Modulation of Resistance Artery Tone by the Trace Amine β-Phenylethylamine: Dual Indirect Sympathomimetic and α₁-Adrenoceptor Blocking Actions

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ABSTRACT

The trace amine β-phenylethylamine (PEA) is normally present in the body at low nanomolar concentrations but can reach micromolar levels after ingestion of drugs that inhibit monoamine oxidase and primary amine oxidase. In vivo, PEA elicits a robust pressor response, but there is no consensus regarding the underlying mechanism, with both vasodilation and constriction reported in isolated blood vessels. Using functional and biochemical approaches, we found that at low micromolar concentrations PEA (1–30 μM) enhanced nerve-evoked vasoconstriction in the perfused rat mesenteric bed but at a higher concentration (100 μM) significantly inhibited these responses. The α₂-adrenoceptor antagonist rauwolscine (1 μM) also enhanced nerve-mediated vasconstriction, but in the presence of both rauwolscine (1 μM) and PEA (30 μM) together, nerve-evoked responses were initially potentiated and then showed time-dependent rundown. PEA (10 and 100 μM) significantly increased noradrenaline outflow from the mesenteric bed as determined by high-pressure liquid chromatography coupled with electrochemical detection. In isolated endothelium-denuded arterial segments, PEA (1 μM to 1 mM) caused concentration-dependent reversal of tone elicited by the α₁-adrenoceptor agonists noradrenaline (EC₅₀ 51.69 ± 10.8 μM; n = 5), methoxamine (EC₅₀ 68.21 ± 1.70 μM; n = 5), and phenylephrine (EC₅₀ 67.74 ± 16.72 μM; n = 5) but was ineffective against tone induced by prostaglandin F₂α, or U46619 (3,11-dideoxy-9α,11α-methanoepoxyprostaglandin F₂α). In rat brain homogenates, PEA displaced binding of both [³H]prazosin (Kᵢ 25 μM) and [³H]rauwolscine (Kᵢ 1.2 μM), ligands for α₁- and α₂-adrenoceptors, respectively. These data provide the first demonstration that dual indirect sympathomimetic and α₁-adrenoceptor blocking actions underlie the vascular effects of PEA in resistance arteries.

Introduction

Synthesized from phenylalanine by the enzyme aromatic L-amino acid decarboxylase (Holt et al., 2008; Ramsay et al., 2011), the trace amine β-phenylethylamine (PEA) is a byproduct of catecholamine biosynthesis in both the central nervous system and the periphery. PEA is metabolized by monoamine oxidase (Huebert et al., 1994) and primary amine oxidase enzymes (Tsuji et al., 1986). Although under normal physiologic conditions PEA is present in the circulation at low nanomolar concentrations (Udenfriend and Cooper, 1953; Berry, 2004), plasma levels can be increased by several hundred-fold in patients taking amine oxidase enzyme inhibitors (Saavedra, 1974; Reynolds et al., 1980; Nazarali et al., 1987). High plasma levels of trace amines have also been recorded after consumption of foods, such as chocolate, alcoholic beverages, and fermented products such as cheese, and have been associated with tachycardia and hypertension (Zucchi et al., 2006). Indeed, the first description of trace amine-associated hypertension was directly demonstrated that at micromolar concentrations PEA both stimulates the release and inhibits the reuptake of noradrenaline in rat brain synaptosomes (Horn and Snyder, 1972; Raiteri et al., 1977) and enhances noradrenaline efflux from adrenergic nerves in isolated rabbit atra (Paton, 1975).

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Abbreviations: HPLC, high-pressure liquid chromatography; L-NNAME, L-Nω-nitroarginine methyl ester; NO, nitric oxide; PEA, amine β-phenylethylamine; PGF₂α, prostaglandin F₂α; TAAR, trace amine-associated receptor; U46619, 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin F₂α; WB4101, 2-[2,6-dimethoxyphenoxyl]aminomethyl-1,4-benzodioxane.
In contrast, studies of isolated preparations have indicated that various other mechanisms may contribute to the vascular actions of PEA. In the rat perfused mesenteric bed, reversal of phenylephrine-induced increases in perfusion pressure by PEA (0.1–1000 nmol) was attributed to the release of endothelium-derived nitric oxide (NO) (Broadley et al., 2009). In rat brain, inhibition of noradrenaline-stimulated increases in inositol trisphosphate production was ascribed to PEA, at millimolar concentrations, acting as a partial agonist at α1-adrenoceptors (Dyck and Boulton, 1989). Identification of specific trace amine-associated receptors (TAARs) has also led to the suggestion that increases in tone in large conduit arteries caused by millimolar concentrations of PEA may be due to a direct interaction with these receptors (Zucchi et al., 2006; Grandy, 2007; Herbert et al., 2008; Broadley et al., 2009; Broadley, 2010; Fehler et al., 2010). However, the lack of selective agonists and antagonists for TAARs means that this conclusion is based only on an association between changes in tone and expression of receptors, and is at odds with data from expression systems showing that activation of TAARs by nanomolar concentrations of PEA is linked to increases in cyclic adenosine monophosphate, a mediator of vasorelaxation (Murray, 1990; Bunzow et al., 2001; Borowsky et al., 2001).

Thus, despite both clinical and experimental evidence that elevated plasma levels of PEA can alter blood pressure and vascular tone, the underlying mechanisms remain unclear. To date, in vitro studies have largely focused on the effects of PEA on conduit arteries rather than on the resistance vessels, which are the primary regulators of blood pressure and tissue perfusion in vivo. In this study, we have tested the hypothesis that PEA modulation of resistance artery tone is mediated by two mechanisms: an indirect sympathomimetic action on perivascular nerves and a direct interaction with α1-adrenoceptors on vascular smooth muscle cells.

**Materials and Methods**

**Effects of PEA on the Rat Perfused Mesenteric Vascular Bed**

Male Sprague-Dawley rats (250–350 g; Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada) were housed and euthanized according to the standards of the Canadian Council on Animal care and protocols approved by the Animal Care Committee of the Faculty of Medicine and Dentistry, University of Alberta. Rats were euthanized by isoflurane inhalation followed by decapitation. The abdomen was opened by a midline incision, and the whole mesenteric bed was removed and placed into ice-cold Krebs buffer containing (in mM): NaCl 119.0, NaHCO3 25.0, KCl 4.7, MgSO4 1.2, KH2PO4 1.18, glucose 11.0, disodium EDTA 0.027 and CaCl2 2.5. The superior mesenteric artery was cleaned of connective tissue, cannulated with a blunted hypodermic needle (20G), and flushed with Krebs buffer to remove blood. In some experiments, the endothelium was eliminated with a blunted hypodermic needle (20G), and flushed with Krebs containing (in mM): NaCl 119.0, NaHCO3 25.0, KCl 4.7, MgSO4 1.2, KH2PO4 1.18, glucose 11.0, disodium EDTA 0.027 and CaCl2 2.5. The endothelium was confirmed by the absence of relaxation in response to acetylcholine (1 μM) after vasoconstriction with methoxamine (1 μM).

**Transmural Stimulation of Perivascular Nerves.** Electrodes were attached to the cannulating needle and to the wire mesh to allow electrical field stimulation using a Grass SD9 stimulator (Grass Instruments Division, Astro-Med, Warwick, RI). After an equilibration period of 30 minutes, a single stimulation (30 Hz, 90 V, pulse width 1 millisecond, 30 seconds) was applied to assess the viability of the preparation. After a further 10 minutes, a frequency-response curve was constructed by stimulating the preparation at 1–40 Hz (90 V, pulse width 1 millisecond, 30 seconds) at 10-minute intervals (Pakdeechote et al., 2007). The effects of PEA (1–100 μM) or the α1-adrenoceptor antagonist rauwolscine (1 μM) on nerve-evoked vasoconstriction were assessed by perfusing the drugs through the lumen of the preparation for 30 minutes before constructing a second frequency-response curve. In some experiments a third frequency-response curve was constructed in the presence of either a higher concentration of PEA or PEA and rauwolscine in combination.

In experiments to determine whether the combination of PEA (30 μM) and rauwolscine (1 μM) caused rundown of nerve-mediated vasoconstriction, the perfused bed was repeatedly stimulated at 15 Hz for 30 seconds at 10-minute intervals in the absence and presence of the two drugs. Time control experiments were performed in which either three consecutive frequency-response curves or two rundown protocols were applied in the absence of any pharmacologic agent.

**Determination of Noradrenaline Overload in the Perfused Mesenteric Bed.** Rat mesenteric vascular beds were perfused with Krebs buffer (2 mL/min at 37°C with 95% O2/5% CO2). The preparations were stimulated at 30 Hz for 1 minute in the absence or presence of perfused PEA (10 and 100 μM). We collected 2 mL of perfusate in prechilled tubes for 1 minute at baseline (B), during stimulation (S), and 1, 4, and 9 minutes after stimulation. The tubes were centrifuged (1000g for 1 minute at 4°C) to remove blood and other particulate matter and stored at −80°C. The noradrenaline content of 50–100 μL samples was determined by high-pressure liquid chromatography (HPLC) coupled with electrochemical detection (Silverstone et al., 2012). In brief, reverse-phase HPLC was used to separate noradrenaline using a 3 μm octadecylsilane column of length 12.5 cm and internal diameter 4.6 mm. The mobile phase (pH 5.0) contained (in mM): sodium acetate (24.4), citric acid (16.1), and octanesulfonic acid (2.3) with methanol added at 13% (v/v), and was pumped through the column at 1 mL/min. Detection was via a dual electrode cell (5014A) coupled to an electrochemical detector (Coulochem II; ESA/Thermo Scientific, Chelmsford, MA) with a potential of −40 mV on one electrode and −250 mV on the second electrode, from which the signal was measured. Under these conditions, noradrenaline eluted as a well defined peak at 5.5 minutes. The amount of noradrenaline in the samples was determined by extrapolation from standard curves that were constructed during each HPLC session.

**Evaluation of Vasorelaxant Effects of PEA.** The potential role of endothelin in the vascular actions of PEA was assessed by constructing dose-response curves to bolus doses of PEA (10–3000 nmol; 20 μl injection volume) in endothelium-intact and endothelium-denuded perfused vascular beds in which tone was raised with methoxamine (30 μM and 1 μM, respectively). In endothelium-intact tissues, the effect of the NO synthase inhibitor l-nitroarginine methyl ester (L-NAME) on PEA-induced vasodilation was also assessed. In these experiments, perfusion pressure was allowed to stabilize between additions of PEA. Control injections of 20 μl of Krebs buffer allowed the injection artifact to be subtracted from responses elicited by PEA. The vasorelaxant effects of bolus doses of PEA were expressed as a percentage reversal of methoxamine-induced increases in perfusion pressure.

**Effects of PEA on Isolated Mesenteric Resistance Arteries**

After removal of the endothelium from the mesenteric bed by perfusion with 0.5% Triton X-100, third-order arteries were removed, cleaned of adhering fat and connective tissue, and cut into segments
(≈2 mm in length). Arterial segments were then mounted between two gold-plated tungsten wires (20 μm diameter) in a Mulvany-Halpern myograph (model 400A; JP Trading, Thisted, Denmark). Changes in isometric tension were recorded via a PowerLab 2/20 computerized data acquisition system using Chart 5.0 software (ADInstruments) (Plant et al., 2001). Tissues were maintained in Krebs buffer gassed with 95% O₂/5% CO₂ at 37°C and were set to a predetermined optimal resting tension of 5 mN. After an equilibration period of 60 minutes, successful removal of the endothelium was confirmed by the absence of relaxation in response to acetylcholine (10 μM) after pretreatment with methoxamine (3 μM; 75% of maximal tone). Cumulative concentration-response curves to PEA (1 μM to 1 mM) were constructed in arteries in which tone was raised to 75% of maximum with noradrenaline (3 μM), methoxamine (3 μM), phenylephrine (3 μM), prostaglandin F₂α (PGF₂α) (10 μM), or U46619 (9,11-dideoxy-9α,11α-methanoepoxyprostaglandin F₂α) (1 μM). The effects of PEA are expressed as a percentage reversal of agonist-induced tone. The EC₅₀ values were determined by fitting data to the Hill equation using GraphPad Prism (v.5.0d; GraphPad Software, San Diego, CA).

Radioligand Binding

Brains from different rats were collected and homogenates were prepared as described elsewhere (Lione et al., 1998), and the membrane pellets were stored at −70°C. Before use, the pellets were thawed and washed 4 times by resuspension in 10 volumes of assay buffer (Tris-HCl, pH 7.4 at 4°C) and repeated centrifugation (32,000g) to remove any endogenous inhibitors of binding. The protein content of the membrane preparations was determined using Coomassie blue and bovine serum albumin as a standard (Bradford, 1976). All binding studies were performed at 25°C in assay buffer (Tris-HCl, pH 7.4 at 25°C). Radioligand binding assays were performed using polypropylene 96-deep well plates.

To assess α₁-adrenoceptor binding, rat brain homogenates were incubated with 25 μl [³H]prazosin (0.84 nM; 2 × K₈), 25 μl water (determinations of total binding; quintuples), or WB4101 [(2-2',6'-dimethoxyphenoxethylamino)methylbenzo-1,4-dioxane] (2.7 μM final concentration; 1000 × K₈) to determine nonspecific binding; triplicates), 25 μl PEA (4.6 μM to 10 mM), and 75 μl brain homogenate (500 μg protein) prepared in the aforementioned assay buffer for α₁-adrenoceptor binding, the homogenates were incubated with 25 μl [³H]rauwolscine (4.0 nM; 1.75 × K₈), 25 μl water (determinations of total binding; triplicates), or yohimbine (1.8 μM final concentration; 1000 × K₈) to determine nonspecific binding; triplicates), 25 μl different concentrations of PEA (46 nM to 100 μM), and 75 μl brain homogenate (500 μg protein) prepared in binding assay buffer. Samples were incubated at 25°C for 30 minutes before rapid filtration through Whatman GF/B filter paper (Brandel Inc., Gaithersburg, MD), presoaked for 1 hour in cold polyethyleneimine (0.5%, v/v), in a Brandel cell harvester. Filters were washed rapidly 3 times with 600 μl of ice-cold assay buffer (pH 7.4) and were counted for tritium content in 3 ml of CytoScint cocktail (MP Biomedicals, Santa Ana, CA). Specific binding data were fitted to the Hill equation by nonlinear regression using GraphPad Prism (v.5.0d). Data were expressed as percentage of control binding, and the Kₛ was obtained from the IC₅₀ using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Drugs

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Radiolabeled prazosin (17-methoxy-³H)prazosin and rauwolscine ([methyl-³H]rauwolscine) were from PerkinElmer Life and Analytical Sciences (Waltham, MA). U46619 and prostaglandin F₂α were from Tocris Bioscience/R&D Systems (Minneapolis, MN) and Calbiochem/EMD Millipore (San Diego, CA), respectively. All drugs were dissolved in Krebs buffer except for rauwolscine, which was dissolved as a stock solution in water, and prazosin, which was dissolved as a stock solution in dimethylsulphoxide. Both drugs were then diluted into Krebs buffer.

Statistical Analysis

All results are expressed as mean ± S.E.M. for n observations, where n represents the number of animals from which tissues were removed. Statistical significance of functional responses and radioligand binding data were determined by Student's paired t test or two-way analysis of variance followed by Bonferroni’s post-hoc test where appropriate. P < 0.05 was considered statistically significant. Paired data for outflow of noradrenaline were analyzed by repeated measures one-way analysis of variance with the Greenhouse-Geisser correction for sphericity, followed by Tukey's multiple comparisons post-hoc test.

Results

PEA Causes Concentration-Dependent Changes in Nerve-Mediated Vasoconstriction. The mean baseline perfusion pressure in the rat perfused mesenteric bed was 5.88 ± 0.45 mm Hg (n = 42) and did not change significantly during any of the experiments, illustrating that PEA had no direct effect on vascular tone. Transmural nerve stimulation elicited frequency-dependent increases in perfusion pressure in endothelium-denuded mesenteric beds, as shown in the representative trace in Fig. 1. Repeated frequency-response curves showed that nerve-evoked responses did not significantly change over the time course of experiments (Fig. 2A). Perfusion with prazosin (100 nM) abolished nerve-evoked increases in perfusion pressure; the response to 40 Hz stimulation was reduced from 105 ± 5.9 to 20.1 ± 2 mm Hg (n = 4; P < 0.05). After perfusion with PEA (1 and 10 μM) concentration-dependent enhancement of nerve-evoked increases in perfusion pressure was observed (Fig. 2, B and C). In the continued presence of PEA (10 μM), addition of prazosin (100 nM) abolished nerve-evoked increases in perfusion pressure, confirming that the enhanced responses could be fully accounted for by the release of noradrenaline (Fig. 2C).

At a higher concentration of PEA (30 μM) the enhancement of nerve-mediated responses was not significantly different from that seen in the presence of 10 μM PEA (Fig. 2D). However, at 100 μM PEA, PEA significantly depressed nerve-evoked increases in perfusion pressure (Fig. 2E).

Autoinhibitory presynaptic α₂-adrenoceptors are key modulators of noradrenaline release, with antagonists at these receptors causing enhancement of noradrenaline release from peripheral nerves (Paton, 1975). In our experiments, the α₂-adrenoceptor antagonist rauwolscine (1 μM) caused a significant enhancement of nerve-mediated vasoconstriction (Fig. 3A). In the presence of both rauwolscine and PEA (30 μM), vasoconstrictor responses to lower frequencies were enhanced to a greater extent than with either agent alone, but the responses to high frequencies of nerve stimulation were depressed (Fig. 3A). To determine whether this depression could be due to time-dependent depletion of noradrenaline from nerve terminals, preparations were repeatedly stimulated at a frequency of 15 Hz for 30 seconds at 10-minute intervals. Under control conditions application of this stimulation protocol elicited reproducible increases in perfusion pressure that did not change over time. However, in the presence of rauwolscine (1 μM) and PEA (30 μM), an initial enhancement of responses was followed by a significant time-dependent attenuation of nerve-mediated vasoconstriction (Fig. 3B).

PEA Increases Outflow of Noradrenaline from the Perfused Mesenteric Bed. The effect of PEA on noradrenaline outflow elicited by stimulation of perivascular nerves
was measured by HPLC coupled with electrochemical detection. Application of a 30 Hz stimulus for 1 minute caused a significant increase in the concentration of noradrenaline in the perfusate, which returned to baseline after 10 minutes. As shown in Fig. 4, PEA (10 and 100 μM) significantly increased noradrenaline outflow (shown as fmol per 50 μl) both before and during nerve stimulation.

**PEA Reverses α₁-Adrenoceptor–Induced Increases in Vascular Tone.** Because it has been recently reported that PEA can cause vasorelaxation via the release of endothelium-derived NO, we investigated its effects on agonist-induced tone. Bolus doses of PEA (10–3000 nmol) were applied to perfused mesenteric preparations in which vasoconstriction was induced by methoxamine. In both endothelium-intact and endothelium-denuded mesenteric beds, bolus doses of PEA caused dose-dependent reversal of methoxamine-induced increases in perfusion pressure. In endothelium-intact preparations, these responses were not altered by prior administration of the NO synthase inhibitor L-NAME (100 μM; Fig. 5).

The ability of PEA to reverse vascular tone induced by other contractile agonists was further assessed in isolated segments of mesenteric artery mounted in a wire myograph. This approach was taken because the level of tone induced by all of the agonists under isometric conditions was more stable than in

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**Fig. 1.** Effect of PEA on nerve-evoked increases in perfusion pressure in the perfused rat mesenteric bed. Representative traces show frequency-dependent increases in perfusion pressure elicited by stimulation of perivascular nerves under control conditions and in the presence of 10 and 100 μM PEA.

**Fig. 2.** PEA has concentration-dependent effects on nerve-evoked vasoconstriction in the perfused rat mesenteric bed. Mean data show (A) repeated frequency-response curves for nerve-evoked increases in perfusion pressure, n = 4 in each case. These data illustrate the reproducibility of the responses. Frequency-response curves are shown for nerve-mediated increases in perfusion pressure in the absence and presence of PEA at (B) 1 μM (n = 6), (C) 10 μM (n = 11), (D) 30 μM (n = 8), and (E) 100 μM (n = 8). Panel C also shows the effect of prazosin (100 nM). *P < 0.05; ***P < 0.01 versus control.
A representative trace showing PEA-evoked relaxation of the perfused mesenteric preparation. PEA (1 μM to 1 mM) caused a concentration-dependent reversal of tone elicited by the α₁-adrenoceptor agonist noradrenaline, methoxamine, and phenylephrine, with EC₅₀ values of 51.7 ± 10.8 μM (n = 5), 68.2 ± 1.7 μM (n = 5), and 67.7 ± 16.7 μM (n = 5), respectively. A representative trace showing PEA-evoked relaxation of noradrenaline-induced tone is shown in Fig. 6A, and the mean data for each agonist are shown in Fig. 6, B–D. Critically, PEA had no effect on increases in vascular tone elicited by the prostanoid receptor agonist PGE₂ or the thromboxane A₂ mimetic U46619 (Fig. 6, E and F), and it should also be noted that PEA (up to 100 μM) had no effect on basal tone (data not shown).

**PEA Is a Ligand for α₁- and α₂-Adrenoceptors.** Because the functional studies showed that PEA could selectively reverse vascular tone induced by α₁-adrenoceptor agonists, the ability of PEA to bind to α-adrenoceptors was investigated. In binding studies using rat brain homogenates, PEA displaced the α₁-adrenoceptor ligand [³H]prazosin and the α₂-adrenoceptor ligand [³H]rauwolscine with Kᵢ values (mean and 95% confidence intervals) of 25.5 μM (17.1 to 37.9 μM) and 1.2 μM (0.8 to 1.9 μM), respectively (Fig. 7, A and B). The Kᵢ values determined in this study for prazosin (0.4 nM) and rauwolscine (23 nM), used to calculate Kᵢ values, were consistent with previously reported values in the literature (Raiteri et al., 1977; Agrawal and Daniel, 1985).

**Discussion**

These data provide the first direct demonstration that PEA can alter the tone of resistance arteries via dual indirect sympathomimetic and α₁-adrenoceptor blocking actions. These dual effects of PEA occur over similar concentration ranges, so the observed net effect of PEA on nerve-mediated vascular contractility is due to a balance between increased availability of noradrenaline due to inhibition of presynaptic autoinhibitory α₂-adrenoceptors and block of postsynaptic smooth muscle α₁-adrenoceptors (Supplemental Fig. 1). For example, at a concentration of 30 μM, PEA enhances nerve-mediated vasoconstriction as the increase in availability of noradrenaline is sufficient to overcome block of postsynaptic α₁-adrenoceptors. However, at a concentration of 100 μM, the degree of block of α₁-adrenoceptors by PEA is sufficient to prevent the contractile actions of the neurotransmitter, even though noradrenaline outflow is significantly increased.

At low micromolar concentrations, PEA (1–30 μM) enhanced nerve-mediated vasoconstriction of the perfused mesenteric vascular bed and caused concentration-dependent displacement of [³H]rauwolscine from α₂-adrenergic receptors in rat brain homogenates. The observed Kᵢ of ~1.2 μM is in line with other recent studies in which PEA was shown to bind to...
α₂-adrenoceptors expressed in Chinese hamster ovary cells ($K_i \sim 8 \mu M$; Ma et al., 2010). The α₂-adrenoceptor antagonist rauwolscine also increased nerve-mediated vasoconstriction in the perfused mesenteric bed and has previously been shown to act as a sympathomimetic agent by increasing noradrenaline outflow in the vasculature (Rump et al., 1992). Thus, we propose that enhancement of nerve-evoked vasoconstriction caused by PEA is due, at least in part, to enhanced release of noradrenaline from sympathetic nerves resulting from block of presynaptic inhibitory α₂-receptors.

Further support for this hypothesis comes from our measurements of noradrenaline levels which show that PEA (10 and 100 μM) enhanced noradrenaline levels in the perfusate during and after nerve stimulation. Furthermore, block of α₁-adrenoceptors by prazosin abolished nerve-evoked vasoconstriction in the presence of PEA, confirming that enhancement of responses was due to the release of noradrenaline and not to some other contractile factor. Although an indirect sympathomimetic action has been implicated in the pressor effect of trace amines on blood pressure in vivo (Meck et al., 2003) and in conduit arteries such as rabbit pulmonary and ear artery (Knoll et al., 1996), these data provide the first direct demonstration that PEA can significantly enhance nerve-mediated vasoconstriction and increase noradrenaline release in resistance arteries.

At lower frequencies of stimulation (5–15 Hz), the combination of rauwolscine and PEA acted synergistically to increase nerve-evoked vasoconstriction above that seen with either agent alone, indicating that other mechanisms may also contribute to the sympathomimetic actions of PEA. In brain synaptosomes, PEA inhibits noradrenaline uptake and also stimulates its release from storage vesicles with an EC₅₀ of around 1–5 μM (Snyder and Coyle, 1969; Coyle and Snyder, 1969; Horn and Snyder, 1972). Similarly, PEA inhibits uptake of noradrenaline in isolated rat heart (Burgen and Iversen, 1965) and directly increases noradrenaline efflux from adrenergic nerves in rabbit atria (Paton, 1975). In our experiments, baseline perfusion pressure in the mesenteric bed was unchanged throughout all experiments, suggesting that PEA did not directly release noradrenaline but rather modulated the release in response to nerve stimulation. Thus, although we did not directly measure the effects of PEA on noradrenaline reuptake into nerve terminals, the enhancement in nerve-mediated responses caused by PEA beyond that caused by antagonism of presynaptic α₂-adrenoceptors was most likely due to inhibition of reuptake into nerve terminals. This possibility was not further explored in this study due to the lack of selectivity of reuptake inhibitors such as desipramine (Bradley and Doggrell, 1985).

Stimulation of sympathetic nerves in the presence of prolonged block of α₂-adrenoceptors and inhibition of noradrenaline reuptake may be predicted to lead to depletion of noradrenaline levels within nerve terminals. Indeed, at 100 μM (4 times the $K_D$ so 80% receptor occupancy) PEA reversed α₁-adrenoceptor-mediated tone by 75–80% but had a greater effect on nerve-mediated vasoconstriction, indicating that depletion may also be a contributory factor. Furthermore, although enhancement of responses to lower frequency stimulation was observed in the presence of the combination rauwolscine and PEA, responses to higher frequencies were significantly depressed. The possibility that PEA may cause depletion of neuronal noradrenaline levels was thus investigated using a repetitive stimulation protocol at 15 Hz, and using this approach we demonstrated that application of both the α₂-antagonist rauwolscine and PEA together caused a time-dependent attenuation of nerve-mediated vasoconstriction, indicative of depletion of neuronal noradrenaline levels.

Fig. 6. PEA reverses α₁-adrenoceptor–induced tone. (A) Representative trace showing concentration-dependent relaxation of noradrenaline-induced tone by PEA in third-order rat mesenteric arterial segment. Mean concentration-response curves are shown for PEA-induced relaxations in arteries prestimulated with (B) noradrenaline ($n = 5$), (C) methoxamine ($n = 5$), (D) phenylephrine ($n = 5$), (E) PGE₂ ($n = 4$), and (F) U46619 ($n = 5$). Data were fitted to the Hill equation; EC₅₀ values thereby determined are indicated in the text.
PEA has previously been reported to act as a partial agonist at α₁-adrenoceptors in rat aorta (Hansen et al., 1980). However, in this study PEA had no effect on basal tone in vessels mounted in either the wire myograph or the perfused preparation and did not increase tone induced by U46619 or PGF₂α, indicating that it does not act as an agonist at α₁-adrenoceptors on vascular smooth muscle cells in mesenteric arteries. In fact, while lower concentrations of PEA (1–30 μM) enhanced nerve-mediated increases in perfusion pressure in the perfused mesenteric bed, in line with a direct sympathomimetic action, a higher concentration of PEA (100 μM) inhibited vasoconstriction in response to nerve stimulation, leading us to investigate whether PEA could in fact act as α₁-receptor antagonist.

We found that PEA is able to displace [³H]prazosin from α₁-adrenoceptors in rat brain homogenates with a Kᵢ around 25 μM, close to the 19 μM reported for PEA displacement of [³H]prazosin from α₁-adrenergic receptors stably expressed in human embryonic kidney 293 cells (Ma et al., 2010). Based on this result, we then used a functional assay to show that PEA could selectively reverse α₁-adrenoceptor–mediated increases in vascular tone. In the endothelium-intact rat perfused mesenteric bed, bolus doses of PEA caused dose-dependent reversal of methoxamine-induced vasoconstriction, an effect that, in contrast to a previous study (Anwar et al., 2012), was independent of the endothelium. The reason for the discrepancy is unclear although in the previous study the removal of the endothelium was not attempted and the concentration of L-NAME applied was relatively high (1 mM), raising the possibility of nonselective effects (Buxton et al., 1993; Wang et al., 1993).

Further confirmation that PEA acts to reverse vascular tone in an endothelium-independent manner via a selective interaction with smooth muscle α₁-adrenoceptors was then obtained from experiments using isolated endothelium-denuded mesenteric arteries mounted under isometric conditions. This approach was used because the increases in tone elicited by the different agonists were more stable under isometric conditions than in the perfused vascular bed, thus allowing a more quantitative assessment of vasorelaxation. PEA caused concentration-dependent relaxations of arterial segments in which tone was raised by three different α₁-adrenergic receptor agonists but was ineffective versus increases in tone induced by the prostaglandin receptor agonist PGF₂α or the thromboxane mimetic U46619. The EC₅₀ value for the relaxant effect of PEA against α₁-adrenoceptor agonist-induced tone was ~60 μM, higher than the ~30 μM observed in the perfused preparation, but this difference is most likely due to the higher concentration of contractile agonist required to raise tone in isolated vessels mounted under isometric conditions (3 μM versus 1 μM).

Although PEA was ineffective against U46619- or PGF₂α–induced tone, the β₂-adrenoceptor agonist isoprenaline was equally effective in causing relaxation of tone elicited by all agonists, thus ruling out a role for β₂-adrenoceptors in PEA-mediated vasorelaxation (data not shown).

Reports that TAARs, G protein–coupled receptors activated by a variety of biogenic amines, amphetamines, and trace amines, are expressed within blood vessels (Borowsky et al., 2001; Bunzow et al., 2001) has created renewed interest in the vascular actions of trace amines such as PEA. Inhibition of the noradrenaline uptake and increased efflux stimulated by PEA were only observed in brain synaptosomes expressing TAARs, suggesting that the sympathomimetic actions of the trace amine may be due to interaction with TAARs (Xie and Miller, 2008). In isolated conduit arteries, PEA-induced increases in tone were ascribed to activation of TAARs largely due to the fact that mRNA for TAARs was found to be expressed in the same vessels, and the large disparity between the millimolar concentrations used to elicit changes in tone and the nanomolar concentrations shown to activate TAARs in expression systems were not taken into account (Broadley et al., 2009; Fehler et al., 2010).

PEA has been suggested to be a partial agonist at rat TAAR1, which means that the Kᵢ would be similar to its EC₅₀ (240 nM; Bunzow et al., 2001), which is consistent with functional data on monoamine transport in the brain (Xie and Miller, 2008). Accordingly, it is likely that in our preparations and in previous studies of conduit vessels (Broadley et al., 2009; Fehler et al., 2010) TAAR1 would be fully occupied across the concentration range of PEA used and that there would be no concentration-dependence to the effects of PEA. We observed clear concentration dependence for responses to PEA, with only the most minor of effects evident at the lowest concentrations of PEA used that would nevertheless give maximal stimulation of any TAAR1.
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**Authorship Contributions**


Wrote or contributed to the writing of the manuscript: Kerr, Holt, Plane, Narang.

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