported.

**(+)Nortenfluramine as a Pressor Agent.** As early as 1974, treatment with (+)-fenfluramine was related to blood pressure increases (Mabadeje, 1974). In 1999, it was reported that (+)-fenfluramine increased systemic blood pressure, and this pressor response was due to an increase in systemic vascular resistance (Michelakis et al., 1999). As the active metabolite of (+)-fenfluramine, (+)-nortenfluramine has a significantly longer half-life (18 versus 30 h) (http://www.mari poisoncenter.com/ctr/9707fenfluramine.html). Based on the results of our in vitro experiments and the long half-life of (+)-nortenfluramine, we speculate that the (+)-fenfluramine pressor response could be caused, at least partially, by (+)-nortenfluramine. Our in vivo experiments are consistent with this possibility. (+)-Nortenfluramine (10–300 μg/kg i.v.) caused a dose-dependent increase in blood pressure in conscious rats (Fig. 2D) without a concomitant change in heart rate. The concentration of (+)-nortenfluramine that elicits contraction and pressor responses are in a dose range considered therapeutic in humans (20 mg t.i.d.; plasma levels approximately 1.7 × 10−6 M) (http://www.mari poisoncenter.com/ctr/9707fenfluramine.html). Thus, the potential for (+)-nortenfluramine to cause these cardiovascular effects in the human is real. In addition, we investigated the sensitivity of (+)-nortenfluramine-induced pressor responses to ketanserin and prazosin in vivo. Compared with untreated normal rats, pretreatment with 3 mg/kg ketanserin nearly abolished the pressor effect of (+)-nortenfluramine (Fig. 2D), whereas prazosin exerted only a modest effect (Fig. 5C). An important point to make is that in different experiments in which small arterial function has been investigated, all point to the suggestion that the 5-HT2A receptor on small arteries is exquisitely sensitive to antagonism. These experiments include the quantitatively greater antagonism exerted by ketanserin and LY53857 in mesenteric resistance arteries. If one were to estimate the pK<sub>H</sub> for ketanserin in resistance arteries by
assuming that a parallel response to 5-HT in ketanserin-incubated tissues was observed in Fig. 2C, the pKᵢ value would be close to 11, significantly different from what has classically been found for 5-HT₂A receptors. This difference in large versus small artery 5-HT₂A receptor pharmacology will continue to be of interest.

Collectively, (+)-norfenfluramine likely directly activates the 5-HT₂A receptor, which mediates contraction in arteries and leads to an increase in blood pressure. In our experiments, we cannot discount the possibility that some of effects of (+)-norfenfluramine are centrally mediated and this is a noted limitation of these studies. Nonetheless, our work demonstrates for the first time that (+)-norfenfluramine is vasoactive and has the potential to increase blood pressure.

In summary, the major finding in this study is that (+)-norfenfluramine, a hepatic metabolite of the anorexigen (+)-fenfluramine, is vasoactive and a pressor agent. In normal rats, the 5-HT₂A receptor is the primary receptor mediating vessel contraction and pressor response, although a modest direct interaction with α₁-adrenergic receptors may occur. Our findings reveal a new and important action of (+)-norfenfluramine and confirm that drug design for body weight control in the future should avoid a pharmacophore similar to (+)-norfenfluramine.

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References


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