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Non-adrenergic vasoconstriction and vasodilatation of guinea-pig aorta by β -phenylethylamine and amphetamine – role of nitric oxide determined with L-NAME and NO scavengers

Kenneth J Broadley and Harrison D Broadley

Division of Pharmacology, Cardiff School of Pharmacy & Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cathays Park, Cardiff Wales, CF10 3NB, UK

Corresponding author: Kenneth J Broadley, Division of Pharmacology, Cardiff School of Pharmacy & Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cathays Park, Cardiff Wales, CF10 3NB, UK. E-mail: BroadleyKJ@Cardiff.ac.uk

ABSTRACT

Sympathomimetic and trace amines, including β -phenylethylamine (PEA) and amphetamine, increase blood pressure and constrict isolated blood vessels. By convention this is regarded as a sympathomimetic response, however, recent studies suggest trace amine-associated receptor (TAAR) involvement. There is also uncertainty whether these amines also release nitric oxide (NO) causing opposing vasodilatation. These questions were addressed in guinea-pig isolated aorta, a species not previously examined. Guinea-pig aortic rings were set up to measure contractile tension. Cumulative concentration-response curves were constructed for the reference α -adrenoceptor agonist, phenylephrine, PEA or d-amphetamine before and in the presence of vehicles, the α_1 -adrenoceptor antagonist, prazosin (1 μ M), the nitric oxide synthase inhibitor, N^o-nitro-L-arginine (L-NAME), or NO scavengers, curcumin and astaxanthin. Prazosin inhibited phenylephrine contractions with low affinity consistent with α_{1L} -adrenoceptors. However, PEA and amphetamine were not antagonised, indicating non-adrenergic responses probably via TAARs. L-NAME potentiated contractions to PEA both in the absence and presence of prazosin, indicating that PEA releases NO to cause underlying opposing vasodilatation, independent of α_1 -adrenoceptors. L-NAME also potentiated amphetamine and phenylephrine. PEA was potentiated by the NO scavenger astaxanthin but less effectively. Curcumin, an active component of turmeric, however, inhibited PEA. Trace amines therefore constrict blood vessels non-adrenergically with an underlying NO-mediated non-adrenergic vasodilatation. This has implications in the pressor actions of these amines when NO is compromised.

Keywords:

Guinea-pig aorta

Amphetamine

β -phenylethylamine

Nitric oxide

NO-scavengers

Curcumin

Chemical compounds studied in this article:

d-Amphetamine hydrochloride (PubChem CID: 5826); astaxanthin (PubChem CID: 5281224); curcumin (PubChem CID: 969516); L-NAME (PubChem CID: 74764530); L-phenylephrine hydrochloride (PubChem CID: 5284443); 2-phenylethylamine hydrochloride (PubChem CID: 1001); prazosin hydrochloride (PubChem CID: 68546).

1. Introduction

Trace amines including β -phenylethylamine (PEA) and tyramine occur in the body in trace amounts and are widespread in our diet (Burchett and Hicks, 2006). They cause vasoconstriction in isolated blood vessels including aortic rings from rats (Maling et al., 1971; Krishnamurty and Grollman, 1972; Fehler et al., 2010) guinea-pigs (Maling et al., 1971) and rabbits (Maling *et al.*, 1971) and porcine coronary arteries (Herbert et al., 2008). This is reflected *in vivo* as a pressor response, oral administration of tyramine to humans increasing blood pressure (Peatfield et al., 1983). In animals, intravenously administered tyramine and PEA increase the blood pressures of rats (Day, 1967; Liles et al., 2006; Khwanchuea et al., 2008), cats (Burn and Rand, 1958; Day, 1967), dogs (Kohli and Goldberg, 1982; Woodman and Pannangpetch, 1994) and rabbits (Du et al., 1992).

The conventionally accepted mechanism for these amines is that they are indirectly acting sympathomimetic amines releasing noradrenaline from sympathetic neurones onto vascular α_1 -adrenoceptors causing vasoconstriction and a rise in blood pressure (Broadley, 2010). However, emerging evidence suggests this mechanism may not entirely explain the vasoconstriction since we have shown that the vasoconstriction by PEA of rat isolated aorta (Fehler et al., 2010; Broadley et al., 2013) and pig coronary artery (Herbert et al., 2008) is not inhibited by the α_1 -adrenoceptor antagonist, prazosin. We proposed that the vasoconstriction was therefore due to an action on trace amine-associated receptors (TAARs) which were identified in the rat aorta (Fehler et al., 2010).

In other isolated blood vessels such as the rat perfused mesenteric bed, tyramine and PEA, cause vasodilatation rather than vasoconstriction (Anwar et al., 2012) which was blocked by the nitric oxide synthase (NOS) inhibitor, N^o-nitro-L-arginine (L-NAME), and attributed to nitric oxide (NO) release (Anwar et al., 2012). Tyramine infusion to humans increased systolic blood pressure but increased forearm blood flow which also indicated a paradoxical vasodilatation (Jacob et al., 2003). This raises the question whether vessels that display a predominant vasoconstriction, such as the aorta, also exhibit an underlying vasodilatation mediated via NO. In an earlier study we found no effect of L-NAME on the vasoconstriction by PEA in rat aorta (Fehler et al., 2010). However, L-NAME potentiated the vasoconstriction by tryptamine in rat mesenteric vascular beds (Anwar et al., 2013) and the pressor response to tyramine in conscious rabbits (Du et al., 1992). Therefore the present study aimed to resolve these discrepancies using guinea-pig aorta, a species not previously employed to study non-adrenergic vascular responses to PE. We examine the hypothesis that there is an underlying NO-mediated vasodilator response to PEA and amphetamine by using both the NOS inhibitor L-NAME and a novel approach of scavenging NO with curcumin and astaxanthin (Sumanont et al., 2004). This study therefore additionally examined curcumin, the active constituent of turmeric (Ravindran, 2007), on the vascular responses to trace amines and α -adrenoceptor agonists. Anti-inflammatory and anti-allergic properties of curcumin are well known (Kurup and Barrios, 2009) but there is little information on its cardiovascular effects.

2. Materials and methods

2.1. *Guinea-pig isolated aortic rings*

Male Dunkin-Hartley guinea-pigs (250-350g) (Charles River, U.K.) were given one week to acclimatise with their new surroundings before commencement of experiments. They were housed in flat bottomed cages with environmental enrichment in the form of cardboard tubes and hay and were given food and water *ad-libitum*. The housing room conditions were: twelve hour light/dark cycles, at 50% humidity and room temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Guinea-pigs were killed by cervical dislocation and exsanguination. The guidelines for the care and use of laboratory animals were followed according to the Animals (Scientific Procedures) Act 1986. The work and its reporting were undertaken according to the principles for transparent reporting and scientific rigour of preclinical research as set out in the Basel Declaration (McGrath et al., 2015).

The thoracic aorta was removed and cut into at least four ring sections approximately 0.5 cm long, through which were passed fixed and mobile hangers. The fixed hanger was secured in a 50 ml organ bath. The bath was filled with pre-warmed (37°C) Krebs-bicarbonate buffer gassed with CO_2/O_2 (5%/95%) (BOC Gases, Guildford, UK). The Krebs bicarbonate buffer was made up in distilled water and had the following composition (mM): NaCl (118), NaHCO_3 (25), glucose (11.7), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.2), KH_2PO_4 (1.2), KCl (4.7) and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.5). Organ baths were maintained at $37 \pm 0.5^{\circ}\text{C}$ by a circulator (type KD Grant Instruments, Cambridge, UK). A suture attached to the upper mobile hanger was connected to an isometric transducer (Dynamometer UF1, 57 g sensitivity range, Pioden Controls Ltd., Canterbury, UK) and a resting tension of 1.5 g was applied. Isometric tension was measured and displayed on a computer (Power Lab, Chart 5, ADInstruments, Chalgrove, Oxfordshire, UK). To check that functional endothelium was not removed by this set-up procedure, in a selection of tissues prior to commencing the protocol, acetylcholine (100 μM) was added to aortic rings precontracted with U46619 (1 μM). Small vasodilator responses of 0.07 ± 0.02 g ($n=6$) were observed, which represented $7.1 \pm 3.0\%$ of the contraction to U46619 (1.33 ± 0.27 g). It could therefore be concluded that a functional endothelium was present, although this was relatively minor compared with rat aorta where acetylcholine relaxed U46619-induced contractions by $72 \pm 4\%$ (Bullock et al., 1986).

2.2. *Experimental protocol*

After 1 hour equilibration, a cumulative concentration-response curve (CRC) for β -phenylethylamine (PEA), amphetamine or phenylephrine was obtained by addition of half logarithmic increments in concentration, each successive concentration being added after the peak effect was reached for the preceding concentration. After the maximum effect, the tissue was washed and again after approximately 15 min to restore baseline. A second CRC was then constructed in the presence of inhibitors, their vehicles or nothing (control). Inhibitors and vehicles were left in contact with the tissue for 15 min before commencing the second CRC. At the end of each experiment, isotonic KCl (60 mM) was routinely added. It was decided to add the KCl without washout to avoid further decline in the baseline before adding

the KCl, which may have affected its response. Also, we wanted to measure the KCl maximum in the presence of the maximum effect of agonist. It must be admitted that the presence of the antagonist, however, may have influenced the KCl maximum. The second CRC was routinely found to be potentiated as described in the results section. However, we elected not to produce three CRCs and discard the first because this would have added another confounding factor and secondly we were interested in distinguishing this potentiating effect from effects of inhibitors.

2.3. *Analysis of results*

Contractions at the plateau response to each concentration of agonist were measured from the baseline before the CRC. These were then expressed as a percentage of the contraction to KCl in each experiment, to normalize each response to the maximum contractility of each tissue. The mean responses (\pm S.E.M.) were then plotted. *n* values are the number of guinea-pigs providing aortae. Maximum responses before and after inhibitors and at individual concentrations were compared by paired Student's *t*-tests. The entire curves before and after inhibitors were compared by repeated-measures two-way analysis of variance (ANOVA). EC₂₀ values were calculated as the molar concentration required to produce 20% of the maximum response to KCl. This was to ensure that values were obtained for all tissues as not all reached 50% of the KCl maximum contraction. These were converted to the $-\log$ EC₂₀ values and the mean values (\pm S.E.M.) calculated. They were compared by Student's paired *t*-tests. Differences were considered significant when $P \leq 0.05$. CRCs obtained before and after prazosin or its vehicle were plotted as a percentage of the first curve maximum response so that dose-ratios for the shifts of CRCs could be calculated from the true EC₂₀ values. The dose-ratio (DR) was calculated as the difference in the $-\log$ EC₂₀ values in the absence and presence of prazosin and the $-\log$ K_D was calculated from the equation: $-\log$ K_D = \log [A] - \log (DR-1), where A is the molar concentration of antagonist.

2.4. *Drugs used*

D-Amphetamine sulphate, astaxanthin, curcumin, N^ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), prazosin hydrochloride, (-)-phenylephrine hydrochloride, β-phenylethylamine hydrochloride (PEA) and U46619 (9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α) were obtained from Sigma-Aldrich, (Poole, Dorset, UK). All chemicals for the Krebs-bicarbonate buffer were of analytical grade and were obtained from Fisher Scientific, Leicestershire, UK. Amphetamine, PEA, L-NAME and phenylephrine were dissolved in distilled water. Prazosin hydrochloride was dissolved in dimethylsulfoxide (DMSO):distilled water (1:10) and further diluted 1 in 10 with DMSO:water (1:10). Curcumin and astaxanthin were dissolved in neat DMSO. The amounts of DMSO in contact with the tissues were 0.04μl and 4μl, respectively.

3. Results

3.1. *Effects of prazosin on contractions to PEA, phenylephrine and amphetamine*

β -Phenylethylamine (PEA) caused concentration-related constriction of the guinea-pig aorta with a $-\log EC_{20}$ of 3.98 ± 0.21 (Fig. 1A). There was a small upwards shift of the CRC in the presence of DMSO (1:10) which was significant at the maximum (Fig. 1B). However, in the presence of the α_1 -adrenoceptor antagonist, prazosin (1 μ M), the CRC was not affected (Fig. 1C). The α -adrenoceptor agonist, phenylephrine, also caused dose-related contractions of the guinea-pig aorta, with a $-\log EC_{20}$ value of 5.61 ± 0.16 . These responses were enhanced on repeating in the control experiments in the presence of 1:10 DMSO (Fig. 2A). In the presence of prazosin (1 μ M), the CRC was displaced to the right (Fig. 2B). The dose-ratio (DR) for the shift of the mean CRCs at the EC_{20} was 30.5 which yielded a $-\log KD$ value of 7.47 ± 0.09 calculated from the individual dose-ratios. Amphetamine caused concentration-related constriction of guinea-pig aortic rings which was not affected in the presence of the DMSO (1:10) vehicle (Fig. 3A). Prazosin (1 μ M) potentiated the responses, significantly shifting the CRC upwards and to the left (Fig. 3B). The mean $-\log EC_{10}$ values in the presence and absence of prazosin were 4.0 ± 0.3 and 3.2 ± 0.3 respectively.

3.2. *Effects of L-NAME on contractions to PEA, amphetamine and phenylephrine*

In control experiments, the PEA CRC was not affected in the presence of the distilled water vehicle (Fig. 4A). However, in the presence of the NO synthase inhibitor, L-NAME (100 μ M), there was a significant upwards shift of the CRC (Fig. 4B). The maximum response was significantly increased from 59.1 ± 3.6 to 87.5 ± 2.8 %KCl. To assess whether L-NAME would still potentiate the response to PEA when α_1 -adrenoceptors were blocked and therefore PEA could not be constricting the aorta through α_1 -adrenoceptors, these experiments were repeated in the presence throughout of prazosin (1 μ M). As before, L-NAME (100 μ M) caused significant potentiation of the vasoconstriction to PEA, the CRC was elevated and the maximum response was significantly increased from 55.3 ± 6.4 to 86.9 ± 3.5 %KCl (Fig. 4C). L-NAME had no effect on the resting tension and in the presence of prazosin the resting tension was 1.45 ± 0.13 g before adding L-NAME, 1.44 ± 0.13 g immediately after L-NAME and 1.42 ± 0.12 g at 15 min after adding L-NAME.

In amphetamine control experiments, there was a small increase in the amphetamine CRC in the presence of distilled water, the maximum increasing from 36.0 ± 3.3 to 44.7 ± 1.1 %KCl (Fig. 5A). However, in the presence of L-NAME (100 μ M), the CRC was substantially raised and the maximum contraction was significantly increased from 42.0 ± 1.0 to 69.0 ± 2.5 %KCl (Fig. 5B).

There was a small but significant increase in the maximum response for phenylephrine in the control experiments in the presence of distilled water from 70.7 ± 4.1 to 78.9 ± 4.9 %KCl (Fig. 6A). In the presence of L-NAME (100 μ M), the maximum response was also significantly

raised from 75.8 ± 3.2 to 94.0 ± 0.8 %KCl (Fig. 6B). This increase was significantly greater than for the control experiments when measured as the mean differences in response between first and second curve, which were 8.1 ± 2.4 %KCl for the control and 18.2 ± 1.9 %KCl for the presence of L-NAME. L-NAME had no effect on the resting tension, which was 1.36 ± 0.09 g before and 1.30 ± 0.09 g at 30 min after adding L-NAME.

3.3 *Effects of curcumin and astaxanthin on contractions to phenylephrine and PEA*

In the presence of the NO scavenger, curcumin ($100 \mu\text{M}$), the contractions of the aorta to phenylephrine were unaffected. In contrast, the contractions to PEA were significantly reduced by this concentration of curcumin from 103.4 ± 4.9 to 74.5 ± 2.7 %KCl at the maximum response (Fig. 7B). The bath turned orange when curcumin was added. In the presence of phenylephrine but not PEA, this faded, suggesting some reaction with phenylephrine. The tissue became coated with an orange deposit in both experiments. Curcumin was dissolved in neat DMSO and the control experiments in the presence of an equivalent volume of neat DMSO showed no effect upon PEA contractions (Fig. 7C). Astaxanthin ($100 \mu\text{M}$), another NO scavenger, exerted a small but significant potentiation of the maximum contraction to PEA from 72.1 ± 3.2 to 81.6 ± 6.2 %KCl, although the whole CRC was not significantly affected (Fig. 7D).

4. Discussion

β -Phenylethylamine (PEA) and amphetamine are traditionally known as sympathomimetic amines (Broadley, 1996). However, they may also be categorized as agonists at trace amine-associated receptors (TAARs) (Bunzow et al., 2001). PEA and amphetamine exerted vasoconstriction of guinea-pig isolated aortic rings, similar to the α -adrenoceptor agonist, phenylephrine. Phenylephrine was antagonised by the α_1 -adrenoceptor antagonist, prazosin. The $-\log K_D$ value for prazosin of 7.47 ± 0.09 is two orders of magnitude less potent than the values of 9.9 and 9.8 obtained previously in rat aorta (Hussain and Marshall, 1997; Kenny et al., 1995). However, it is similar to the value of 7.83 obtained by Yamamoto and Koike (1999) in guinea-pig thoracic aorta. Therefore the α -adrenoceptor subtype mediating contraction of the guinea-pig aorta is similar pharmacologically to α -adrenoceptors in human lower urinary tract and rabbit mesenteric artery and urethra where it has been designated as an α_{1L} -adrenoceptor subtype (Flavahan and Vanhoutte, 1986). In the rat aorta, in contrast, the receptors belong to the α_{1D} -subtype (Kenny et al., 1995; Hussain and Marshall, 1997).

While prazosin shifted the concentration-response curve for phenylephrine, it did not antagonise PEA. This demonstrates for the first time in guinea-pig aorta that the vasoconstriction to PEA is not mediated via α -adrenoceptors and that it is not acting as a sympathomimetic amine. Thus, the vasoconstriction cannot be explained by the classical indirect sympathomimetic action of noradrenaline release from noradrenergic neurones onto α -adrenoceptors. This observation confirms our previous studies where the vasoconstriction

by PEA of rat isolated aorta (Fehler et al., 2010; Broadley et al., 2013) and pig coronary artery (Herbert et al., 2008) was not inhibited by prazosin. Narang et al. (2014) have shown that PEA binds to both α_1 - and α_2 -adrenoceptors in rat brain homogenates and propose that it is an antagonist at these receptors. Thus, the contraction to PEA is unlikely due to agonist activity at α_2 -adrenoceptors. Indeed, we have eliminated α_2 -adrenoceptors, since the contractions of rat aorta were not inhibited by yohimbine, which antagonised contractions to clonidine (Broadley et al., 2013). We conclude that the response is mediated via TAARs which we have shown to be present in rat aorta (Fehler et al., 2010). In the case of amphetamine, rather than exert no effect on the contractions, prazosin *potentiated* the contractions. We did not observe this effect previously on rat aorta (Broadley et al., 2013), where prazosin was not used alone but in combination with cocaine, pargyline and ICI-118,551 to block neuronal uptake, monoamine oxidase and β_2 -adrenoceptors, respectively. The reason for this potentiation is unclear. It must arise from blockade by prazosin of an opposing inhibitory action of amphetamine. This is unlikely to be an α_1 -adrenoceptor-mediated response since this would be contractile not vasodilator. It is possible that amphetamine releases a vasodilator substance in the vascular wall, such as nitric oxide (NO), which is inhibited by prazosin. However, there is no evidence in the literature for prazosin having nitric oxide synthase inhibitory properties. Could it be a NO scavenger similar to curcumin and astaxanthin which we discuss later? The degree of potentiation is substantially greater than observed with astaxanthin (see later) at a concentration 100-fold greater. It is therefore unlikely to be a NO scavenging effect. Further work would be required to explain this interesting property of amphetamine.

Having established that PEA and amphetamine constrict guinea-pig aorta by a non-adrenergic mechanism, we then examined whether these amines cause an opposing vasodilatation by using the NOS inhibitor, L-NAME. In the presence of L-NAME, both PEA and amphetamine were significantly potentiated. This result is at variance with our previous studies in which L-NAME had no effect on the vasoconstriction by PEA in rat aorta (Fehler et al., 2010). However, the vasoconstriction of rat mesenteric bed by tryptamine was potentiated by L-NAME (Anwar et al., 2013) and Du et al. (1992) showed that L-NAME enhanced the pressor response to tyramine in conscious rabbits. The previous study in rat aortae was probably not sufficiently robust to enable the potentiation to be detected. For example, only single dose-response curves were constructed in each preparation either in the absence or presence of L-NAME. The between-tissue variance was therefore too great to permit identification of the potentiation. Furthermore, in the previous study we were not aware that the non-adrenergic responses to PEA and other trace amines develop more slowly than the α -adrenoceptor-mediated responses to phenylephrine (Broadley and Richards, 2015). It was therefore necessary to allow each concentration to fully contract the tissue which was usually about 20 min. The potentiating action of L-NAME was confirmed with amphetamine. Both amines therefore release NO which exerts an opposing vasodilator action which, when prevented by nitric oxide synthesis inhibition, allows the full vasoconstrictor action to occur.

The question arises whether this release of NO is due to α -adrenoceptor stimulation since it is known that α -adrenoceptor agonists release NO, probably from the endothelium. L-NAME

potentiated the contractions to phenylephrine of rat aortic rings with intact endothelium. There was also a small significant potentiation of the constrictions to phenylephrine in the control experiments. However, the potentiation by L-NAME was significantly greater. This potentiation has been attributed to inhibition by L-NAME of NO release from the endothelium via α_1 -adrenoceptors (Tabernero et al., 1996). Similarly, in rat cremaster arterioles, L-NAME potentiates the vasoconstriction by phenylephrine through inhibition of the α_1 -adrenoceptor-mediated release of NO from the endothelium (Tuttle and Falcone, 2001). In other blood vessels, such as the rabbit isolated pulmonary artery, NO can be released from the endothelium by α_2 -adrenoceptor stimulation (MacLean *et al.*, 1993). Thus, did L-NAME potentiate PEA because PEA stimulates NO release from the endothelium via α_1 -adrenoceptors? To answer this question L-NAME was examined in the presence of prazosin to block α_1 -adrenoceptors. The vasoconstriction was still potentiated to the same extent, thus eliminating a role for α_1 -adrenoceptors in NO release by PEA. Whether TAARs are involved in the response is a matter for further study. The potentiation of these three amines by L-NAME was not due to a common inhibition of baseline NO production because addition of L-NAME to the tissues did not cause any increase in baseline tension. Further support for this conclusion was the observation that the potentiation of contractions by L-NAME was not by a parallel shift of the dose-response curves but by increases only towards the maximum. If it was due to increases in baseline, the lower doses of agonist would also be potentiated.

The potentiation of phenylephrine on repeating a second CRC in control experiments is a phenomenon that has been observed before (Demirel and Türker, 1989; Ford and Broadley, 1999). Although Demirel and Türker (1989) attributed this potentiation to the presence of the endothelium, it is generally regarded as due to an increase in the myofilament sensitivity to calcium induced by the α_1 -adrenoceptor stimulation during the first exposure (Nishimura et al., 1989) and is known as Ca^{2+} sensitization (Somlyo and Somlyo, 1993). This effect was not observed with PEA when distilled water was the vehicle, but there was a potentiation when 1 in 10 DMSO was the vehicle. It is not clear why this discrepancy occurred but does suggest that PEA may also cause Ca^{2+} sensitization under certain circumstances.

Next we examined an alternative mode of inhibiting levels of NO; by use of the NO scavengers curcumin and astaxanthin. This would identify whether such an approach was appropriate for studying the role of NO in pharmacological responses. Both curcumin, a component of turmeric powder, and astaxanthin, the pigment providing colour to salmon and shrimp, are NO scavengers with *in vitro* IC_{50} values of 20.39 ± 4.10 and 3.42 ± 0.50 μM , respectively (Sumanont et al., 2004). We used 100 μM of both substances, which was therefore well in excess of their IC_{50} values. Curcumin had no effect on phenylephrine contractions and therefore did not mimic NOS inhibition by L-NAME. However, curcumin unexpectedly caused inhibition of the contractions to PEA. One possibility is that curcumin is relatively unspecific and scavenges other reactive oxygen species (ROS) such as superoxide anion. ROS degrade NO to form peroxynitrite thus lowering its levels. Indeed, the free radical scavenger, edaravone, increases the levels of NO in the microcirculation rather than reducing it (Yamashita et al., 2013). To further illustrate the complex interactions that may

occur between NO and other reactive oxygen species, it has been shown that L-DOPA can inhibit NO-dependent vasorelaxation and therefore potentiate phenylephrine contractions of the aorta by generating ROS which scavenge the NO (Yanhua et al., 2009). Thus, a non-specific scavenger such as curcumin may exert the opposite effect to what was expected. However, this explanation seems unlikely because the same did not occur with contractions to phenylephrine, which like PEA was potentiated by L-NAME. It is tempting to suggest that we have identified an antagonist of PEA at the TAAR. Further studies on the selectivity of this antagonism are indicated but the present studies have shown for the first time an action of curcumin on vascular responses. Astaxanthin showed a small significant potentiation of the PEA contraction but was still not as effective as L-NAME. Astaxanthin fed to hypertensive rats lowered blood pressure (Hussein *et al.*, 2005), which was attributed to normalization of sympathetic sensitivity, although our results would suggest an opposing vasoconstriction through NO scavenging. It is possible that a further increase in our concentration of astaxanthin may have yielded greater potentiation, but clearly this method is not as effective as NOS inhibition. It also suffers from possible non-selective effects arising from scavenging other radicals and inhibitory actions at several other sites. It is therefore not recommended for studying NO scavenging activity.

5. Conclusions

The trace amine, β -phenylethylamine (PEA), and amphetamine exerted vasoconstriction in guinea-pig aorta which was not inhibited by prazosin. PEA and amphetamine do not therefore exert vasoconstriction of guinea-pig aorta through α_1 -adrenoceptors but most likely through trace amine-associated receptors (TAARs). The responses to PEA and amphetamine were potentiated by L-NAME indicating an opposing NO-mediated vasodilatation also not via α_1 -adrenoceptors. Nitric oxide scavengers were not an effective means of demonstrating the role of NO in these vascular responses. The underlying vasodilatation by these trace amines is consistent with dominant vasodilatation in other vascular beds such as the rat isolated mesentery (Anwar et al., 2012). The relative importance of vasoconstriction and vasodilatation to trace amines clearly depends upon the location and type of blood vessel. Since the vasodilator response is NO-mediated, any disruption of the NO pathways would have implications in the overall responses to trace amines. For example, in inflammatory conditions generating excessive reactive oxygen species, NO could be removed to form peroxynitrite and expose enhanced vasoconstriction.

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Author contributions

K.J.B. contributed the original concept and experimental design, manuscript preparation. K.J.B. and H.D.B made equal contributions to data acquisition, analysis, interpretation and presentation, and to revision of the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Figure Legends

Fig. 1. Contractions of guinea-pig aorta to β -phenylethylamine (PEA). A. Typical cumulative concentration-response curve for PEA with KCl (60 mM) added at the maximum dose. B. Control experiments with PEA concentration-response curves added before (■) and repeated in the presence of DMSO (1 in 10) (◆)(n=4). C. Concentration-response curves for PEA before (■) and in the presence of prazosin (1 μ M, ◆) (n=4). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between with and without DMSO, $P \leq 0.05$. CRCs were significantly different before and after DMSO (1:10) by two-way ANOVA.

Fig. 2. Effect of prazosin (1 μ M) on the contractions of guinea-pig aorta to phenylephrine. A. Control experiments with phenylephrine concentration-response curves added before (■) and repeated in the presence of DMSO (1 in 10) (◆) (n=4). B. Concentration-response curves for phenylephrine before (■) and in the presence of prazosin (1 μ M, ◆) (n=4). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction of the first concentration-response curve. * Significant difference between with and without prazosin $P \leq 0.05$. CRCs were significantly different before and after DMSO (1:10) and before and after prazosin by two-way ANOVA.

Fig. 3. Effect of prazosin (1 μ M) on the contractions of guinea-pig aorta to d-amphetamine. A. Control experiments with d-amphetamine concentration-response curves added before (■) and repeated in the presence of DMSO (1 in 10) (◆) (n=4). B. Concentration-response curves for d-amphetamine before (■) and in the presence of prazosin (1 μ M, ◆) (n=4). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between with and without prazosin $P \leq 0.05$. CRCs before and after prazosin were significantly different by two-way ANOVA.

Fig. 4. Effect of L-NAME (100 μ M) on the contractions of guinea-pig aorta to β -phenylethylamine (PEA). A. Control experiments with PEA concentration-response curves added before (■) and repeated in the presence of vehicle (distilled water, ◆) (n=6). B. Concentration-response curves for PEA before (■) and in the presence of L-NAME (◆) (n=6). C. Concentration-response curves for PEA before (■) and in the presence of L-NAME (◆) (n=6), both curves in the presence of prazosin (1 μ M). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between with and without L-NAME $P < 0.05$. CRCs before and after distilled water not significantly different but CRCs before and after L-NAME with and without prazosin were significantly different by two-way ANOVA.

Fig. 5. Effect of L-NAME (100 μ M) on the contractions of guinea-pig aorta to d-amphetamine. A. Control experiments with PEA concentration-response curves added before (■) and repeated in the presence of vehicle (distilled water, ◆) (n=4). B. Concentration-response curves for d-amphetamine before (■) and in the presence of L-NAME (◆) (n=4). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between with and without L-NAME $P \leq 0.05$. CRCs before and after distilled water and before and after L-NAME were significantly different by two-way ANOVA.

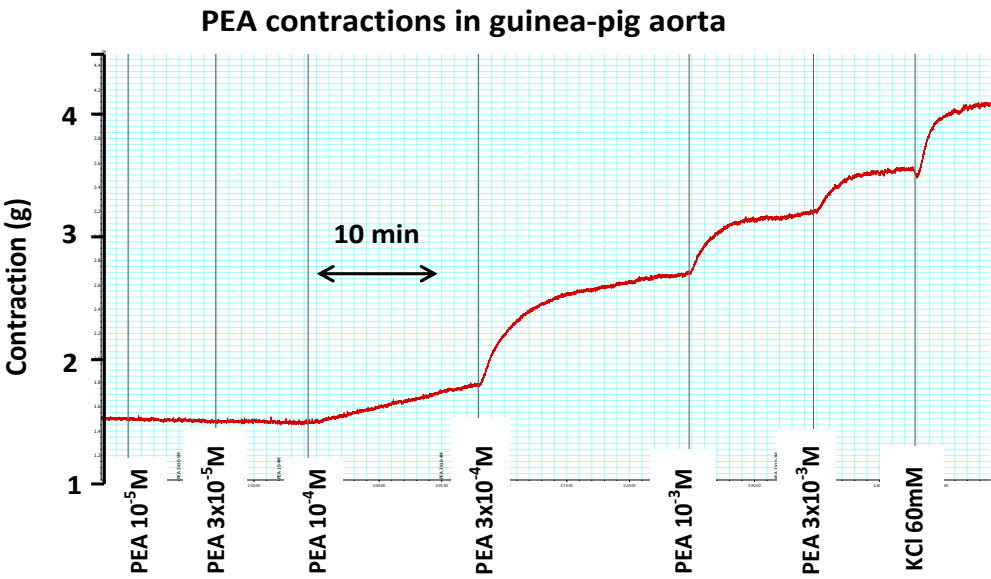
Fig. 6. Effect of L-NAME (100 μ M) on the contractions of guinea-pig aorta to phenylephrine. A. Control experiments with phenylephrine concentration-response curves added before (1st curve, ■) and repeated in the presence of vehicle (distilled water, 2nd curve ◆) (n=6). B. Concentration-response curves for phenylephrine before (■) and in the presence of L-NAME (◆) (n=5). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between 1st and 2nd curve or between with and without L-NAME $P \leq 0.05$. CRCs before and after distilled water were not significantly different but CRCs before and after L-NAME were significantly different by two-way ANOVA.

Fig. 7. Effects of curcumin (100 μ M) and astaxanthin (100 μ M) on the contractions of guinea-pig aorta to phenylephrine or β -phenylethylamine (PEA). A. Concentration-response curves for phenylephrine before (■) and in the presence of curcumin (◆) (n=5). B. Concentration-response curves for PEA before (■) and in the presence of curcumin (◆) (n=6). C. Control experiments with PEA concentration-response curves added before (■) and repeated in the presence of neat DMSO (◆) (n=5). D. Concentration-response curves for PEA before (■) and in the presence of astaxanthin (◆) (n=5). Responses are plotted as the increase in tension expressed as a percentage of the maximum contraction to KCl (60 mM). * Significant difference between with and without curcumin or astaxanthin $P \leq 0.05$. CRCs for PEA before and after curcumin were significantly different but CRCs for phenylephrine before and after curcumin, for PEA before and after DMSO and for PEA before and after astaxanthin were not significantly different by two-way ANOVA.

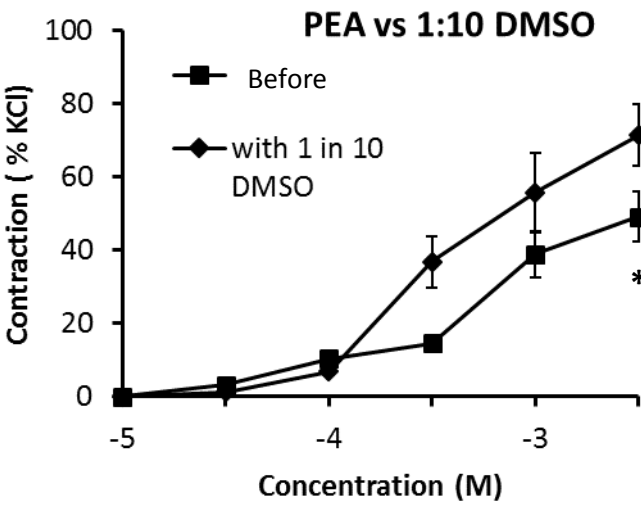
Figure 1

Fig 1

A



B



C

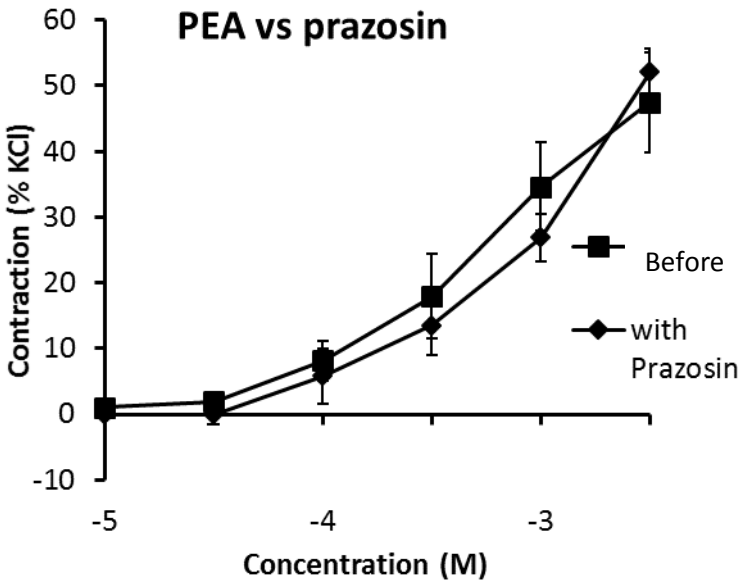
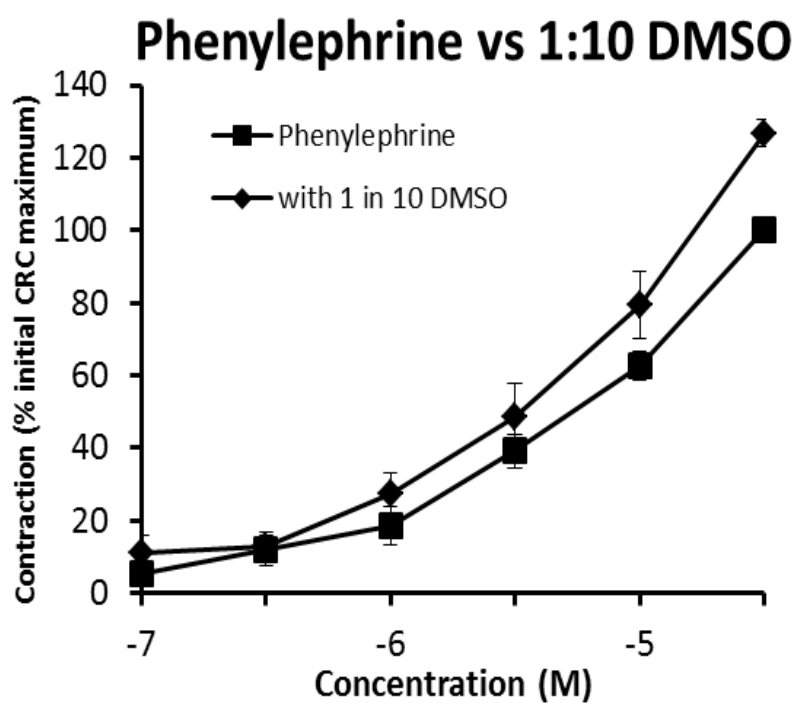


Figure 2

Fig 2

A



B

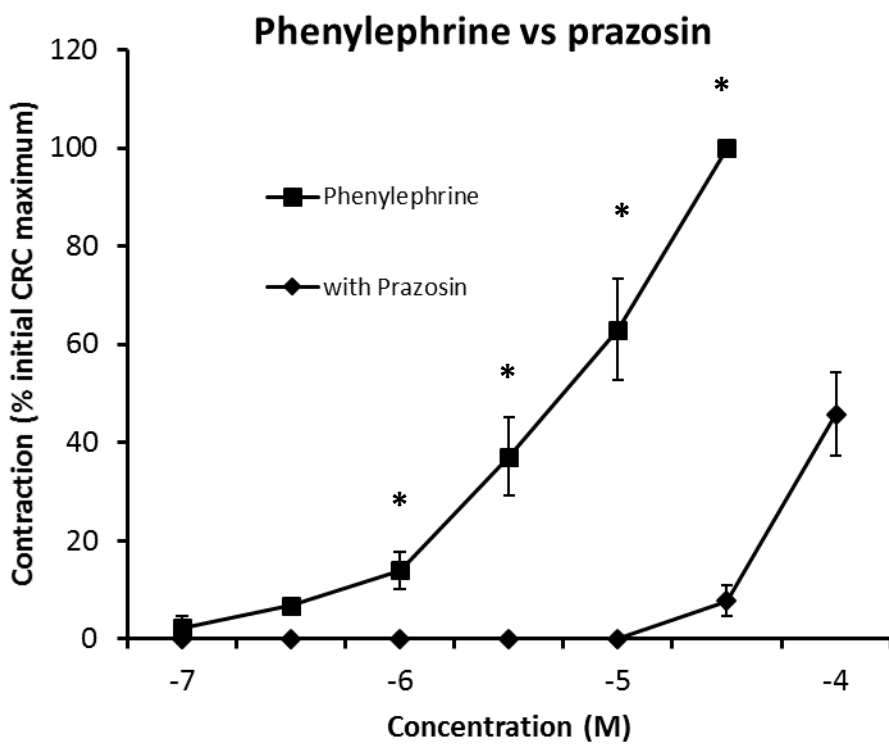
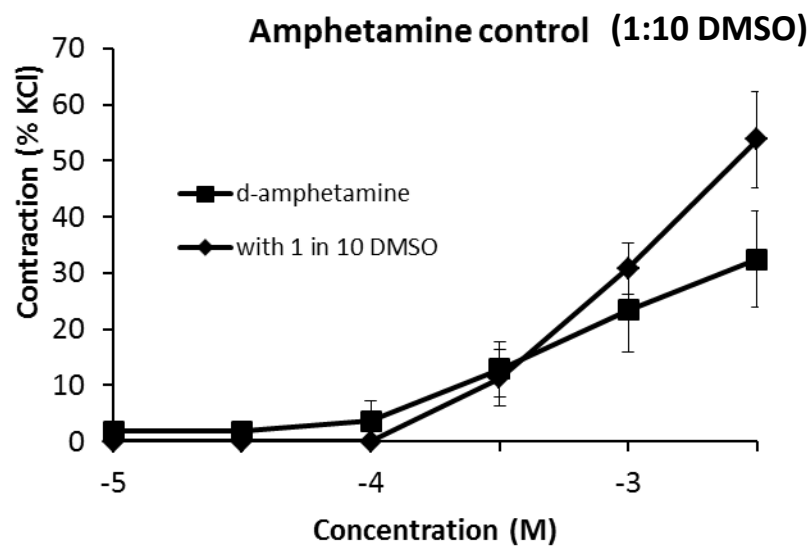


Figure 3

Fig 3

A



B

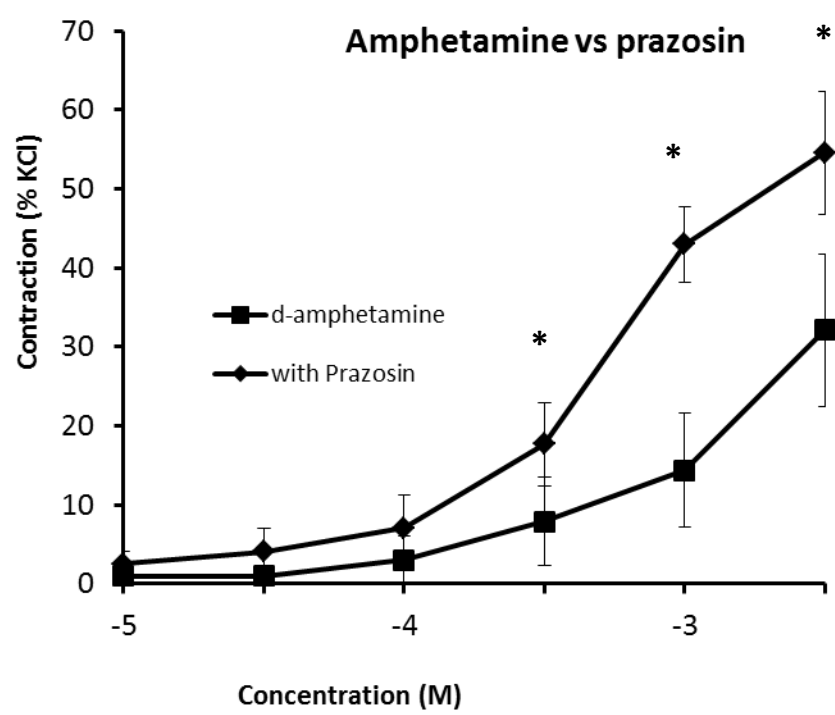
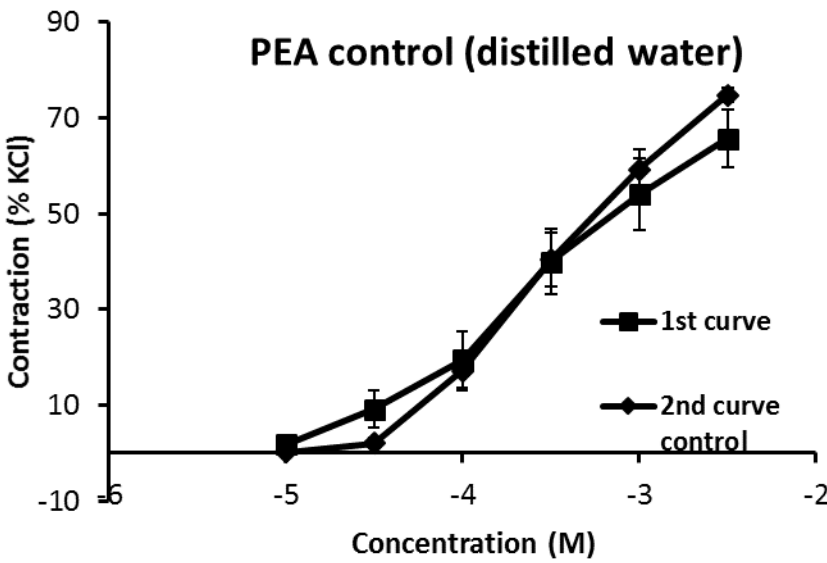


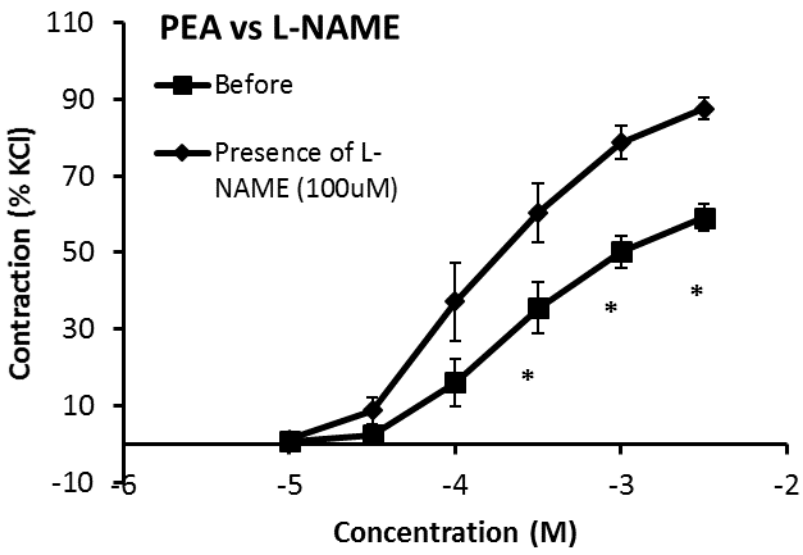
Figure 4

Fig 4

A



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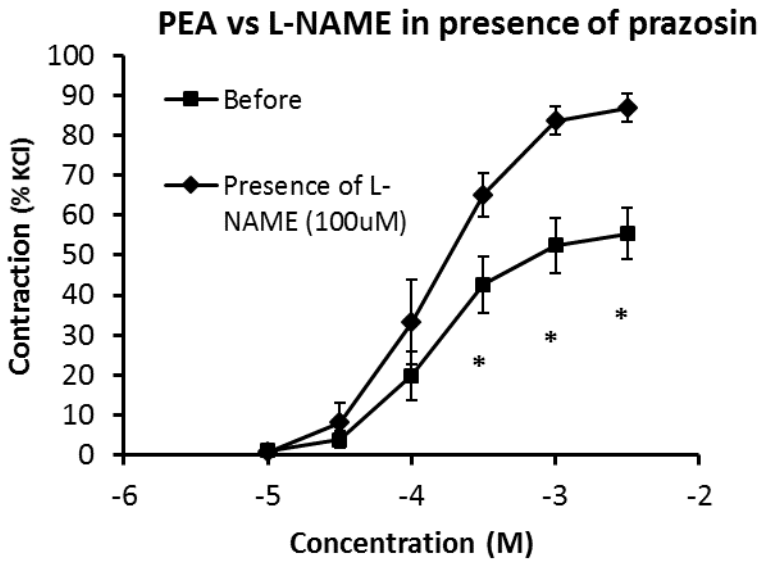
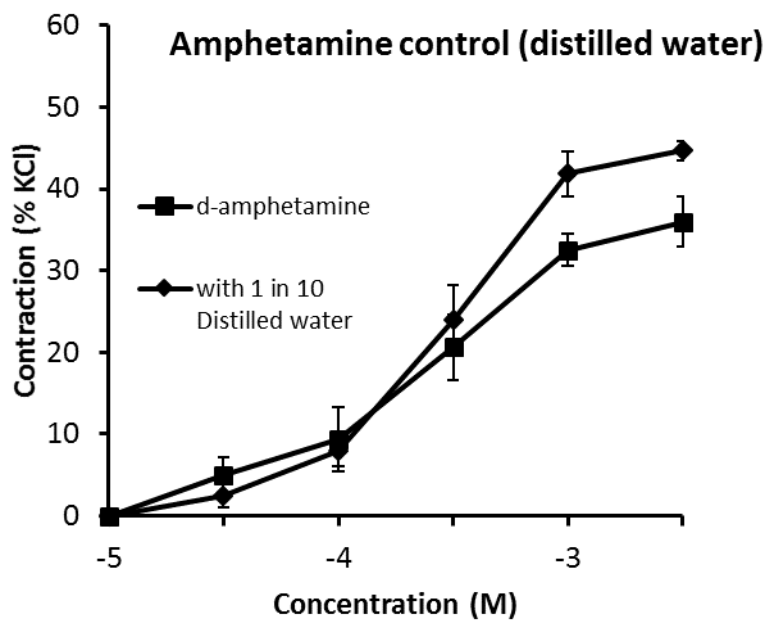


Figure 5

Fig 5

A



B

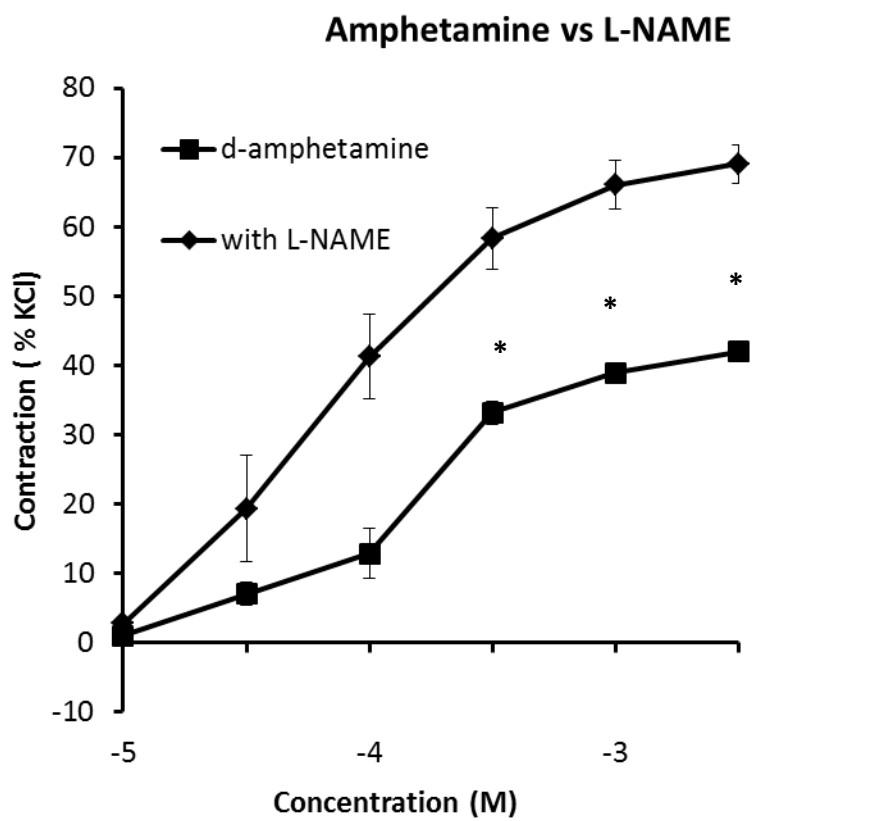
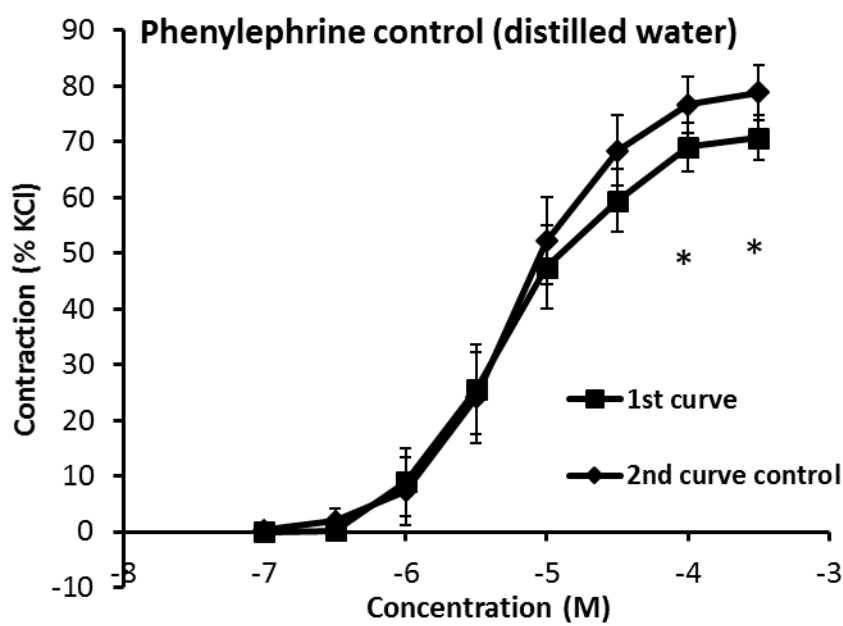


Figure 6

Fig 6

A



B

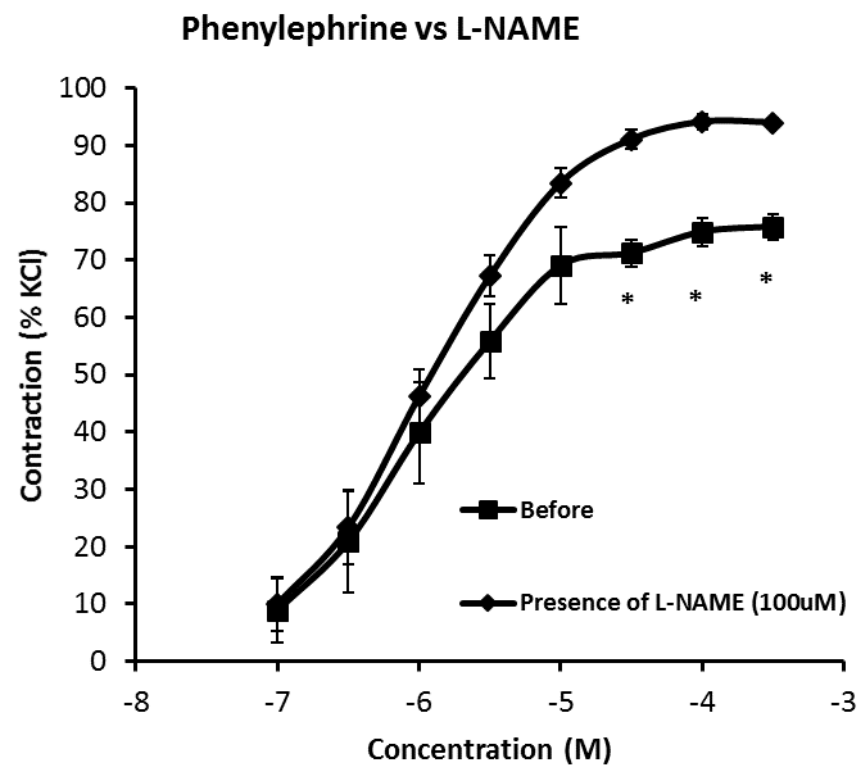
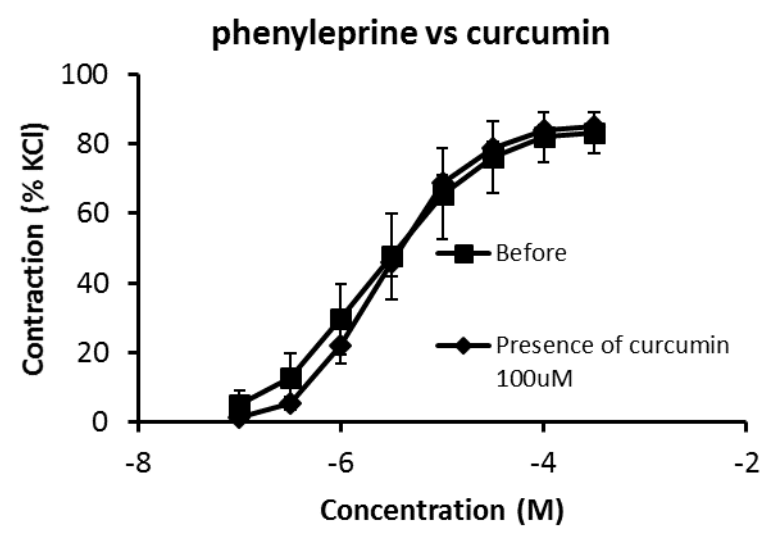
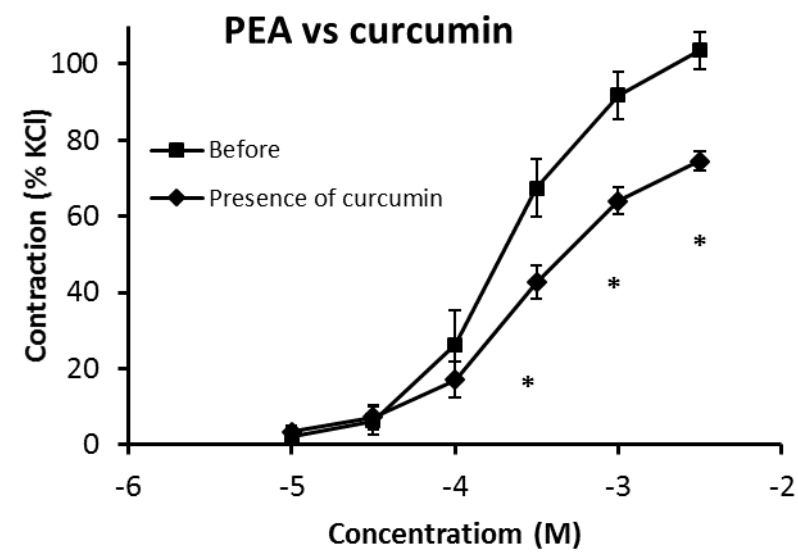


Fig 7

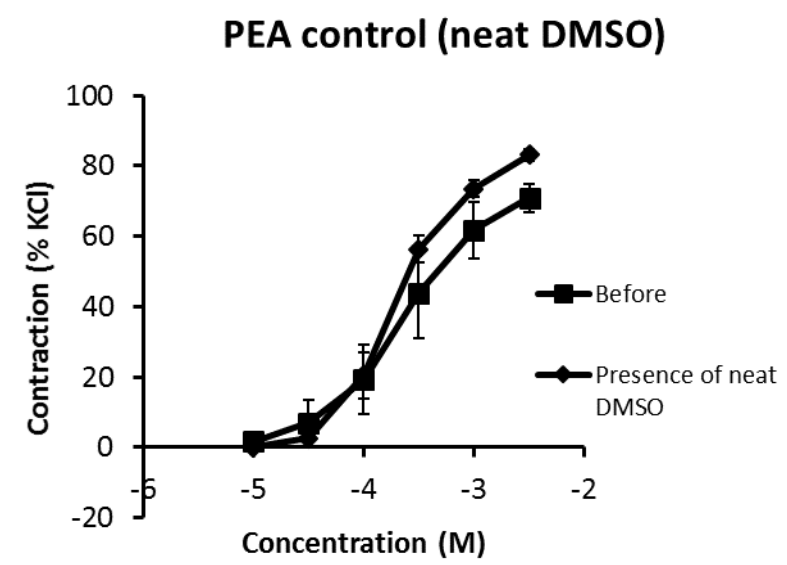
A



B



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D

