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Short communication

Dexfenfluramine-induced contraction of human and rat isolated pulmonary arteries

Larry A. Patnaude [a], Bradley J. Undem [b], Stephen T. O'Rourke [a,*]

[a] *Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58105, USA*
[b] *Allergy and Clinical Immunology Division, Johns Hopkins Asthma and Allergy Center, Baltimore, MD 21224, USA*

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Abstract

Mechanisms of dexfenfluramine-induced vasoconstriction were studied in isolated pulmonary arteries suspended in organ baths for isometric tension recording. Dexfenfluramine (10^{-7}-10^{-4} M) caused concentration-dependent contractions in rat and human pulmonary arteries with and without endothelium. In pulmonary arteries of the rat, the response to dexfenfluramine was nearly abolished by treatment with the α-adrenoceptor antagonists, phentolamine (10^{-6} M) or prazosin (10^{-7} M). In human pulmonary arteries, the concentration-response curve to dexfenfluramine was unaltered by the presence of phentolamine (10^{-6} M), prazosin (10^{-7} M), ketanserin (10^{-6} M), or indomethacin (3×10^{-6} M). The results suggest that dexfenfluramine causes contraction of pulmonary vascular smooth muscle by multiple mechanisms, one of which involves activation of α-adrenoceptors within the blood vessel wall. The mechanisms by which dexfenfluramine causes pulmonary vasoconstriction may differ between rat and human pulmonary arteries. © 2000 Published by Elsevier Science B.V.

Keywords: Pulmonary arteries; Dexfenfluramine; Phentolamine; Prazosin; Ketanserin; Indomethacin

1. Introduction

Dexfenfluramine is an appetite suppressant used in the treatment of obesity. Patients treated with dexfenfluramine and related anorectic drugs are at increased risk of developing primary pulmonary hypertension (Pouwels et al., 1990; Brenot et al., 1993; Abenhaim et al., 1996), a serious disorder associated with high patient morbidity and mortality (Rubin, 1997). The pathogenesis of primary pulmonary hypertension is poorly understood, but intense vasoconstriction of the pulmonary vasculature is recognized as a hallmark characteristic (Rubin, 1997). The mechanism by which anorectic drugs constrict pulmonary arteries has not yet been determined. Such an effect could be due to a direct action on the pulmonary arteries, or could be mediated indirectly via activation of cardiovascular reflexes or other neurohumoral pathways. In order to assess whether dexfenfluramine can directly constrict pulmonary arteries, the present study was performed in isolated blood vessels, in order to minimize the influence of other neurohumoral mechanisms that could indirectly alter pulmonary vasomotor tone in vivo.

2. Materials and methods

2.1. Tissue preparation

All animal studies were approved by the Institutional Animal Care and Use Committee of North Dakota State University. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) aged 7-10 weeks, were euthanized by CO_2 inhalation. The heart and lungs were rapidly excised and placed in cold physiologic salt solution (PSS) of the following composition (in mM): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1.

* Corresponding author. Tel.: +1-701-231-7836; fax: +1-701-231-7606.
E-mail address: orourke@badlands.nodak.edu (S.T. O'Rourke).

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Macroscopically normal human lung tissue was obtained from anonymous organ donors (supplied by the International Institute for the Advancement of Medicine, Exton, PA or the Anatomical Gift Foundation, Woodbine, GA), with the approval of the Johns Hopkins University Institutional Review Board. Organ donor specimens were mainly from victims of head trauma or cerebral vascular accidents. Lungs from donors with documented pulmonary pathology were not used in these studies. Organ donor specimens were placed in cold Eagle's minimum essential medium and transferred to the laboratory within 24 h. Upon reaching the laboratory, tissues were immediately placed in 4 l of PSS and aerated with 95% O_2 and 5% CO_2 at 4°C.

Rat and human intralobar pulmonary arteries (approximate diameter: 1 mm (rat) and 5 mm (human)) were dissected free of surrounding parenchymal lung tissue and cut into rings 2-4 mm in length. Blood vessel rings were suspended in water-jacketed (37°C) organ baths filled with PSS and aerated with a mixture of 95% O_2/5% CO_2. Each ring was suspended horizontally by two wire clips passed through the lumen; one clip was anchored inside the organ bath, the other connected to a force transducer (Model FT03, Grass Instrument, Quincy, MA). Isometric tension was measured and recorded on a Grass polygraph. The arterial preparations were suspended under an initial resting tension of either 1 g (rat) or 5 g (human). In preliminary length-tension experiments, these initial tensions were found to be optimal for eliciting contractile responses in these arteries. The tissues were maintained at their initial resting tension throughout a 60-min equilibration period, during which they were washed every 15 min with fresh PSS.

In some experiments, the endothelium was removed by gently rubbing the intimal surface prior to placing the tissues in the organ baths. The presence or absence of intact endothelium was determined by testing the ability of acetylcholine (10^{-6} M) to cause endothelium-dependent relaxation of vascular rings contracted with norepinephrine (10^{-7} M).

2.2. Experimental protocols

Concentration–response curves for dexfenfluramine, serotonin, or phenylephrine (10^{-8}-10^{-4} M) were obtained in quiescent pulmonary arterial rings. Cumulative concentration–response curves were performed by increasing the concentration of the agonist in the organ bath by approximately three-fold, only after the response to the previous concentration had reached its maximum (Van Rossum, 1963). All experiments were performed in the presence of propranolol (10^{-6} M), in order to inhibit activation of β-adrenoceptors. In some experiments, contractile responses were obtained in the presence of the α-adrenoceptor antagonists, phentolamine (10^{-6} M) or prazosin (10^{-7} M), the 5-HT-receptor antagonist, ketanserin (10^{-6} M), or the cyclooxygenase inhibitor, indomethacin (3×10^{-6} M). In these experiments, the inhibitors were equilibrated with the tissues for at least 30 min prior to the addition of the agonist and remained in the organ bath for the duration of the experiment. Several adjacent pulmonary artery ring segments from the same origin were studied in parallel and only one concentration–response curve was obtained from each blood vessel ring.

2.3. Data analysis

Contractile responses were normalized by expressing them as a percentage of the maximal contraction evoked by norepinephrine (10^{-4} M) (rat pulmonary arteries) or $BaCl_2$ (30 mM) (human pulmonary arteries), which were added to the organ bath at the end of the experiment. For each dexfenfluramine concentration–response curve, both the maximal effect (E_{max}) and the concentration of dexfenfluramine necessary to produce 50% of its own maximal response (EC_{50}) were determined. The EC_{50} values were converted to the negative logarithms and expressed as $-\log$ molar EC_{50}. The pK_B values (negative logarithm of the apparent dissociation constant) for phentolamine and ketanserin were determined from the standard equation, $pK_B = \log(\text{dose ratio} - 1) - \log B$, where B is the concentration of the antagonist. Dose ratios were calculated by using the concentrations of the agonist that produced 50% of the maximal response in the absence and presence of antagonists. Results are expressed as the mean ± S.E.M., and n refers to the number of animals or human lung specimens from which blood vessels were taken. Values were compared by Student's t test for paired or unpaired observations and were considered to be significantly different when $P < 0.05$.

2.4. Drugs and solutions

The following drugs were used in this study: acetylcholine chloride (Sigma, St. Louis, MO), dexfenfluramine HCl (Research Biochemicals International, Natick, MA), indomethacin (Sigma), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium), L-norepinephrine bitartrate (Sigma), phentolamine mesylate (RBI), L-phenylephrine HCl (Sigma), prazosin HCl (Sigma), DL-propranolol HCl (Sigma), and serotonin creatinine sulfate (Sigma). Drug solutions were prepared daily, kept on ice, and protected from light until used. All drugs were dissolved initially in distilled water, with the exception of indomethacin and prazosin, which were dissolved in ethanol and dimethylsulfoxide, respectively, before further dilution in distilled water. Drug concentrations are reported as final molar concentration in the organ bath.

3. Results

3.1. Rat pulmonary arteries

Dexfenfluramine caused concentration-dependent contractions in endothelium-intact and denuded rat isolated pulmonary arteries (Fig. 1, top). The concentration–response curve was shifted to the left ($-\log EC_{50} = 5.38 \pm 0.1$ (intact) vs. 5.97 ± 0.2 (denuded); $P < 0.05$) and the maximal response was increased ($E_{max} = 82 \pm 5$ (intact) vs. $96 \pm 3\%$ (denuded); $P < 0.05$) in rings without endothelium. Incubation of the tissues with the nonselective α-adrenoceptor antagonist, phentolamine (10^{-6} M), significantly inhibited the contractile response to dexfenfluramine in rat pulmonary arterial rings (Fig. 1, bottom). Similar results were obtained in the presence of the selective α_1-adrenoceptor antagonist, prazosin (10^{-7} M) (Fig. 1, bottom).

3.2. Human pulmonary arteries

Dexfenfluramine also caused concentration-dependent contractions in endothelium-intact and denuded human

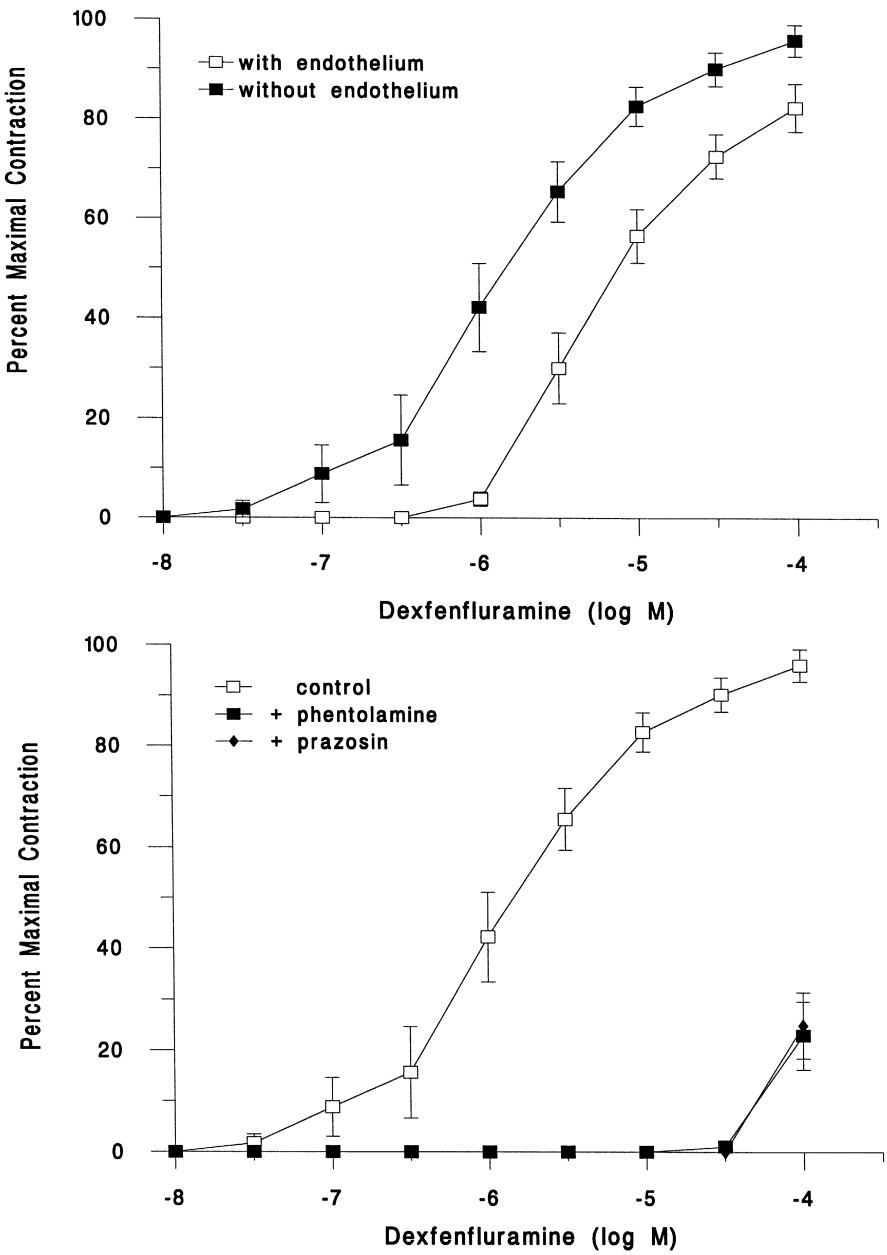

Fig. 1. Top: concentration–response curves for dexfenfluramine in contracting rat pulmonary arteries, with and without endothelium ($n = 8$). Bottom: concentration–response curves for dexfenfluramine in contracting rat pulmonary arteries without endothelium in the absence (control) and presence of phentolamine (10^{-6} M; $n = 6$) or prazosin (10^{-7} M; $n = 6$). The data are expressed as a percentage of the maximal contraction evoked by norepinephrine (10^{-4} M). Each point represents the mean ± S.E.M.

isolated pulmonary arteries (Fig. 2, top). Removal of the endothelium caused a modest, but nonsignificant, leftward shift in the dexfenfluramine concentration–response curve ($-\log\ EC_{50} = 4.60 \pm 0.1$ (intact) vs. 4.90 ± 0.1 (denuded)). The maximal effect of dexfenfluramine was similar in rings with and without endothelium ($E_{max} = 78 \pm 5$ (intact) vs. $80 \pm 4\%$ (denuded)). In contrast to the results obtained in rat pulmonary arteries, the contractile response to dexfenfluramine in human pulmonary arterial rings was unaffected by the presence of phentolamine (10^{-6} M) (Fig. 2, bottom) or prazosin (10^{-7} M) (not shown). Moreover, incubation with the cyclooxygenase inhibitor, indomethacin (3×10^{-6} M), or the 5-HT_2-receptor antagonist, ketanserin (10^{-6} M), had no effect on the concentration–response curve to dexfenfluramine in human isolated pulmonary arteries (Fig. 2, bottom).

Serotonin was an effective contractile agonist in human isolated pulmonary arteries. The $-\log$ molar EC_{50} for serotonin was 6.32 ± 0.1 and the maximum contractile response was $79 \pm 5.1\%$ ($n = 3$). Ketanserin (10^{-6} M)

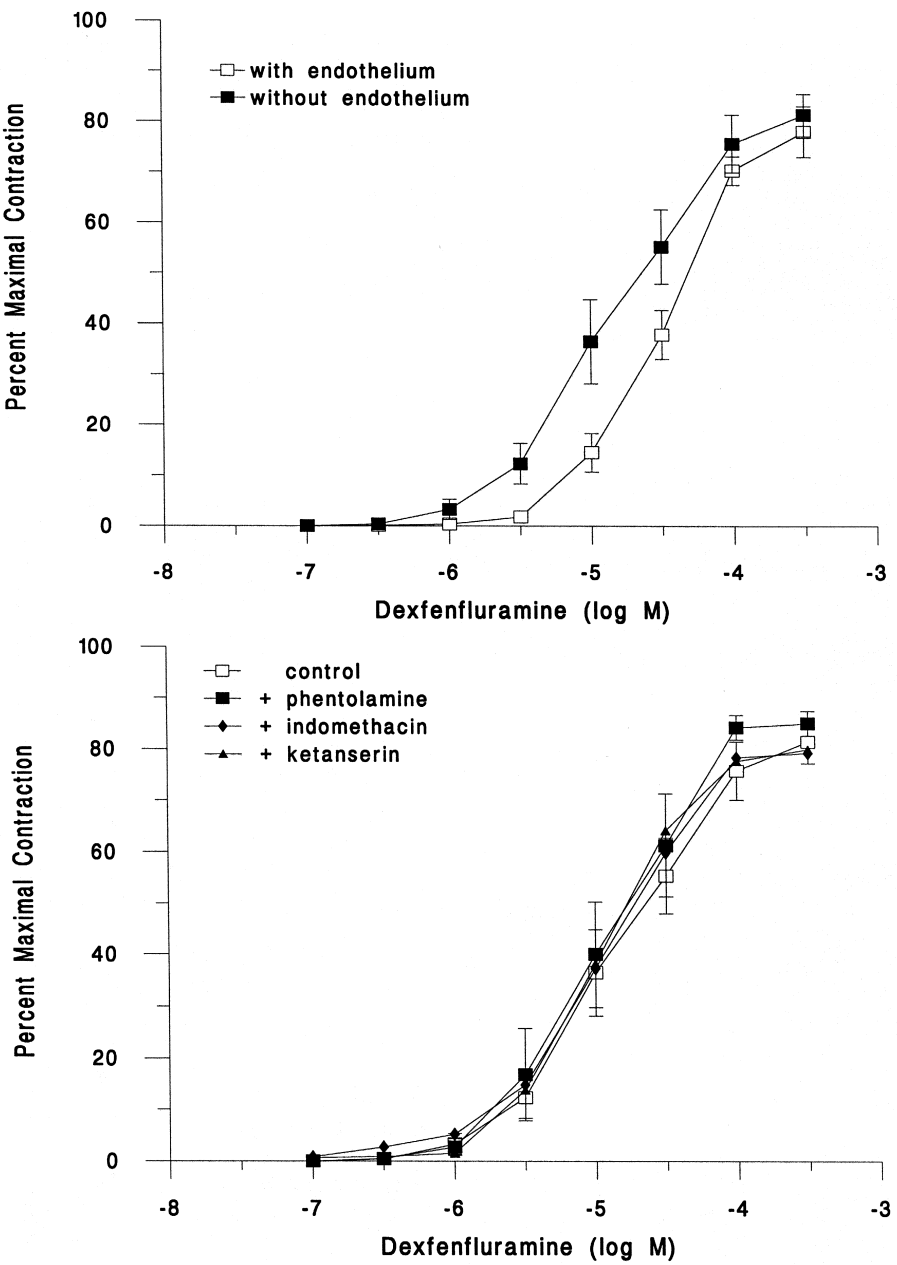

Fig. 2. Top: concentration–response curves for dexfenfluramine in contracting human pulmonary arteries with and without endothelium ($n = 4$). Bottom: concentration–response curves for dexfenfluramine in contracting human pulmonary arteries in the absence (control) and presence of phentolamine (10^{-6} M; $n = 3$), ketanserin (10^{-6} M; $n = 4$), or indomethacin (3×10^{-6} M; $n = 3$). The data are expressed as a percentage of the maximal contraction evoked by $BaCl_2$ (30 mM). Each point represents the mean ± S.E.M.

shifted the concentration–response curve to serotonin to the right in a parallel fashion. The estimated pK_B for ketanserin was 7.32 ± 0.1 ($n = 3$).

Phenylephrine was also an effective contractile agonist in human isolated pulmonary arteries. In two experiments, the EC_{50} values for phenylephrine were 1×10^{-6} and 2×10^{-6} M, and the respective maximum phenylephrine-induced contractions were 74.5% and 70%. Phentolamine (10^{-6} M) shifted the concentration–response curves to phenylephrine to the right in a parallel manner. The calculated pK_B values for phentolamine were 6.70 and 6.80.

4. Discussion

Patients treated with dexfenfluramine and related anorectic drugs are at increased risk of developing primary pulmonary hypertension (Pouwels et al., 1990; Brenot et al., 1993; Abenhaim et al., 1996), a serious disorder characterized by intense vasoconstriction of the pulmonary vasculature (Rubin, 1997). The mechanism by which anorectic drugs increase the risk of primary pulmonary hypertension is not known.

The results of the present study demonstrate for the first time, that dexfenfluramine, when applied directly to isolated pulmonary arteries, causes contraction of human pulmonary vascular smooth muscle. The contractile effect of dexfenfluramine was endothelium-independent, inasmuch as the dexfenfluramine concentration–response curve was similar in arterial preparations with and without intact endothelium. Thus, it is likely that the effect of dexfenfluramine is the result of an action directly on the pulmonary vascular smooth muscle and does not involve the release of a contractile factor from endothelial cells (Pearson and Vanhoutte, 1993). Moreover, since the experiments were performed on isolated blood vessels the results indicate that dexfenfluramine itself, independent of other physiologic mechanisms that may be operative in vivo, causes constriction of human pulmonary arteries.

It is generally held that the anorectic effect of dexfenfluramine is due to enhanced serotonergic activity (Davis and Faulds, 1996). Dexfenfluramine stimulates serotonin release from nerve endings and inhibits neuronal reuptake of serotonin (Samanin and Garattini, 1993). Since serotonin, via 5-HT_2-receptor activation, is a potent vasoconstrictor in the pulmonary circulation (Selig et al., 1988; Le Roux and Syce, 1989), it is conceivable that dexfenfluramine-induced pulmonary vasoconstriction may be mediated by activation of vascular 5-HT-receptors. However, a role for 5-HT-receptors in the observed response to dexfenfluramine is unlikely, since the potent 5-HT_2-receptor antagonist, ketanserin, at a concentration that inhibited significantly the contractile response to serotonin, did not inhibit dexfenfluramine-induced contractions in human pulmonary arteries. Likewise, ketanserin had no effect on the contractile response to dexfenfluramine in porcine and canine pulmonary arteries (Desta et al., 1998; Naeije et al., 1995).

Concentration-dependent contractions to dexfenfluramine also occur in porcine isolated coronary arteries (Desta et al., 1996). In this blood vessel, indomethacin abolishes the response to dexfenfluramine (Desta et al., 1996), suggesting that cyclooxygenase metabolites of arachidonic acid mediate the contractile effect. Since indomethacin had no effect on the contractile response to dexfenfluramine in human pulmonary arteries, it is unlikely that prostaglandins or other products of the cyclooxygenase pathway play a role in the response.

The present study demonstrates that dexfenfluramine also causes contraction of rat isolated pulmonary arteries, confirming an earlier report in which dexfenfluramine produced vasoconstriction in perfused rat lungs (Weir et al., 1996). As observed with human pulmonary arteries, removal of the endothelium from pulmonary arteries of the rat failed to inhibit the response to dexfenfluramine, a finding that is consistent with a direct effect of dexfenfluramine on vascular smooth muscle. Indeed, the presence of an intact endothelium appears to inhibit the contractile effect of dexfenfluramine in rat pulmonary arteries, inasmuch as the concentration–response curve to dexfenfluramine was shifted to the left in preparations without endothelium. The inhibitory effect of the endothelium may be due to nitric oxide, released either spontaneously from endothelial cells (Pearson and Vanhoutte, 1993) or in response to dexfenfluramine.

A major finding of the present study is that the α-adrenoceptor antagonists, phentolamine and prazosin, had no effect on the contractile response to dexfenfluramine in human pulmonary arteries but markedly suppressed the response to dexfenfluramine in pulmonary arteries of the rat. In human pulmonary arteries, the α-adrenoceptor agonist, phenylephrine, caused contractions that were inhibited in a competitive manner by phentolamine, indicating that the lack of effect of the α-adrenoceptor antagonists on the response to dexfenfluramine is not due to the absence of α-adrenoceptors in this preparation. These data strongly suggest that dexfenfluramine-induced contractions are mediated by α-adrenoceptors in rat, but not human pulmonary arteries. That responses to dexfenfluramine may be due to α-adrenoceptor stimulation is supported by the recent observation that dexfenfluramine binds to and activates α_1-adrenoceptors in rat hepatocytes (Comte et al., 1997).

Irrespective of the mechanism by which dexfenfluramine causes pulmonary vasoconstriction, the difference in pharmacology between rat and human pulmonary arteries has implications regarding the choice of animal models in the study of anorexigen-induced pulmonary hypertension. Indeed, the results of the present study suggest that the rat pulmonary vasculature may not be the most appropriate model for investigating the mechanism(s) by which dexfenfluramine causes vasoconstriction in human pulmonary arteries.

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