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Vascular Effects of Dietary Supplement BMPEA on Mesenteric Porcine Vasculature

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Abstract

β-methylphenylethylamine (BMPEA) was first synthesized in the 1930's as a potential stimulant and amphetamine replacement. It was expected to act as an indirect sympathomimetic agent that binds to peripheral α- and β- adrenergic receptors to control vascular responses in peripheral sites. The effects of BMPEA were never studied in humans, but due to its structural similarity to amphetamines as well as the effects noticed in animal studies in the 1920's and 1930's, BMPEA was assumed to work as a vasoconstrictor. In the current study, porcine mesenteric arteries were used as a model following confirmation of arterial viability with the known constrictor, KCl, and the known dilator, nitroprusside. Using an isolated vascular ring protocol, arteries were exposed to increasing concentrations ($1x10^{-7}$M to $1x10^{-3}$M) of BMPEA to determine changes in vascular reactivity. The results were compared to constriction in response to the known α-adrenergic agonist phenylephrine ($1x10^{-7}$M to $1x10^{-4}$M), and to dilation in response to the known β-adrenergic agonist isoproterenol ($1x10^{-7}$M to $1x10^{-4}$M). No significant change in arterial tension was noted when BMPEA was added as a potential constrictor, or when it was added as a potential dilator after pre-constriction with KCl. Phenylephrine induced a significant increase in tension, serving as a positive control for α-adrenergic-mediated vasoconstriction. However, isoproterenol did not show a decrease in tension with administration of increasing concentrations as expected. The presence of an α-adrenergic receptor-mediated response in the tested porcine vasculature was confirmed by incubation with $1x10^{-5}$M of the α-adrenergic antagonist phentolamine. This was effective in blocking the phenylephrine-induced constriction. BMPEA caused no change in tension when administered following phentolamine incubation. Propranolol ($1x10^{-5}$M) was used to block β-adrenergic receptors with the addition of BMPEA and isoproterenol. BMPEA showed no change in arterial tension for any of these experiments, and it

was concluded that it does not act as a vasoactive agent to constrict or dilate arteries in porcine mesenteric vasculature. Further studies are needed to determine whether BMPEA elicits a vascular response in other organ systems, or if it indeed demonstrates no vascular effect in this species.

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Introduction

Amphetamines and Sympathetic Action. Amphetamines are a group of compounds that act on α and β adrenergic receptors and that are similar in action to dopaminergic, catecholaminergic, and serotonergic agonists. As such, they are stimulators of both the central and peripheral nervous systems and can elicit vascular effects. As catecholaminergic agonists, they can induce tachycardia and vasoconstriction with a concomitant increase in blood pressure. Amphetamines also mimic serotonergic actions that result in vasoconstriction (Handley, 2016).

One pathway through which amphetamines work to elicit these responses is by targeting vascular smooth muscles in peripheral sites. Smooth muscles are found in blood vessel walls, the digestive tract and associated organs such as the gall bladder. Smooth muscles are also found in tissues of the urinary tract such as the bladder and ureter linings, in airways, in reproductive organs and in the ocular muscles of the eye. They contract in response to electrical signals, chemical signals, or both, and are regulated by the autonomic nervous system. The receptors for these signals in smooth muscles are not localized to specific regions, as is the case for skeletal muscle. Instead, they are located along the entire length of the smooth muscle. Most smooth muscles do not receive parasympathetic signals and only induce sympathetic effects, including vascular smooth muscle contraction. These contractions are achieved via an influx of calcium from the extracellular fluid or from the sarcoplasmic reticulum, resulting in a phosphorylation cascade that ends in phosphorylation of myosin light chains and activation of myosin ATPase.

Sympathetic nerves also innervate cardiac muscle to increase heart rate, contractility, conduction velocity, and rate of relaxation. In blood vessels, the sympathetic nerves elicit vasoconstriction of arteries and arterioles, resulting in increased friction between the vessel and

the blood, and increased resistance to the blood flow through the constricted artery, as well as a decrease in distal blood flow. Ohm's law states that the current through two points of a conductor is directly proportional to the voltage across the two points, and is inversely proportional to the resistance through the system. An analogue of this law is used to describe blood flow, and shows that blood flow is equal to the difference in pressure between two points in the vasculature (also known as driving pressure, pressure gradient and perfusion pressure) divided by the resistance given by the blood vessel. Thus the increased resistance due to constriction results in increased arterial pressure.

$$F = \frac{\Delta P}{R} = \frac{(P_A - P_V)}{R}$$

Changes in blood pressure can have many health ramifications. Persistent high blood pressure, or hypertension, can cause weakened areas in vessel walls that can result in ruptures and bleeding into surrounding tissues. This is particularly problematic when it occurs in arteries in the brain, as it can result in hemorrhagic stroke and loss of neurological function. Should the rupture occur in large arteries in the abdomen, the large amount of blood lost can result in a rapid loss in pressure. This can make it difficult for the blood flow to overcome gravity, resulting in decreased oxygen to the brain. Large aneurysms such as these can be fatal.

The prolonged use of amphetamines and the resulting vasoconstriction, increased heart rate, and increased blood pressure can have major health ramifications, and long term use of amphetamines are associated with dilated cardiomyopathy, and myocardial infarction. In addition, the abuse of amphetamines and their analogs can result in seizures, hypertension, psychosis, tachycardia, stroke, myocardial infarction, or death. Unfortunately, such abuse and prolonged use is common in the United States, Europe and Australasia. (Handley et al., 2016). Trace amounts of amines and other analogs of amphetamine can also be found in the body

naturally due to ingestion of herbs (Broadley et al., 2010). These analogs include tyramine, β-phenylethylamine (β-PEA), octopamine, tryptamine and other compounds based on phenylethylamine. Many of these cause vasoconstriction and elevated blood pressure (Broadley et al., 2010).

Adrenergic Receptors. Sympathetic agents such as catecholamines, and amphetamines act on adrenergic receptors to achieve a myriad of sympathetic actions. There are subclasses of adrenergic receptors, and the action elicited by activation of these adrenergic receptors is dependent on the subtype acted upon. This can result in different sympathetic actions at different locations due to the ratio of the adrenergic receptor subtypes present.

α-adrenergic receptors induce vasoconstriction of veins and decreased motility of intestinal smooth muscle. These receptors can be further subcategorized into α_1 and $_2$ receptors, and further yet into $\alpha_{1A,B}$ and $_D$, and into $\alpha_{2A,B}$ and $_C$ receptors. Activation of α_1 receptors results in smooth muscle contraction, including vasoconstriction in the skin, intestines, kidney and brain, and smooth muscles associated with the genitourinary systems. Actions of α_2 receptor activation include inhibition of insulin and glucagon release in the pancreas, and contraction of gastrointestinal sphincters.

β adrenergic receptors include β_1, β_2, and β_3 subcategories. Specific actions of β_1 receptors included increase in heart rate, conductivity and stroke volume resulting in increased cardiac output. Activation of β_2 receptors results in smooth muscle relaxation. This includes relaxation of bronchial and gastrointestinal smooth muscles, as well as vasodilation of blood vessels. Activation of β_3 adrenergic receptors results in the increased lipolysis of adipose tissue.

Amphetamine Analog BMPEA. Amphetamines and their analogs act on both α and β adrenergic receptors throughout the body, and it is the increased expression of specific subtypes

over the others that determines which effects are elicited. One analog of amphetamine is β-methylphenylethylamine (BMPEA), a substance that was first synthesized in the 1930's as a potential stimulant and amphetamine replacement (Hartung et al., 1931). It was expected to act as an indirect sympathomimetic agent that would act on peripheral α- and β- adrenergic receptors to control the vascular response in peripheral sites. Due to its structural similarity to amphetamines, it was expected to have many of the same vascular effects. However, the physiological effects and efficacy of BMPEA have never been systematically studied and remain unconfirmed (Cohen et al., 2016). Structural similarities between BMPEA, amphetamine, and other common amphetamine analogs are shown in Figure 1.

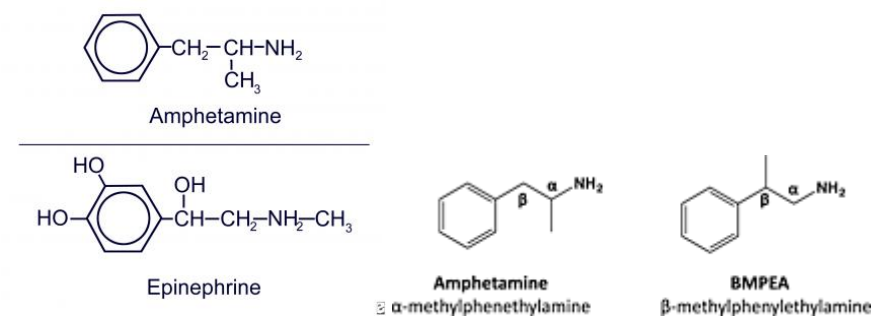


Figure 1. Structure of Amphetamine, BMPEA, and Epinephrine (Cohen et al., 2016; Neal et al, 2016)

BMPEA in Animal Studies. While the effect of BMPEA in humans is unknown, a few animal studies have been performed. In the 1930s and 1940s, BMPEA was studied in dogs and cats where it was shown to increase blood pressure and heart rate in these animals (Graham et al., 1944; Tainter et al., 1943; Warren et al., 1943). It was also shown to cross the blood-brain barrier in rats (Mosnaim et al., 2013). Further study is needed to determine whether BMPEA has similar effects in humans. Another study of BMPEA analogs α -MPEA and BPEA in Swiss

albino mice showed elevated locomotor activity, hyperexcitability and increased fighting behavior upon administration of these compounds (Mosnaim et al., 2015).

BMPEA and Stroke. One anecdotal study of BMPEA in humans does exist. A study by Cohen et al. (2015) indicated that exercise in combination with BMPEA may have caused hemorrhagic stroke in a 53-year-old woman. The patient in this case study had no previous history of stroke, but had taken 13g of a sports supplement for the first time immediately prior to the onset of her stroke. She was taking no other medications. The supplement that the patient took was not labeled with BMPEA or *Acacia rigidula,* a proposed natural source of BMPEA, but upon testing the supplement, it was determined to have 290mg of BMPEA per dose (Cohen et al., 2015). Given the increased risk of stroke with amphetamine overdose (Handley et al., 2016), and considering the structural similarity of BMPEA and amphetamines, the proposed link between this woman's stroke and BMPEA is not unexpected. However, this case was anecdotal, and further study would be needed to confirm a causative effect. Thus the effect of BMPEA in humans is still unknown.

BMPEA as a Supplement. The presence of BMPEA in dietary supplements such as that taken by the woman in the aforementioned study is not uncommon. Due to the fact that the effects of BMPEA in humans were never studied, it has never been introduced as a pharmaceutical drug. However, in 2013 the FDA identified BMPEA in supplements that contained *Acacia rigidula*, a shrub native to Texas (Cohen et al., 2016). Cohen et al. demonstrated the continued presence of BMPEA in these supplements following this FDA finding. Twenty-one supplements containing *Acacia rigidula* available for sale in the United States were included in the study. Three of these also listed a synonym for BMPEA. These supplements where marketed to consumers to enhance athletic performance, weight loss, or to

increase cognition. The research team found BMPEA in eleven of the supplements tested, in amounts from 2.9mg to 93.3mg per maximum recommended dose of supplement (Cohen et al., 2016). Despite these findings, and despite marketing of BMPEA as an extract of *Acacia rigidula*, BMPEA has never been extracted from any natural source, including *Acacia rigidula* or any other plant. Cohen et al. suggests that the presence of BMPEA in *Acacia rigidula* containing supplements is instead indicative that BMPEA is made synthetically and added to supplements to produce the targeted physiologic effects of said supplements (Cohen et al., 2016).

Due to its unknown effects, consumers should be wary of consuming supplements that may contain BMPEA, including supplements that list *Acacia rigidula* on the label. This is particularly important for athletes who use "natural" supplements. Dietary supplements are not controlled by the FDA, so many athletes are not aware that the supplements they are taking may contain substances that are recognized as doping substances (de Hon et al., 2007). This has often led to athletes failing drug tests following the implementation of new detection methods by testing laboratories. Due to its similarity to amphetamine, BMPEA has been classified by the World Anti-Doping Agency as one of these banned stimulants (Chołbińsk et al., 2014), and many athletes have failed doping urine tests due to the lack of public association of BMPEA with *Acacia rigidula* labels (Cohen et al., 2016).

BMPEA and This Study. As an amphetamine analog, consumption of BMPEA is risky. Anecdotal evidence suggests increased risk of hemorrhagic stroke, and preliminary animal studies indicate that BMPEA might have vascular effects. These potential effects, combined with the general lack of information on the effects of BMPEA in human subjects, makes the presence of BMPEA in dietary supplements concerning. BMPEA is banned by drug-screening agencies, and it can be present without disclosure on product labels. There is concern for athletes and the

common consumer alike as the adverse effects of BMPEA are not known. In this study, we intend to study the vascular effects of BMPEA to determine whether it is likely to be harmful for human consumption.

Specific aim number 1. Determine whether BMPEA has any effect on vascular reactivity in the mesenteric arteries. To determine whether BMPEA elicits a vasoconstrictive effect, it was administered to vascular rings for comparison to the constriction caused by a known α-adrenergic agonist. To determine whether it instead elicits a vasodilation response, it was added after pre-constriction with a known constrictor, and compared to the action of a known β-adrenergic dilator. Due to the vasoconstrictive effects of amphetamine and the animal studies that indicate that BMPEA may increase blood pressure, it was expected that BMPEA would engage α-adrenergic receptors to elicit a vasoconstrictive effect.

Specific aim number 2. Confirm the α/β adrenergic response in porcine mesenteric vasculature via pharmacologic techniques. Amphetamines work on α and β adrenergic receptors to elicit either vasoconstriction or vasodilation responses respectively. BMPEA is thus expected to act along similar pathways. To confirm that α and β adrenergic responses in the porcine vessels studied are intact, α and β antagonists were used to block these receptors before administration of BMPEA and positive controls. Should the controls or BMPEA elicit a vasoactive response via these receptors, the administration of the antagonists should block said response.

Drugs Used in Experimentation. In order to test the effects of BMPEA in the selected vasculature, it was tested against known vasoconstrictors, vasodilators, and known α and β agonists. In addition, α and β antagonists were used to block action and to confirm the method of action. Table 1 illustrates all drugs used in this study, and their respective purpose/action.

Table 1. Drugs Used in Study

Drug/supplement/chemical	Purpose/action
BMPEA	Supplement of study; potential α/β adrenergic agonist and vasoactive substance
KCl	Known vasoconstrictor. Agent used for viability testing
Sodium Nitroprusside	Known vasodilator. Agent used for viability testing
Phenylephrine	Known vasoconstrictor. Nonspecific α-adrenergic agonist and positive control
Isoproterenol	Known vasodilator. Nonspecific β-adrenergic agonist and positive control
Phentolamine	Nonspecific α-antagonist
Propranolol	Nonspecific β-antagonist

Isoproterenol was used as a positive control for β-adrenergic receptor-mediated vasodilation. Isoproterenol is a sympathomimetic amine that has been a frequently used pulmonary vasodilator since the early 1960s. It acts as a β-adrenergic agonist and acts on β-adrenergic receptors throughout the body while leaving α-adrenergic receptors unaffected. It has been shown to increase cardiac output via intrinsic effects, and it has been shown to cause peripheral vasodilation (Palevsky et al., 1985). Phentolamine was used as a nonspecific α-adrenergic blocker (Eason et al., 1992) as a $1x10^{-5}$M solution, a concentration that has been shown to be effective in blocking the action of α-adrenergic agonists after an hour-long incubation (Duckles et al., 1976). Aliquots were prepared and stored in the freezer. Propranolol

was used as a nonspecific β-adrenergic antagonist (Nayler et al., 1967) that leaves α-adrenergic receptors unaffected (Propranolol Hydrochloride Injection).

Methods

Model used. Pigs are one of the major non-rodent animal models for translational research, surgical and pharmaceutical testing (Swindle et al., 2012). We chose to use the porcine mesenteric vasculature as a pre-clinical model to determine whether additional studies of BMPEA on the human vasculature would be warranted. Although our lab is investigating the effects of BMPEA in multiple organ systems of the pig, the catecholaminergic response to amphetamines suggests that the mesenteric arteries would be a sensitive site for studying the effects of BMPEA as well.

Blood to the abdomen is supplied via the abdominal aorta. The superior mesenteric artery branches from the aorta to provide blood to the small intestines. Jejunal and ileal arteries branch off along the length of the superior mesenteric artery before branching in to anastomotic loops (arcades) and then from these loops to the arteriae rectae (straight arteries) to the loops of the jejunum and ilium. Arteries in this study were dissected from the mesenteric fascia and were determined to be either a segment of anastomotic loop or of the arteriae rectae.

General Procedure. Porcine small intestines were obtained from DeVries Meats in Coopersville, Michigan. To reduce tissue deterioration and cell death and the amount of time between the sacrifice of the animals and subsequent experimentation, the organs were obtained directly from the production line. The intestines were placed directly in bags, and then on ice for travel. Segments of anastomotic loops or arteriae rectae mesenteric arteries of approximately 1-5mm in diameter were dissected from the intestines upon arrival at the lab. These arteries were adhering parenchymal tissue without damaging the arteries to reduce any potential confounding vascular response originating from surrounding tissues. The ends of the dissected arteries were cut off to remove any potentially damaged tissue, resulting in arterial ring segments

approximately 4mm long. The dissected arterial segments were then placed in Krebs- Henseleit buffer solution. This is a common buffer used to mimic normal physiological conditions when studying the vasculature *ex vivo,* and has a pH of 7.4 when equilibrated with 95% O_2/5% CO_2 (Bailey et al., 1978). Approximate arterial ringlet size is illustrated in Figure 2.

Figure 2. Porcine Mesenteric Arterial Ring Size Comparison.

Isolated Vascular Ring Protocol. Vascular response of the arteries was tested using the isolated vascular ring protocol. This is a standard procedure used to measure the effects of constrictive and dilative agents on the vasculature *in vitro*. It can be used to produce dose-response curves in response to potential vasoactive substances. The procedure eliminates many confounding variables such as circulating hormones, changes in blood flow, or modulation via the nervous system and allows for the potential vasoactivity of the vessels to be measured in precisely defined conditions. These *in vitro* measurements thus indicate the constrictive or dilative responses of the vessels to the tested substance, which in turn may provide evidence justifying further study *in vivo* (Yildiz et al., 2013).

The set up for the isolated vascular ring procedure is illustrated in Figure 3. The organ baths were filled with 25mL of Krebs-Henseleit buffer and maintained at 37ºC. Two hooks were carefully placed through the lumen of the arterial rings. One of these hooks allowed for manual adjustment of vessel tension, and the other was connected to an isometric force transducer. This allowed for adjustment of the artery to a controlled resting tension, and for the measurement of tension changes associated with contraction and dilation.

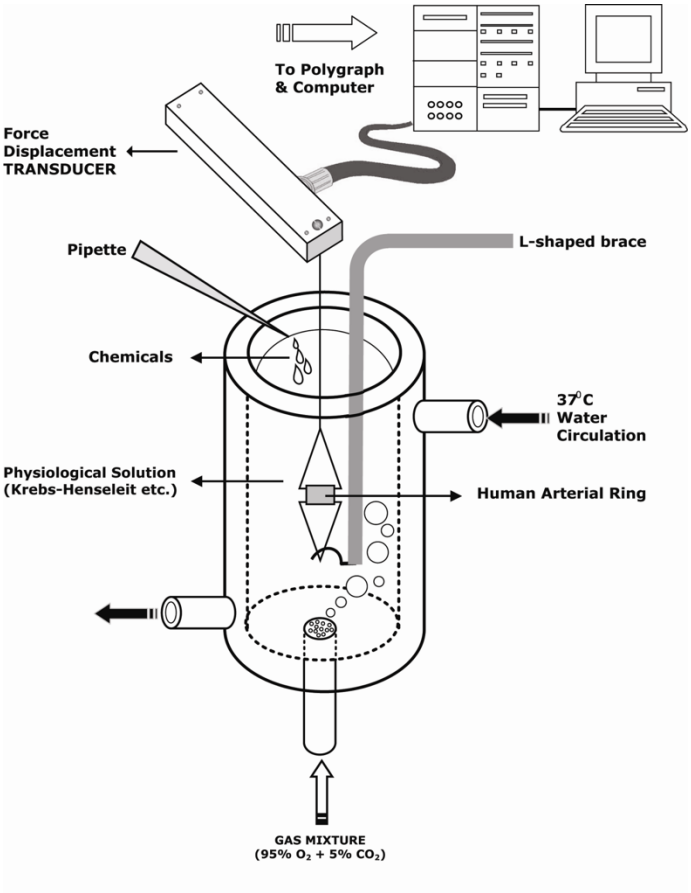


Figure 3. Experimental Set Up. The artery was suspended in Krebs-Henseleit buffer. Arterial resting tension was manipulated with the micromanipulator hook and changes in arterial tension were recorded with a force transducer. Organ baths were maintained at 37°C. Data was collected via iWorx Amplifier (Yildiz et al., 2013)

The Labscribe program produced by iWorx was used to visualize the force measured by the force transducers (Grass Instruments), which were calibrated with 40g weights prior to each experiment. Arterial segments from four different pigs were used for each lab session, one segment for each organ bath. Each bath was filled with 25mL of Krebs buffer, and 95% O_2/5% CO_2 was bubbled into the buffer. Passive tension on the arteries was adjusted to 7g, and was adjusted every 10 minutes for 45-60minutes, or until the recorded tension remained unchanged within a ten-minute interval. The buffer was changed three times during the calibration to avoid accumulation of metabolic waste.

Artery Viability. To check the viability of the arteries, each artery was treated with increasing concentrations of the vasoconstrictor KCl. A stock solution of 0.601M KCl was prepared by adding 2.24g of KCl to 50mL of deionized water. Increasing volumes of stock solution were added every 5 minutes to create total concentrations of 0.015M, 0.030M, 0.045M and 0.060M. Prior to each addition of KCl, an equivalent volume of buffer was removed to maintain calculated concentrations. If the arteries were viable, they exhibited a stepwise increase in tension as illustrated in Figure 4. If vessels were unresponsive, they were washed three times with fresh buffer to remove residual KCl and were adjusted to 7g of passive tension. Following this the arteries were again treated with 0.015M, 0.030M, 0.045mM and 0.060M KCl to achieve the stepwise increase in tension.

The ability of the mesenteric arteries to dilate was confirmed by treatment with sodium nitroprusside (SNP). A $1x10^{-2}$ M stock solution of SNP was prepared by adding 0.149g SNP to 50 mL of filtered water. Following pre-constriction with KCl, increasing concentrations of SNP were added to each organ bath.

Vessels that did not show an increased tension of at least 3g in response to KCl were excluded from this study. Arteries that showed an increased constriction with KCl, and a return to a lower tension with dilator SNP, were considered viable.

Specific Aim 1. To determine if BMPEA is a vasoconstrictor, the addition of BMPEA was compared to the addition of phenylephrine, an α-adrenergic receptor agonist and potent vasoconstrictor (Berthelsen et al., 1977). To eliminate any confounding effects, BMPEA and phenylephrine organ baths were washed with 25mL of Krebs buffer three times for 5 minutes before testing the other compound. This would result in a return to a passive tension of 7g. The temperature of the buffer bath was measured at the end of the 5 minute intervals to confirm that the bath temperature was maintained at 37 °C. The administration of BMPEA and phenylephrine was randomized to minimize an ordering effect.

The viability of the arteries was tested with KCl and SNP. Following this, the arteries were adjusted to 7g and contracted with KCl prior to being tested with BMPEA. 45mM of KCl was administered to each bath and was left to equilibrate for 45-60 minutes. At this point, the arteries would reach an approximately asymptotic line at a constricted tension. BMPEA was then administered in five minute intervals to reach final concentrations of $1x10^{-7}$M, $1x10^{-6}$M, $1x10^{-5}$M, and $1x10^{-4}$M.

Isoproterenol was used as a positive control for vasodilation. Arteries were pre-constricted with 45mM KCl, and were left to equilibrate for 45-60 min. Following this, $1x10^{-7}$M, $1x10^{-6}$M, $1x10^{-5}$M and $1x10^{-4}$M concentrations of isoproterenol were administered at 5 minute intervals.

Specific Aim 2. For each procedure in specific aim 2, an n of 6-8 viable arteries was obtained. Following viability confirmation, phentolamine was added to each organ bath to create

a final concentration of $1x10^{-5}$M. The vessels incubated for an hour before administration of BMPEA and phenylephrine. The arteries were not washed prior to administration of BMPEA and phenylephrine. BMPEA and phenylephrine were administered to separate arteries in five minute intervals to achieve final concentrations of $1x10^{-7}$-$1x10^{-4}$ M and $1x10^{-7}$-$1x10^{-3}$ M for BMPEA and phenylephrine respectively.

A final propranolol concentration of $1x10^{-5}$M was used and was incubated for an hour while the arteries were pre-constricted with 45mM KCl. Following pre-constriction and propranolol incubation, BMPEA and isoproterenol were administered to separate arteries in 5 minute intervals to achieve final concentrations of $1x10^{-7}$-$1x10^{-4}$ M and $1x10^{-7}$-$1x10^{-3}$ M respectively.

Analysis

All viable arterial tensions were charted in an Excel file. This can be seen in Appendix 1. Average tensions were calculated for every dose of the drugs tested. Standard error of the mean was calculated for these values by dividing the standard deviation by the square root of n. The standard error was calculated instead of standard deviation because it gives the standard error after adjusting for small samples sizes. This is typical for physiology experiments. JMP statistical analysis software was used for statistical analysis. A one-way ANOVA was used to determine whether there was any significant difference in arterial tension across different doses of a given drug. If significant differences were observed, a Tukey-Kramer test was used for post hoc analysis. An α of 0.05 was used for all statistical analysis. Please see statistical outputs in Appendix 2. A minimum n of 8 was obtained for each set of experiments in specific aim 1and an n of 6-8 was obtained for each set of experiments in specific aim 2.

Results

Viability of the arteries was tested with known constrictor KCl, and with known vasodilator SNP. Arteries that did not show an increase in tension of at least 3g with the administration of KCl, and then a subsequent decrease in tension with the administration of SNP were not included in the study. A total of 62 arteries were considered viable for this study. Artery tensions for administration of all substances were monitored using Labscribe outputs generated from a force transducer. Concentrations of the drugs administered were noted by inserting labels in the Labscribe program, and each row in the output indicates a different artery and buffer bath. Sample Labscribe outputs are exhibited in Figure 4 and 5 for validity testing with KCl and SNP respectively.

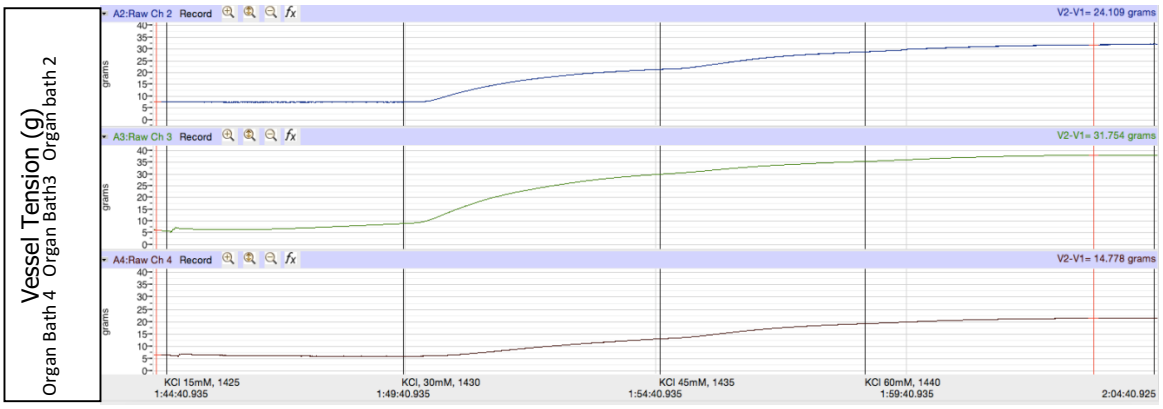


Figure 4. Addition of 0.015M, 0.030M, 0.045M and 0.060M of KCl to Mesenteric Arteries in 5 Minute Increments.

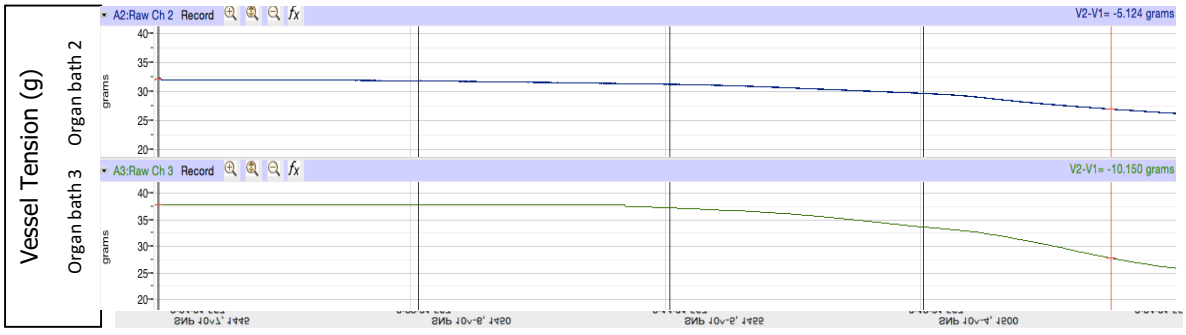


Figure 5. The Response of Mesenteric Arteries to Increasing Concentrations of SNP.

Average tensions for KCl at each concentration, as well as average tensions for SNP, with standard error, are shown in Tables 2a and 2b respectively. Administration of 0.060M KCl yielded an average increase in tension to 26.91g, indicating that KCl was successful as a vasoconstrictor. In turn, SNP mediated an average decrease to 18.82g at 10^{-4}M, with an average change of 6.9g. Thus, all arteries included in the study proved to be viable, and capable of vasoconstriction and vasodilation.

Table 2a. The Response of Mesenteric Arteries to Increasing Concentrations of KCl

n	Concentration	Average tension (g)	Standard Error
62	0.015M	7.37	0.33
62	0.030M	19.26	2.05
62	0.045M	25.45	2.14
62	0.060M	26.91	1.99

Table 2b. The Response of Mesenteric Arteries to Increasing Concentrations of SNP

n	Concentration (M)	Average tension (g)	Standard Error
62	$1x10^{-7}$	25.72	2.02
62	$1x10^{-6}$	25.09	1.94
62	$1x10^{-5}$	23.59	1.83
62	$1x10^{-4}$	18.82	1.43

Graphical representation of these average tensions indicates an increased average arterial tension with increased concentrations of KCl as illustrated in Figure 6a. A one-way ANOVA indicated that the mean tensions at different KCl concentrations were significantly different with

a significance value less than 0.0001. Tukey-Kramer post hoc analysis showed significance between 0.015M and 0.030M, 0.015M and 0.045M, and 0.015M and 0.060M with p value <0.0001 for all three sets, and significance between 0.030M and 0.060M with a p-value of 0.0110. The difference between the mean tensions at 0.030M and 0.045M, as well as between mean tensions at 0.045M and 0.060M were not significant however, with p-values of 0.0775 and 0.8883 respectively.

Similarly, average tensions decreased with increasing concentrations of SNP as illustrated in Figure 6b, with the average tension decreasing by 6.89g between 10^{-7} M and 10^{-4}M SNP. A one-way ANOVA was significant with a value of 0.0359. Tukey-Kramer indicated significance between SNP concentrations of $1x10^{-7}$M and $1x10^{-4}$M with a p-value of 0.0419.

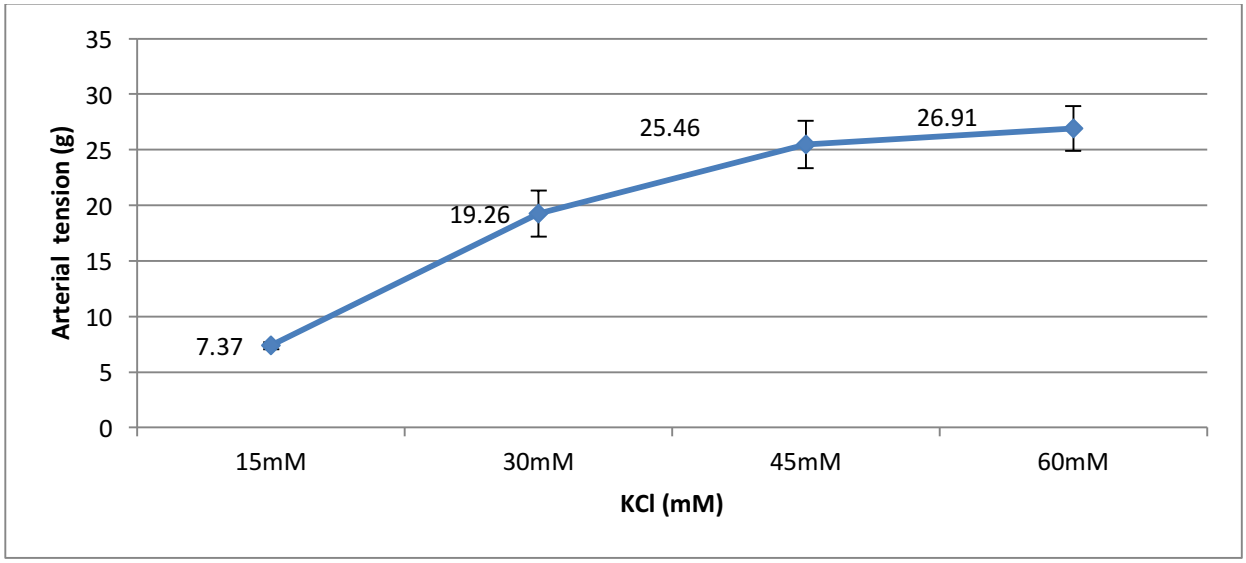


Figure 6a. Average Mesenteric Arterial Tension with Administration of KCl. Graphical representation indicates a visual increase in tension between 0.015M and 0.030M, as well as between 0.030M and 0.060M. One way ANOVA confirms this and showed significant changes in average tension between 0.015M and 0.030M, 0.015M and 0.045M, 0.015M and 0.060M and between 0.030M and 0.060M with p values of <0.0001, <0.0001, <0.0001, and 0.0110 respectively. The mean tensions between 0.030M and 0.045M, and between 0.045M and 0.060M were not significant, α=0.05.

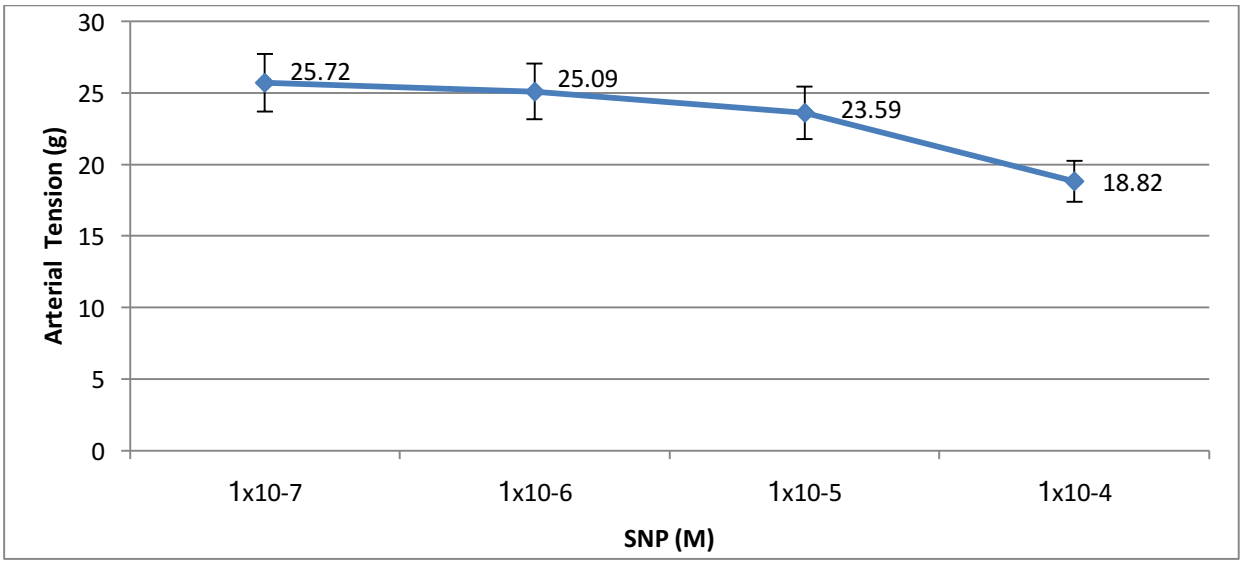


Figure 6b. Average Mesenteric Arterial Tension with Administration of SNP. Graphical representation indicates a visual decrease in tension between $1x10^{-7}$ and $1x10^{-4}$ M. One way ANOVA confirms this and showed significance of 0.0359, and in particular a significant difference in average tension between $1x10^{-7}$ M and $1x10^{-4}$ M with a p value of 0.0412, α=0.05.

The working hypothesis for this study is that BMPEA acts on α-adrenergic receptors to mediate vasoconstriction in the porcine mesenteric vasculature studied. Thus BMPEA was administered in increasing concentrations and compared to the effects of the known α-adrenergic agonist phenylephrine. Average arterial tensions at different BMPEA and phenylephrine concentrations are shown in Table 3a and 3b respectively. The average arterial tension with the administration of BMPEA stayed within 1g of an average tension of 6g, indicating no obvious increase in tension. However, administration of phenylephrine yielded an increase in average tension from 6.62g to 16.37g, indicating that it was successful as a positive control for vasoconstriction.

Table 3a. The Response of Mesenteric Arteries to Increasing Concentrations of BMPEA as a Potential Vasoconstrictor

n	Concentration (M)	Average tension (g)	Standard Error
10	$1x10^{-7}$	6.61	0.18
10	$1x10^{-6}$	6.21	0.20
10	$1x10^{-5}$	5.99	0.23
10	$1x10^{-4}$	5.86	0.24
10	$1x10^{-3}$	5.86	0.25

Table 3b. The Response of Mesenteric Arteries to Increasing Concentrations of Phenylephrine as a Positive control for Vasoconstriction

n	Concentration (M)	Average tension (g)	Standard Error
10	$1x10^{-7}$	6.62	0.19
10	$1x10^{-6}$	9.94	1.33
10	$1x10^{-5}$	18.29	2.01
10	$1x10^{-4}$	16.37	2.18

Graphical representation supports these findings, and indicates no difference in average arterial tension at different concentrations of BMPEA as indicated by Figure 7. This is confirmed with a one-way ANOVA with an F Ratio of 2.0336 and a prob>F of 0.1057. Likewise, graphical representation supports the findings that phenylephrine was successful as a vasoconstrictor (Figure 7). A one-way ANOVA confirmed a significant difference between arterial tensions with a significance value of less than 0.0001. Tukey-Kramer post hoc analysis specifically indicated significant differences between arterial tensions at phenylephrine concentrations of $1x10^{-7}$M and $1x10^{-5}$M, $1x10^{-7}$M and $1x10^{-4}$M, $1x10^{-6}$ M and $1x10^{-5}$M, and $1x10^{-6}$M and $1x10^{-4}$M with p values of <0.0001, 0.0008, 0.0046 and 0.0391 respectively.

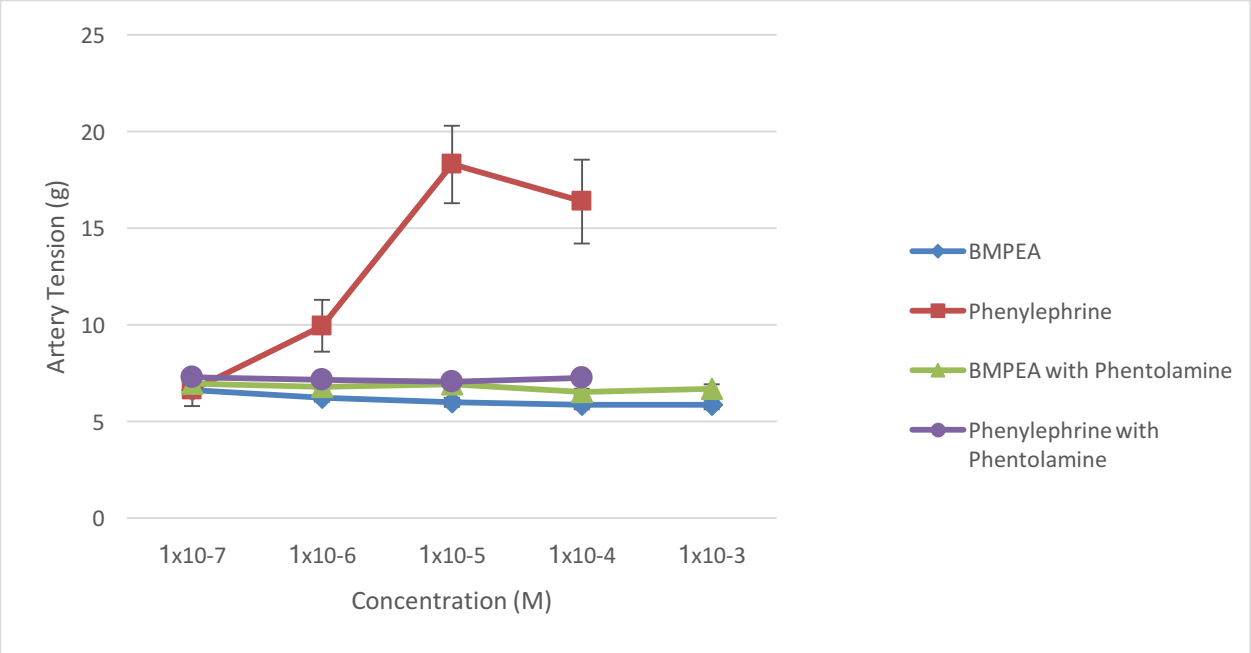


Figure 7. Average Mesenteric Arterial Tension with Administration of BMPEA, Phenylephrine, BMPEA with Phentolamine Blockade, and Phenylephrine with Phentolamine Blockade. BMPEA: Graphical representation indicates no visual difference between mean artery tensions at different concentrations of BMPEA. This is confirmed with a one-way ANOVA with a p-value 0.1057, α=0.05. Phenylephrine: Graphical representation indicates a visual increase in mean artery tension with the administration of phenylephrine. One way ANOVA indicated significant difference in mean artery tensions with a significance of less than 0.0001. Tukey-Kramer post hoc analysis specifically indicated significant difference between artery tensions at phenylephrine concentrations of $1x10^{-7}$M and $1x10^{-5}$M, $1x10^{-7}$M and $1x10^{-4}$M, $1x10^{-6}$ M and $1x10^{-5}$M and $1x10^{-6}$M and $1x10^{-4}$M with p values of <0.0001, 0.0008, 0.0046 and 0.0391 respectively. There was no significant difference between mean tensions at $1x10^{-5}$ M and $1x10^{-4}$M with p-value of 0.8373, α=0.05. BMPEA following incubation with phentolamine as an α-adrenergic antagonist: Graphical representation indicates no visual difference between mean artery tensions at different concentrations of BMPEA. One-way ANOVA confirms that artery tensions are not statistically different (p=0.6319, α=0.05). Phenylephrine following incubation with phentolamine as an α-adrenergic antagonist: Graphical representation indicates no visual difference between mean artery tensions at different concentrations of phenylephrine. One-way ANOVA confirms that artery tensions are not statistically different (p=0.7509, α=0.05).

As graphical and statistical analysis did not indicate that BMPEA acts as a vasoconstrictor, it was tested as a potential vasodilator following pre-constriction with KCl. Average tensions at different BMPEA concentrations are shown in Table 4a. These do not indicate a change in tension with the administration of BMPEA. Isoproterenol was used as an

established positive control for β-adrenergic receptor-mediated vasodilation. However, artery tensions with increasing concentrations of isoproterenol do not indicate a decrease in tension. The average isoproterenol arterial tensions are presented in Table 4b.

Table 4a. The Response of Mesenteric Arteries to Increasing Concentrations of BMPEA as a Potential Vasodilator

n	Concentration (M)	Average tension (g)	Standard Error
10	$1x10^{-7}$	19.85	1.99
10	$1x10^{-6}$	20.02	2.01
10	$1x10^{-5}$	20.08	2.01
10	$1x10^{-4}$	20.43	2.03
10	$1x10^{-3}$	21.24	2.08

Table 4b. The Response of Mesenteric Arteries to Increasing Concentrations of Isoproterenol as Vasodilation Positive Control

n	Concentration (M)	Average tension (g)	Standard Error
10	$1x10^{-7}$	33.63	2.49
10	$1x10^{-6}$	33.34	2.49
10	$1x10^{-5}$	33.01	2.57
10	$1x10^{-4}$	33.21	2.67

Graphical representation of BMPEA concentration-dependent mean arterial tensions following pre-constriction supports the observation that there was no decrease in tension as shown in figure 8a. This is confirmed with a one-way ANOVA which indicated no significant difference between the mean tensions with an F Ratio of 0.0750 and a Prob>F of 0.9894. Likewise, graphical representation of isoproterenol tensions is represented in Figure 8b, and indicates no visibly noticeable decrease in tension with administration of isoproterenol. This is

again confirmed with a one-way ANOVA which indicated no change in mean tensions with an F ratio of 0.0104 and a Prob>F of 0.9985.

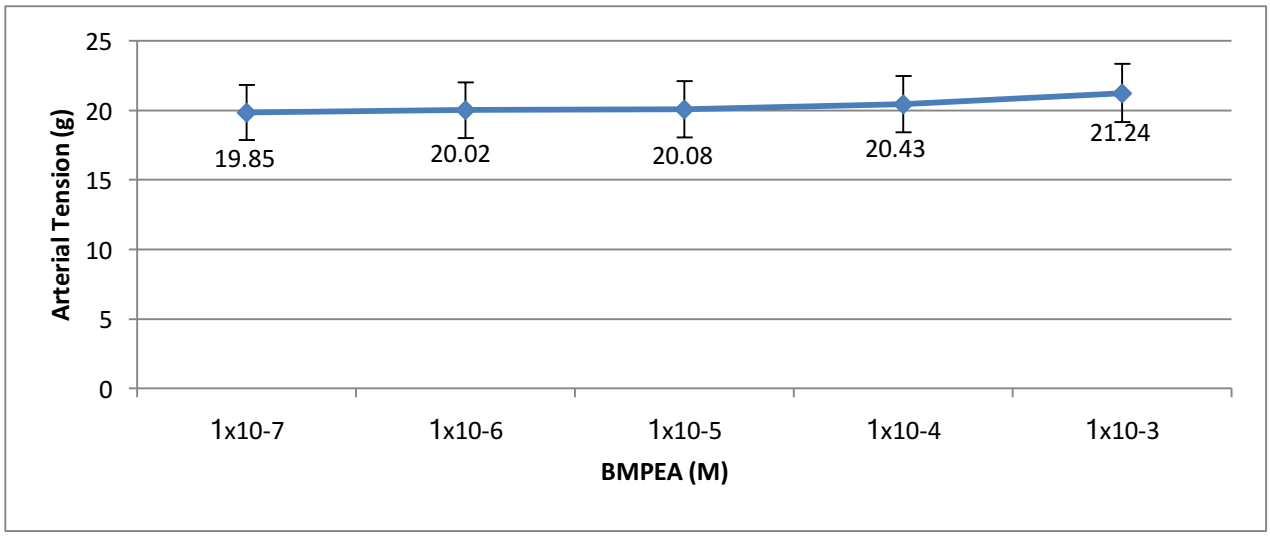


Figure 8a. Average Mesenteric Arterial Tension with Administration of BMPEA as a Potential Vasodilator after Pre-constriction with KCl. Graphical representation indicates no visual difference between mean artery tensions at different concentrations of BMPEA. This was confirmed with a one-way ANOVA with a p-value of 0.9894 α=0.05.

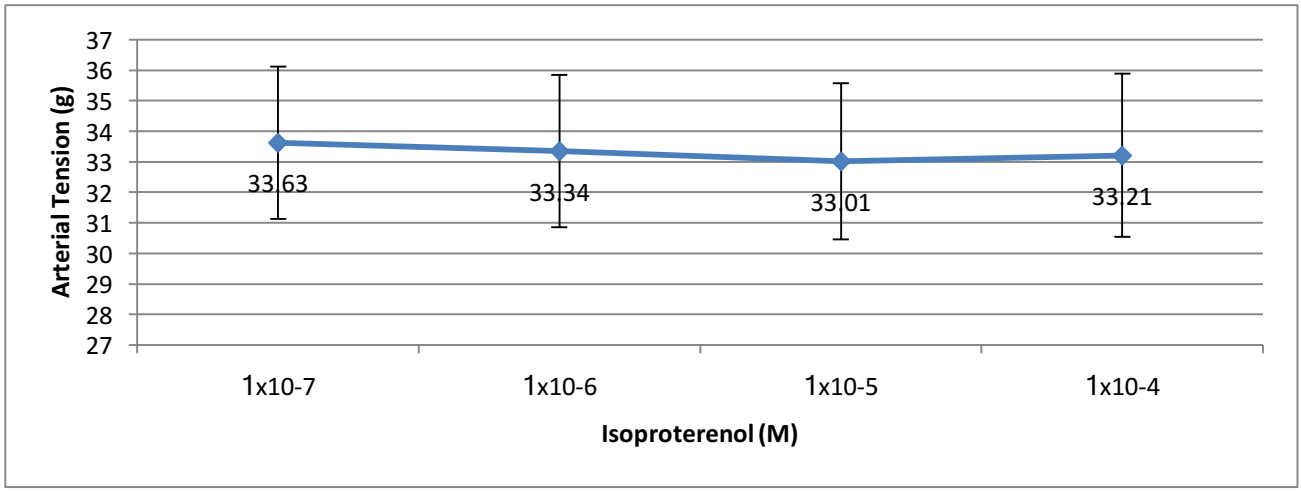


Figure 8b. Average Mesenteric Arterial Tension with Administration of Isoproterenol as a Potential Vasodilator after Pre-constriction with KCl. Graphical representation indicates no visual difference between mean artery tensions at different concentrations of isoproterenol. This was confirmed with a one-way ANOVA with a p-value of 0.9985, $\alpha = 0.05$.

To confirm the response of α-adrenergic receptors in the mesenteric porcine vasculature studied, BMPEA and phenylephrine were administered in increasing concentrations following incubation with α-adrenergic antagonist phentolamine. Concentration dependent mean arterial tensions for BMPEA and phenylephrine following incubation with phentolamine are shown in Table 5a and 5b respectively. Graphical representation indicates no change in mean arterial tension with administration of increasing concentrations of BMPEA and phenylephrine with phentolamine blockade as indicated in Figure 7. The lack in tension change is confirmed with a one-way ANOVA which showed no significant difference between artery tensions for increasing concentrations of BMPEA ($p=0.6319$, $\alpha=0.05$) or for increasing concentrations of phenylephrine ($p=0.7509$, $\alpha=0.05$).

Table 5a. The Response of Mesenteric Arteries to Increasing Concentrations of BMPEA Following Incubation with Phentolamine

n	Concentration (M)	Average tension (g)	Standard Error
8	$1x10^{-7}$	6.94	0.08
8	$1x10^{-6}$	6.77	0.09
8	$1x10^{-5}$	6.91	0.34
8	$1x10^{-4}$	6.53	0.17
8	$1x10^{-3}$	6.69	0.23

Table 5b. The Response of Mesenteric Arteries to Increasing Concentrations of Isoproterenol Following Incubation with Phentolamine

n	Concentration (M)	Average tension (g)	Standard Error
8	$1x10^{-7}$	7.29	0.11
8	$1x10^{-6}$	7.14	0.14
8	$1x10^{-5}$	7.06	0.15
8	$1x10^{-4}$	7.26	0.25

BMPEA and isoproterenol were also tested in the presence of β-adrenergic antagonist propranolol following pre-constriction with KCl. The average arterial tensions for these are shown in Tables 6a and 6b respectively. These findings indicate no change in tension with the administration of BMPEA or isoproterenol following incubation with propranolol. This is not surprising as neither BMPEA nor isoproterenol precipitated a change in arterial tension, and there was no action to block with propranolol.

Table 6a. The Response of Mesenteric Arteries to Increasing Concentrations of BMPEA Following incubation with Propranolol

n	Concentration (M)	Average tension (g)	Standard Error
6	$1x10^{-7}$	16.4	1.55
6	$1x10^{-6}$	16.51	1.57
6	$1x10^{-5}$	16.61	1.55
6	$1x10^{-4}$	16.68	1.39
6	$1x10^{-3}$	16.63	1.19

Table 6b. The Response of Mesenteric Arteries to Increasing Concentrations of Isoproterenol Following Incubation with Propranolol

n	Concentration (M)	Average tension (g)	Standard Error
6	$1x10^{-7}$	14.26	2.97
6	$1x10^{-6}$	14.69	2.24
6	$1x10^{-5}$	16.42	1.61
6	$1x10^{-4}$	17.07	1.73

Graphical analysis indicates no visibly noticeable change in average tensions for increasing concentrations of BMPEA or isoproterenol following propranolol incubation. These results are shown in Figures 9a and 9b respectively. The lack of change in arterial tension is confirmed with one-way ANOVA with an F ratio of 0.2597, Prob>F of 0.9009, and an F ratio of 0.3396, Prob>F of 0.8486 for BMPEA and isoproterenol respectively.

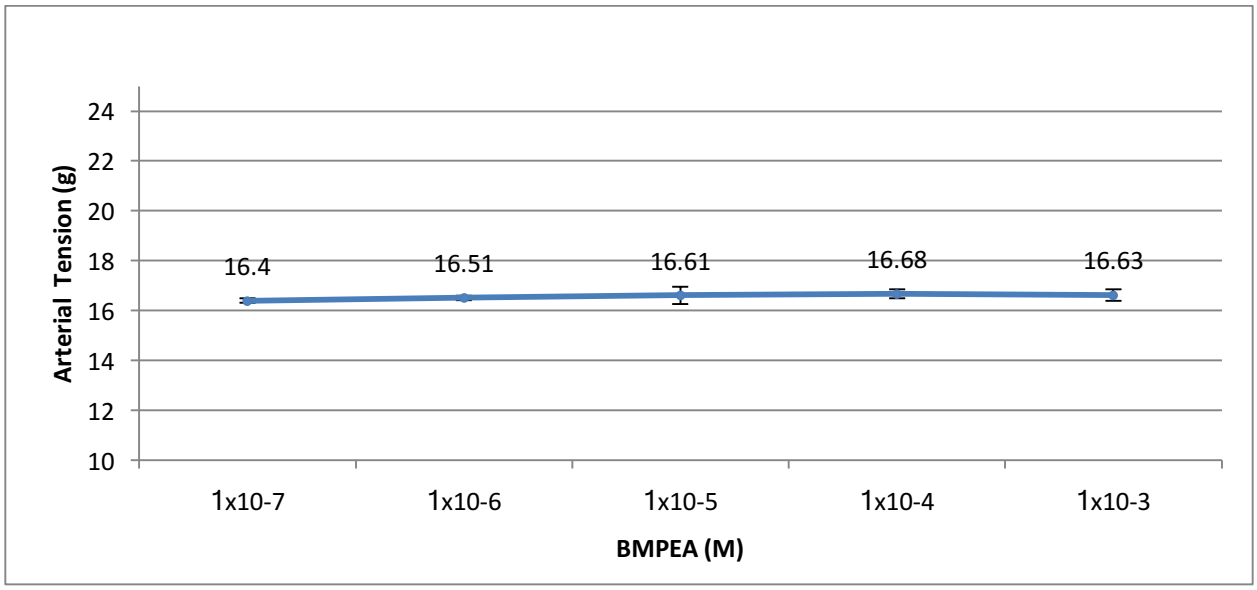


Figure 9a. Average Mesenteric Arterial Tension with Administration of BMPEA Following Incubation with Propranolol as a β-adrenergic Antagonist. Graphical representation indicates no visual difference among mean artery tensions at different concentrations of phenylephrine. One-way ANOVA confirms that artery tensions are not statistically different (F ratio of 0.2597, Prob>F of 0.9009α=0.05).

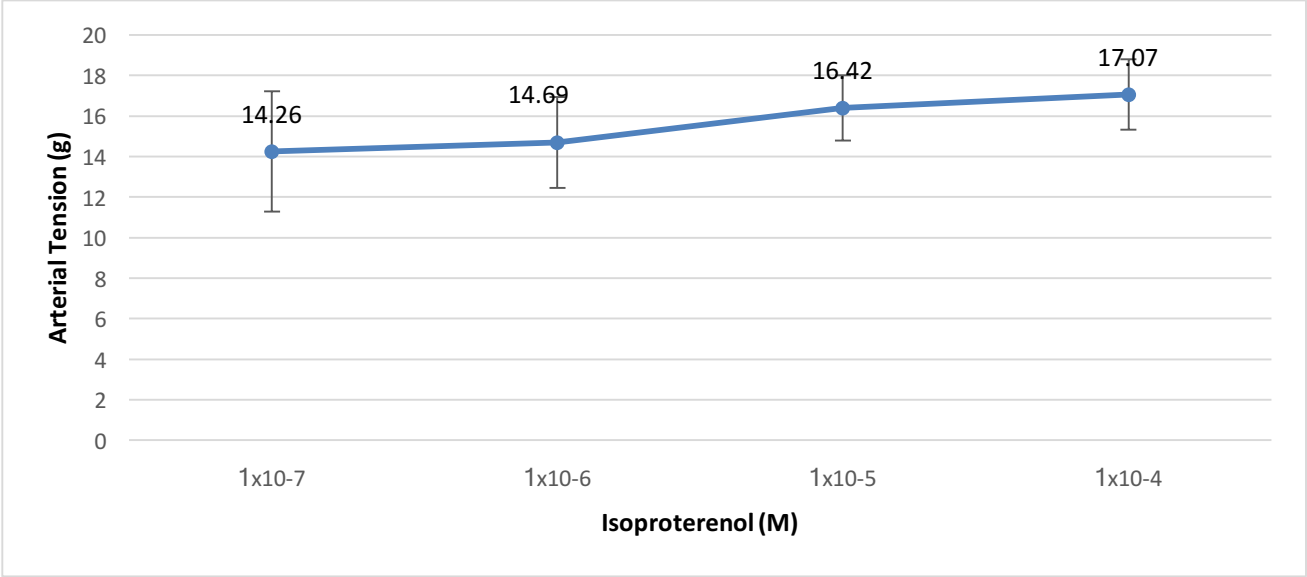


Figure 9b. Average Mesenteric Arterial Tension with Administration of Isoproterenol Following Incubation with Propranolol as a β-adrenergic Antagonist. Graphical representation indicates no visual difference between mean artery tensions at different concentrations of phenylephrine. One-way ANOVA confirms that artery tensions are not statistically different (F ratio of 0.3396, Prob>F of 0.8486, α=0.05).

Discussion

As a structurally similar, potential amphetamine replacement, BMPEA was expected to work on adrenergic receptors in the peripheral vasculature to elicit vasoactive responses. In particular, amphetamines have been shown to act on α-adrenergic receptors to elicit vasoconstrictive responses and BMPEA was expected have a similar response. This hypothesis was supported by animal studies that indicated BMPEA may play a role in increasing blood pressure and heart rate through vasoconstriction (Graham et al., 1944; Tainter et al., 1943; Warren et al., 1943).

Surprisingly, BMPEA did not elicit a statistically significant change in arterial tension when administered to viable arteries in concentrations between $1x10^{-7}$ M and $1x10^{-3}$ M (Prob>F of 0.1057). This was compared to the known α-adrenergic constrictor, phenylephrine, which showed a statistically significant increase in arterial tension that was dose-dependent (Prob>F less than 0.0001). While phenylephrine did not show a significant change in tension at the lowest concentrations ($1x10^{-7}$M and $1x10^{-6}$M, p= 0.4829) there was significant change between $1x10^{-7}$M and $1x10^{-5}$M with an average increase in tension of 11.67g ($p<0.0001$). Graphical analysis demonstrates the dose-dependent increase in arterial tension with a peak at $1x10^{-5}$M, which then dropped slightly at $1x10^{-4}$M. However, this decrease in tension was not statistically significant.

Lack of constriction with BMPEA may indicate that it does not act on α-adrenergic receptors to elicit a sympathetic response. However, some drugs are known to differ in their effects, depending on the area of the body and the associated affinity or number of receptors in that organ. Thus the lack of BMPEA-mediated constriction may have been due to a decreased number of α-adrenergic receptors in porcine mesenteric vasculature, and constriction by

phenylephrine could be mediated through a different pathway. This did not turn out to be the case however, as the presence of α-adrenergic receptors in the tested vasculature, as well as their role in phenylephrine constriction was confirmed using the α-adrenergic antagonist phentolamine. Incubation with phentolamine effectively blocked the action of phenylephrine, resulting in no statistical increase in artery tension with administration of phenylephrine.

Therefore, the lack of BMPEA-mediated vessel constriction would not seem to be indicative of a lack of α-adrenergic receptors in porcine mesenteric vasculature. It would instead indicate that BMPEA does not act on α-adrenergic receptors. While amphetamines act on α-adrenergic receptors to achieve vasoconstriction in sympathetic pathways, they are also known to bind β-adrenergic receptors to induce vasodilation. BMPEA may also act on β-adrenergic receptors in select organ systems to cause vasodilation rather than vasoconstriction as hypothesized. However, administration of increasing concentrations of BMPEA to constricted vasculature did not yield a significant change in tension (F Ratio of 0.0750 and a Prob>F of 0.9894), and no decrease in tension was noted upon graphical analysis. Likewise, there was no significant change in tension with administration of isoproterenol (F ratio of 0.0104 and a Prob>F of 0.9985).

Lack of response to isoproterenol warrants investigation as this is a commonly used, well established β-adrenergic vasodilator. Upon examination after completion of experimentation, it was determined that the drug used was 8 years old. Further investigation is needed to determine whether the lack of response was due to the age of the drug. Lack of dilation may also be due to a lower affinity of β-adrenergic receptors in porcine mesenteric vasculature relative to other sites, or to a lower number of receptors. Another explanation may lie in the procedural plan, which utilizes pre-constriction with KCl. Our procedure did not allow for the buffer baths to be washed

following pre-constriction as this would result in a return to resting tension. Thus there may have been confounding effects resulting from KCl being present in the buffer bath, and it may have had a stronger constrictive effect than the dilation effect produced by isoproterenol. However, KCl works on ion-gated channels to change membrane potential, which thus opens calcium channels resulting in smooth muscle contraction, while isoproterenol works on ligand-gated channels. Thus, KCl should not act as a competitive inhibitor. Additionally, nitroprusside was able to dilate arteries in the presence of KCl during viability testing. Nitroprusside mediates vasodilation by converting to NO and activating guanylate cyclase to increase intracellular production of cGMP, which in turn results in a decrease of calcium in the smooth muscle. This results in muscle relaxation. Further study is needed to determine which, if any, of these theories explains why isoproterenol addition did not result in vasodilation.

Despite the lack of dilation in response to isoproterenol and BMPEA, these substances were still tested in the presence of the β-adrenergic antagonist propranolol. Confounding effects are not uncommon, and it was possible that BMPEA acts on both α and β receptors. If this were the case, the constrictive and dilative responses would cancel each other for a net result of zero change in tension. However, administration of BMPEA and isoproterenol after pre-incubation with propranolol and KCl yielded no statistically significant change in artery tension. Since isoproterenol did not produce a decrease in tension when treated without propranolol, and since no confounding effects were noted with the addition of propranolol incubation, further study is needed to confirm the presence of β- adrenergic receptors in the tested vasculature. This could be done by finding a different β agonist and establishing a vasodilation response with subsequent blocking with propranolol. Or, a western blot could be done to definitively establish the presence

of the β- adrenergic receptor. Further study is also needed to determine the affinity of those receptors for β-agonists such as isoproterenol, if the receptors are shown to be present.

Despite the lack of isoproterenol response, it can at least be said that BMPEA does not elicit a statistically significant response when administered as a potential dilator. Nor did it elicit a change in tension as a potential constrictor, indicating that BMPEA has no vascular effects in porcine mesenteric vasculature in the concentrations tested. It should be noted that BMPEA was administered in higher concentrations than would normally be present under normal physiologic conditions, and thus if any effects were present with normal, oral administration of this supplement, they would also be noted in our experimentation. Consequently, it can be stated with confidence that the aforementioned conclusion is correct, and that BMPEA does not have any vascular effects in swine mesenteric arteries. Unfortunately, there are no previous isolated vascular ring studies of BMPEA with which to compare these results, or indeed any vascular studies at all. Before it can be said that BMPEA elicits no vascular effects at all, further study is needed in other organ systems because BMPEA could still elicit a vascular response in other areas of the body. As a possible sympathetic agent, BMPEA should also be tested for effects on gut motility, bronchial constriction and dilation, and cardiac action to determine whether it has any adverse effects.

Appendix 1: Excel Output Data

Vessel tension in g																
	3-Oct-16				6-Oct-16				10-Oct-16				17-Oct-16			
Drug (dose)	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4
KCL	KCl				KCL				KCL				KCL			
15mM		7.53	7.54	6.12	6.79	4.32	6.47	5.12	6.78	7.73	11.36		6.48	5.75		7.09
30mM		20.97	29.35	12.63	11.42	9.89	6.85	10.07	7.31	24.52	21.26		20.34	7.54		7.44
45mM		28.2	34.93	18.75	27.8	27.16	10.57	24.95	9.07	31.54	23.52		26.34	13.53		9.03
60mM		31.75	37.94	21.4	32.06	32.058	12.16	28.05	10.13	32.52	23.81		27.98	15.69		10.04
SNP	SNP				SNP				SNP				SNP			
$1x10^{-7}$ M		31.9	37.8	21.6	28.86	15.7	10.63	16.23	10.44	31.19	23.02		27.56	15.4		9.97
$1x10^{-6}$M		31	36.8	21.64	28.71	15.23	10.53	15.44	10.19	30.37	21.41		26.99	15.24		9.98
$1x10^{-5}$M		29.6	33.39	21.52	27.42	14.12	10.03	15.01	9.28	28.15	18.1		25.97	14.79		9.94
$1x10^{-4}$M		26.01	25.46	20.89	24.95	10.21	8.91	12.42	7.5	19.85	13.01		21.54	12.42		9.7
Phenylephrine	Phenylephrine				Phenylephrine				Phenylephrine							
$1x10^{-7}$ M		7.34	6.37	5.66	7.503	6.78	7.12	6.77	6.55	6.24	5.9					
$1x10^{-6}$M		19.4	9.5	12.9	13.765	6.87	8.45	6.67	6.7	8.5	6.6					
$1x10^{-5}$M		20.1	24.32	23.03	24.28	13.99	19.19	9.38	12.79	26.14	9.68					
$1x10^{-4}$M		12.34	13.42	20.93	31.7	15.98	14.2	10.15	15.17	21.83	7.98					
BMPEA constriction	BMPEA constriction				BMPEA constriction				BMPEA constriction							
$1x10^{-7}$ M		6.98	7.21	6.65	6.62	5.93	6.52	6.33	6.31	5.84	7.69					
$1x10^{-6}$M		6.9	7.1	5.73	6.33	5.49	6.16	5.94	5.96	5.38	7.13					
$1x10^{-5}$M		6.86	7	5.08	6.18	5.32	5.91	5.73	5.81	5.17	6.88					
$1x10^{-4}$M		6.74	6.97	4.83	6.08	5.23	5.83	5.56	5.6	5.05	6.67					
$1x10^{-3}$M		6.75	7.06	4.79	5.99	5.18	5.81	5.45	5.68	5.05	6.87					
Isoproterenol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA dilation													BMPEA dilation			
$1x10^{-7}$ M													21.16	14.26		7.74
$1x10^{-6}$M													21.25	14.27		7.87
$1x10^{-5}$M													21.31	14.28		7.86
$1x10^{-4}$M													21.48	14.36		7.88
$1x10^{-3}$M													22.37	14.7		7.93
Phenelephrine with Phentolamine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA with Phentolamine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
BMPEA with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																

Vessel tension in g																
	20-Oct-16				27-Oct-16				3-Nov-16				7-Nov-16			
Drug (dose)	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4
KCL	KCL				KCL				KCL				KCL			
15mM	7	7.9	7.08	7.56	6.68		6.45	12.7	6.67		8.04	8.54		6.48	5.65	7.02
30mM	6.91	21.13	19.97	28.58	5.8		23.55	25.89	15.19		36.76	35.96		13.24	26.96	27.75
45mM	11.79	25.46	24.47	34.68	8.927		24.48	27.14	34.09		43.73	42.78		15.07	43.86	31.5
60mM	16.9	26.67	25.26	35.58	13.9		25.78	26.95	35.18		42.58	43.81		15.46	44.49	31.56
SNP	SNP				SNP				SNP				SNP			
$1x10^{-7}$ M	17.28	25.62	23.82	34.64	14.31		25.35	26.77	34.38		41.77	43.68		15.49	42.7	30.97
$1x10^{-6}$M	16.95	24.79	23.23	33.81	14.41		24.3	25.68	33.7		40.67	43.6		15.29	39.37	29.98
$1x10^{-5}$M	15.86	22.42	21.25	30.64	14.37		22.19	22.94	32.4		38.58	42		14.7	35.82	27.86
$1x10^{-4}$M	13.53	15.86	13.94	22.99	12.64		18.09	19.42	29.17		21.82	37.58		13.4	29.52	23.44
Phenylephrine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA constriction																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol									Isoproterenol				Isoproterenol			
$1x10^{-7}$ M									35.97		28.74	42.45		24.94	46.15	35.36
$1x10^{-6}$M									36.79		27.81	42.42		24.86	45.89	35.48
$1x10^{-5}$M									37.41		26.29	42.42		24.93	45.9	35.61
$1x10^{-4}$M									37.81		25.52	42.56		25.45	46.6	37.31
BMPEA dilation	BMPEA dilation				BMPEA dilation											
$1x10^{-7}$ M	15.57	20.12	18.96	29.68	20.92		27.14	22.92								
$1x10^{-6}$M	15.74	20.33	19.3	29.8	21.1		27.5	23.05								
$1x10^{-5}$M	16.09	19.7	19.7	29.94	21.12		27.53	23.26								
$1x10^{-4}$M	16.95	20.04	19.7	29.97	21.61		27.68	24.58								
$1x10^{-3}$M	17.95	21.74	21.74	31.01	21.85		28.22	24.91								
Phenelephrine with Phentolamine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA with Phentolamine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
BMPEA with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																

Vessel tension in g																
	10-Nov-16				9-Feb-17				16-Feb-17				23-Feb-17			
Drug (dose)	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4
KCL	KCL				KCL				KCL				KCL			
15mM	8.23	5.59	10.21	10.31	6.314	6.945	7.318	7.009	6.007	4.022	6.57	6.79	7.03	6.76	11.34	6.58
30mM	8.7	6.44	47.52	37.94	11.01	20.871	40.157	18.05	5.61	10.83	35.05	19.01	7.69	8.19	21.82	9.14
45mM	9.94	12.32	48.47	39.59	35.106	25.039	44.379	24.179	5.83	26.53	38.64	16.37	8.87	15.12	24.33	10.86
60mM					35.53	25.41	42.06		9.16	28.106	38.706	16.86	10.33	17.09	24.82	11.71
SNP	SNP				SNP				SNP				SNP			
$1x10^{-7}$ M	12.16	15.937	46.83	39.45	33.65	24.9	40.74	24.76	9.578	27.53	38.37	16.73	10.34	17.11	24.81	11.73
$1x10^{-6}$M	12.816	16.08	45.51	39.15	32.38	24.37	39	24.19	9.851	26.49	37.97	16.59	10.33	17.12	24.81	11.64
$1x10^{-5}$M	12.57	15.49	43.84	37.52	29.01	21.9	31.47	22.55	8.94	23.72	37.31	16.03	8.9	16.04	21.83	10.34
$1x10^{-4}$M	10.7	10.49	30.5	28.78	18.94	18.94	23.2	20.64	7.307	19.63	35.71	14.812	7.42	15.23	19.23	8.01
Phenylephrine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA constriction																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA dilation																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Phenelephrine with Phentolamine					Phenelephrine with Phentolamine				Phenelephrine with Phentolamine				Phenelephrine with Phentolamine			
$1x10^{-7}$ M						7.53		6.8			7.75	6.99	7.2	7.26		
$1x10^{-6}$M						7.54		6.61			7.74	6.8	6.9	6.95		
$1x10^{-5}$M						7.36		6.69			7.75	6.76	6.66	6.71		
$1x10^{-4}$M						7.26		7.76			8.34	6.74	6.45	6.77		
BMPEA with Phentolamine					BMPEA with Phentolamine				BMPEA with Phentolamine				BMPEA with Phentolamine			
$1x10^{-7}$ M					6.88		7.15		6.98	7.06					7.105	6.48
$1x10^{-6}$M					6.72		7		6.88	6.93					6.78	6.26
$1x10^{-5}$M					6.53		6.92		6.76	8.86					6.57	6.09
$1x10^{-4}$M					6.45		6.9		7.03	6.75					6.42	5.62
$1x10^{-3}$M					6.42		7.46		6.94	6.73					7.18	5.63
BMPEA with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol with propranolol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																

Vessel tension in g																
	2-Mar-17				22-Mar-17				22-Mar-17				2-Mar-17			
Drug (dose)	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	Column 1	Column 2	Column 3	Column 4	23-Mar	Column 2	Column 3	Column 4
KCL	KCL															
15mM	7.68	7.28	8.5	7.25												
30mM	16.6	7.27	19.52	10.12												
45mM	20.77	7.25	22.57	11.64												
60mM	22.94	7.22	23.07	11.89												
SNP	SNP															
$1x10^{-7}$ M	22.6	7.22	22.36	11.88												
$1x10^{-6}$M	21.56	7.23	20.25	11.84												
$1x10^{-5}$M	20.4	7.21	16.8	11.75												
$1x10^{-4}$M	14.715	7.18	11.59	10.37												
Phenylephrine																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA constriction																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Isoproterenol																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
BMPEA dilation																
$1x10^{-7}$ M																
$1x10^{-6}$M																
$1x10^{-5}$M																
$1x10^{-4}$M																
$1x10^{-3}$M																
Phenelephrine with Phentolamine	Phenelephrine with Phentolamine															
$1x10^{-7}$ M			7.24	7.53												
$1x10^{-6}$M			7.08	7.47												
$1x10^{-5}$M			7.01	7.57												
$1x10^{-4}$M			7.04	7.74												
BMPEA with Phentolamine	BMPEA with Phentolamine															
$1x10^{-7}$ M	6.93															
$1x10^{-6}$M	6.79															
$1x10^{-5}$M	6.64															
$1x10^{-4}$M	6.57															
$1x10^{-3}$M	6.5															
BMPEA with propranolol					BMPEA with propranolol				BMPEA with propranolol				BMPEA with propranolol			
$1x10^{-7}$ M					16.78	22.86			14.26	18.08			12.11	14.31		
$1x10^{-6}$M					16.76	22.98			14.65	18.44			12.13	14.12		
$1x10^{-5}$M					16.58	22.87			15.03	18.83			12.21	14.14		
$1x10^{-4}$M					16.56	21.57			15.78	19.53			12.29	14.33		
$1x10^{-3}$M					16.55	21.56			16.52	18.73			12.09	14.32		
Isoproterenol with propranolol					Isoproterenol with propranolol				Isoproterenol with propranolol				Isoproterenol with propranolol			
$1x10^{-7}$ M							20.3	10.29			17.58	16.49			19.59	1.32
$1x10^{-6}$M							20.21	10.29			17.57	6.72			19.92	13.43
$1x10^{-5}$M							20.18	10.26			17.8	16.55			20.27	13.44
$1x10^{-4}$M							21.55	10.58			18.27	16.88			21.16	14

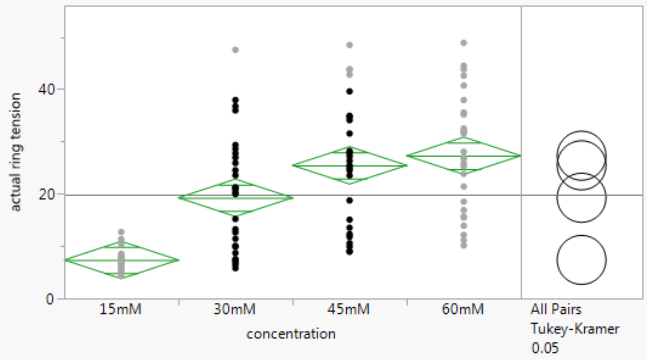
Vessel tension in g		
Drug (dose)		
KCL	Avg tension:	Standar error of the mean
15mM	7.273586957	0.248085585
30mM	18.23517391	1.590412379
45mM	23.93847826	1.719099621
60mM	27.67958621	1.883469698
SNP	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	25.9925	1.75387608
$1x10^{-6}$M	25.36071053	1.686700129
$1x10^{-5}$M	23.65	1.569095063
$1x10^{-4}$M	19.0505	1.257054202
Phenylephrine	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	6.6233	0.189967252
$1x10^{-6}$M	9.9355	1.329753204
$1x10^{-5}$M	18.29	2.007423445
$1x10^{-4}$M	16.37	2.175466744
BMPEA constriction	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	6.608	0.17940519
$1x10^{-6}$M	6.212	0.202883656
$1x10^{-5}$M	5.994	0.227567817
$1x10^{-4}$M	5.856	0.235306892
$1x10^{-3}$M	5.863	0.252441632
Isoproterenol	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	35.60166667	2.526320183
$1x10^{-6}$M	35.54166667	2.568416957
$1x10^{-5}$M	35.42666667	2.669169659
$1x10^{-4}$M	35.875	2.761973751
BMPEA dilation	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	19.847	1.997616385
$1x10^{-6}$M	20.021	2.008528732
$1x10^{-5}$M	20.079	2.01275458
$1x10^{-4}$M	20.425	2.028776451
$1x10^{-3}$M	21.242	2.083114549
Phenelephrine with Phentolamine	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	7.2875	0.109246608
$1x10^{-6}$M	7.13625	0.141584623
$1x10^{-5}$M	7.06375	0.154572423
$1x10^{-4}$M	7.2625	0.226059016
BMPEA with Phentolamine	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	6.940714286	0.084883874
$1x10^{-6}$M	6.765714286	0.091751661
$1x10^{-5}$M	6.91	0.339130541
$1x10^{-4}$M	6.534285714	0.174804069
$1x10^{-3}$M	6.694285714	0.225175668
BMPEA with propranolol	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	16.4	1.54973331
$1x10^{-6}$M	16.51333333	1.570608516
$1x10^{-5}$M	16.61	1.549991398
$1x10^{-4}$M	16.67666667	1.386284402
$1x10^{-3}$M	16.62833333	1.350075101
Isoproterenol with propranolol	Avg tension:	Standar error of the mean
$1x10^{-7}$ M	14.26166667	2.966986593
$1x10^{-6}$M	14.69	2.240432994
$1x10^{-5}$M	16.41666667	1.609423637
$1x10^{-4}$M	17.07333333	1.730313395

Appendix 2: Statistical Outputs

KCl one-way ANOVA and Tukey post hoc testing:

Untitled - Fit Y by X of actual ring tension by concentration - JMP Pro

Oneway Analysis of actual ring tension By concentration



Missing Rows 6

Oneway Anova

Summary of Fit

Rsquare	0.391588
Adj Rsquare	0.375853
Root Mean Square Error	9.879524
Mean of Response	19.84721
Observations (or Sum Wgts)	120

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
concentration	3	7287.214	2429.07	24.8868	<.0001*
Error	116	11322.179	97.60		
C. Total	119	18609.393			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.60667	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	60mM	45mM	30mM	15mM
60mM	-6.649	-4.809	1.385	13.275
45mM	-4.809	-6.649	-0.456	11.434
30mM	1.385	-0.456	-6.649	5.240
15mM	13.275	11.434	5.240	-6.649

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
60mM	A	27.296933
45mM	A B	25.456233
30mM	B	19.262667
15mM	C	7.373000

Levels not connected by same letter are significantly different.

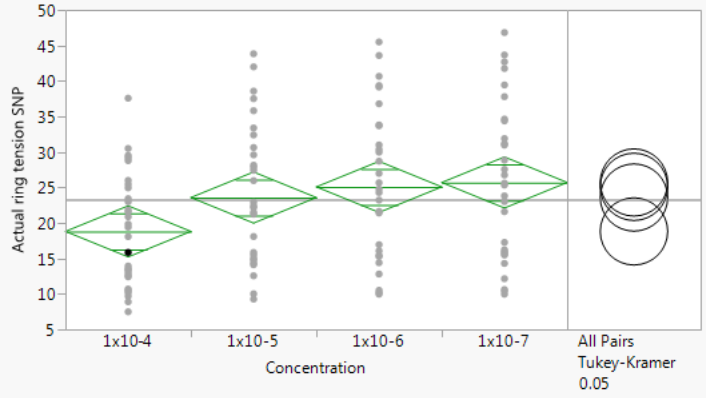
Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
60mM	15mM	19.92393	2.550882	13.2746	26.57324	<.0001*
45mM	15mM	18.08323	2.550882	11.4339	24.73254	<.0001*
30mM	15mM	11.88967	2.550882	5.2404	18.53898	<.0001*
60mM	30mM	8.03427	2.550882	1.3850	14.68358	0.0110*
45mM	30mM	6.19357	2.550882	-0.4557	12.84288	0.0775
60mM	45mM	1.84070	2.550882	-4.8086	8.49001	0.8883

SNP one-way ANOVA and Tukey post hoc testing:

Untitled - Fit Y by X of Actual ring tension SNP by Concentration - JMP Pro

Oneway Analysis of Actual ring tension SNP By Concentration



Oneway Anova

Summary of Fit

Rsquare	0.070783
Adj Rsquare	0.046751
Root Mean Square Error	9.951856
Mean of Response	23.30694
Observations (or Sum Wgts)	120

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Concentration	3	875.135	291.712	2.9454	0.0359*
Error	116	11488.575	99.039		
C. Total	119	12363.710			

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.60667	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10-7	1x10-6	1x10-5	1x10-4
1x10-7	-6.6980	-6.0783	-4.5751	0.1926
1x10-6	-6.0783	-6.6980	-5.1948	-0.4271
1x10-5	-4.5751	-5.1948	-6.6980	-1.9303
1x10-4	0.1926	-0.4271	-1.9303	-6.6980

Positive values show pairs of means that are significantly different.

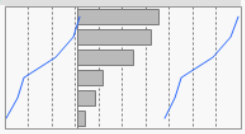
Connecting Letters Report

Level		Mean
1x10-7	A	25.715233
1x10-6	A B	25.095533
1x10-5	A B	23.592333
1x10-4	B	18.824667

Levels not connected by same letter are significantly different.

Ordered Differences Report

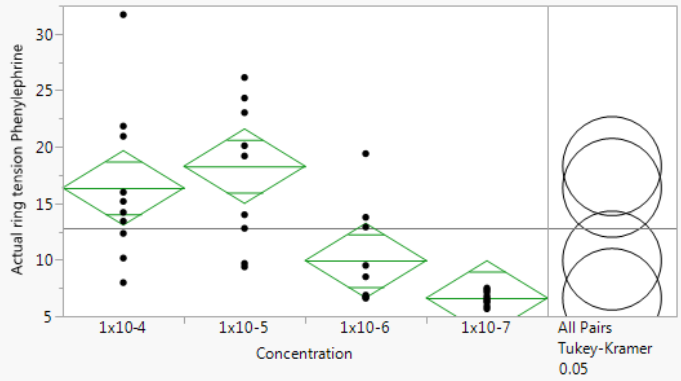
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10-7	1x10-4	6.890567	2.569558	0.19257	13.58856	0.0412*
1x10-6	1x10-4	6.270867	2.569558	-0.42713	12.96886	0.0752
1x10-5	1x10-4	4.767667	2.569558	-1.93033	11.46566	0.2531
1x10-7	1x10-5	2.122900	2.569558	-4.57509	8.82089	0.8420
1x10-6	1x10-5	1.503200	2.569558	-5.19479	8.20119	0.9364
1x10-7	1x10-6	0.619700	2.569558	-6.07829	7.31769	0.9950



Phenylephrine one-way ANOVA and Tukey post hoc testing:

Untitled - Fit Y by X of Actual ring tension Phenylephrine by Concentration - JMP Pro

Oneway Analysis of Actual ring tension Phenylephrine By Concentration



Missing Rows 80

Oneway Anova

Summary of Fit

Rsquare	0.484109
Adj Rsquare	0.441118
Root Mean Square Error	5.139731
Mean of Response	12.8047
Observations (or Sum Wgts)	40

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Concentration	3	892.4189	297.473	11.2607	<.0001*
Error	36	951.0062	26.417		
C. Total	39	1843.4252			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10-4	10	16.3700	1.6253	13.074	19.666
1x10-5	10	18.2900	1.6253	14.994	21.586
1x10-6	10	9.9355	1.6253	6.639	13.232
1x10-7	10	6.6233	1.6253	3.327	9.920

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.69323	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10-5	1x10-4	1x10-6	1x10-7
1x10-5	-6.1905	-4.2705	2.1640	5.4762
1x10-4	-4.2705	-6.1905	0.2440	3.5562
1x10-6	2.1640	0.2440	-6.1905	-2.8783
1x10-7	5.4762	3.5562	-2.8783	-6.1905

Positive values show pairs of means that are significantly different.

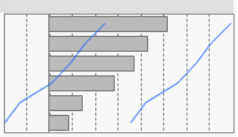
Connecting Letters Report

Level		Mean
1x10-5	A	18.290000
1x10-4	A	16.370000
1x10-6	B	9.935500
1x10-7	B	6.623300

Levels not connected by same letter are significantly different.

Ordered Differences Report

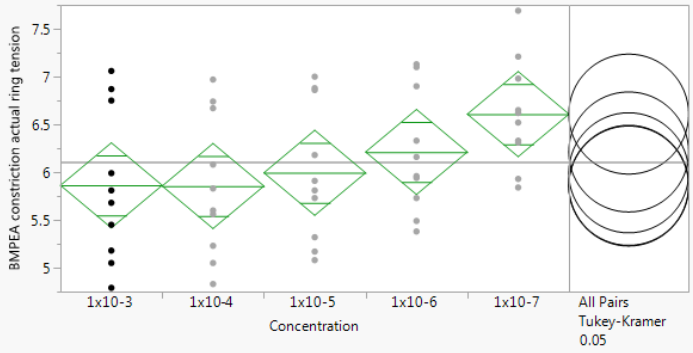
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10-5	1x10-7	11.66670	2.298558	5.47616	17.85724	<.0001*
1x10-4	1x10-7	9.74670	2.298558	3.55616	15.93724	0.0008*
1x10-5	1x10-6	8.35450	2.298558	2.16396	14.54504	0.0046*
1x10-4	1x10-6	6.43450	2.298558	0.24396	12.62504	0.0391*
1x10-6	1x10-7	3.31220	2.298558	-2.87834	9.50274	0.4829
1x10-5	1x10-4	1.92000	2.298558	-4.27054	8.11054	0.8373



BMPEA constrictor one-way ANOVA and Tukey post hoc testing:

Untitled - Fit Y by X of BMPEA constriction actual ring tension by Concentration - JMP Pro

Oneway Analysis of BMPEA constriction actual ring tension By Concentration



Missing Rows 80

Oneway Anova

Summary of Fit

Rsquare	0.15309
Adj Rsquare	0.077809
Root Mean Square Error	0.698901
Mean of Response	6.1066
Observations (or Sum Wgts)	50

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Concentration	4	3.973312	0.993328	2.0336	0.1057
Error	45	21.980810	0.488462		
C. Total	49	25.954122			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10-3	10	5.86300	0.22101	5.4179	6.3081
1x10-4	10	5.85600	0.22101	5.4109	6.3011
1x10-5	10	5.99400	0.22101	5.5489	6.4391
1x10-6	10	6.21200	0.22101	5.7669	6.6571
1x10-7	10	6.60800	0.22101	6.1629	7.0531

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.84145	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10-7	1x10-6	1x10-5	1x10-3	1x10-4
1x10-7	-0.88812	-0.49212	-0.27412	-0.14312	-0.13612
1x10-6	-0.49212	-0.88812	-0.67012	-0.53912	-0.53212
1x10-5	-0.27412	-0.67012	-0.88812	-0.75712	-0.75012
1x10-3	-0.14312	-0.53912	-0.75712	-0.88812	-0.88112
1x10-4	-0.13612	-0.53212	-0.75012	-0.88112	-0.88812

Positive values show pairs of means that are significantly different.

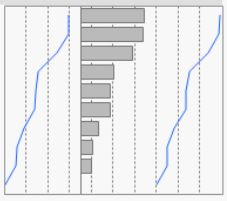
Connecting Letters Report

Level		Mean
1x10-7	A	6.6080000
1x10-6	A	6.2120000
1x10-5	A	5.9940000
1x10-3	A	5.8630000
1x10-4	A	5.8560000

Levels not connected by same letter are significantly different.

Ordered Differences Report

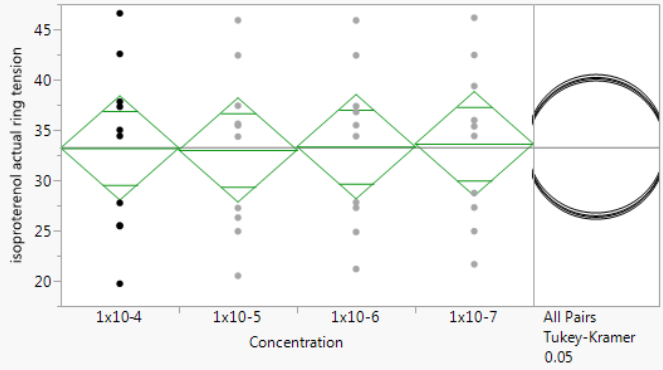
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10-7	1x10-4	0.7520000	0.3125580	-0.136119	1.640119	0.1324
1x10-7	1x10-3	0.7450000	0.3125580	-0.143119	1.633119	0.1386
1x10-7	1x10-5	0.6140000	0.3125580	-0.274119	1.502119	0.2997
1x10-7	1x10-6	0.3960000	0.3125580	-0.492119	1.284119	0.7124
1x10-6	1x10-4	0.3560000	0.3125580	-0.532119	1.244119	0.7851
1x10-6	1x10-3	0.3490000	0.3125580	-0.539119	1.237119	0.7970
1x10-6	1x10-5	0.2180000	0.3125580	-0.670119	1.106119	0.9560
1x10-5	1x10-4	0.1380000	0.3125580	-0.750119	1.026119	0.9918
1x10-5	1x10-3	0.1310000	0.3125580	-0.757119	1.019119	0.9933
1x10-3	1x10-4	0.0070000	0.3125580	-0.881119	0.895119	1.0000



Isoproterenol one-way ANOVA and Tukey post hoc testing:

Untitled - Fit Y by X of isoproterenol actual ring tension by Concentration - JMP Pro

Oneway Analysis of isoproterenol actual ring tension By Concentration



Missing Rows 90

Oneway Anova

Summary of Fit

Rsquare	0.000865
Adj Rsquare	-0.0824
Root Mean Square Error	8.078082
Mean of Response	33.29975
Observations (or Sum Wgts)	40

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Concentration	3	2.0342	0.6781	0.0104	0.9985
Error	36	2349.1945	65.2554		
C. Total	39	2351.2287			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10-4	10	33.2120	2.5545	28.031	38.393
1x10-5	10	33.0110	2.5545	27.830	38.192
1x10-6	10	33.3440	2.5545	28.163	38.525
1x10-7	10	33.6320	2.5545	28.451	38.813

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.69323	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10-7	1x10-6	1x10-4	1x10-5
1x10-7	-9.7296	-9.4416	-9.3096	-9.1086
1x10-6	-9.4416	-9.7296	-9.5976	-9.3966
1x10-4	-9.3096	-9.5976	-9.7296	-9.5286
1x10-5	-9.1086	-9.3966	-9.5286	-9.7296

Positive values show pairs of means that are significantly different.

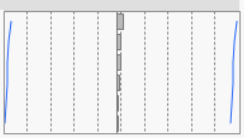
Connecting Letters Report

Level		Mean
1x10-7	A	33.632000
1x10-6	A	33.344000
1x10-4	A	33.212000
1x10-5	A	33.011000

Levels not connected by same letter are significantly different.

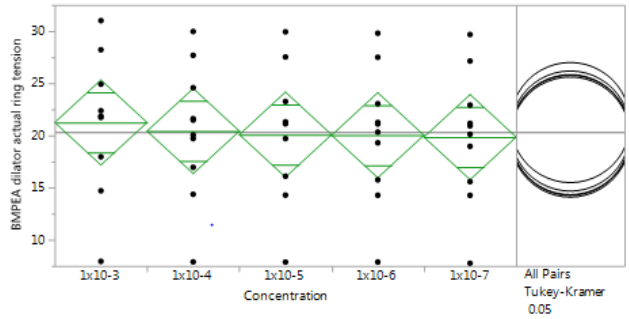
Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10-7	1x10-5	0.6210000	3.612628	-9.10863	10.35063	0.9982
1x10-7	1x10-4	0.4200000	3.612628	-9.30963	10.14963	0.9994
1x10-6	1x10-5	0.3330000	3.612628	-9.39663	10.06263	0.9997
1x10-7	1x10-6	0.2880000	3.612628	-9.44163	10.01763	0.9998
1x10-4	1x10-5	0.2010000	3.612628	-9.52863	9.93063	0.9999
1x10-6	1x10-4	0.1320000	3.612628	-9.59763	9.86163	1.0000



BMPEA dilator one-way ANOVA and Tukey post hoc testing:

Oneway Analysis of BMPEA dilator actual ring tension By Concentration



Missing Rows 80

Oneway Anova

Summary of Fit

Rsquare	0.006625
Adj Rsquare	-0.08168
Root Mean Square Error	6.407986
Mean of Response	20.3228
Observations (or Sum Wgts)	50

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Concentration	4	12.3228	3.0807	0.0750	0.9894
Error	45	1847.8026	41.0623		
C. Total	49	1860.1254			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10-3	10	21.2420	2.0264	17.161	25.323
1x10-4	10	20.4250	2.0264	16.344	24.506
1x10-5	10	20.0790	2.0264	15.998	24.160
1x10-6	10	20.0210	2.0264	15.940	24.102
1x10-7	10	19.8470	2.0264	15.766	23.928

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.84145	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10-3	1x10-4	1x10-5	1x10-6	1x10-7
1x10-3	-8.1429	-7.3259	-6.9799	-6.9219	-6.7479
1x10-4	-7.3259	-8.1429	-7.7969	-7.7389	-7.5649
1x10-5	-6.9799	-7.7969	-8.1429	-8.0849	-7.9109
1x10-6	-6.9219	-7.7389	-8.0849	-8.1429	-7.9689
1x10-7	-6.7479	-7.5649	-7.9109	-7.9689	-8.1429

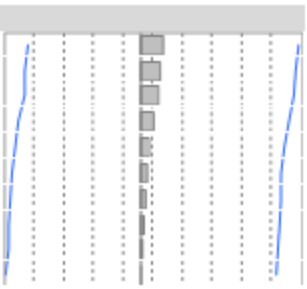
Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level		Mean
1x10-3	A	21.242000
1x10-4	A	20.425000
1x10-5	A	20.079000
1x10-6	A	20.021000
1x10-7	A	19.847000

Ordered Differences Report

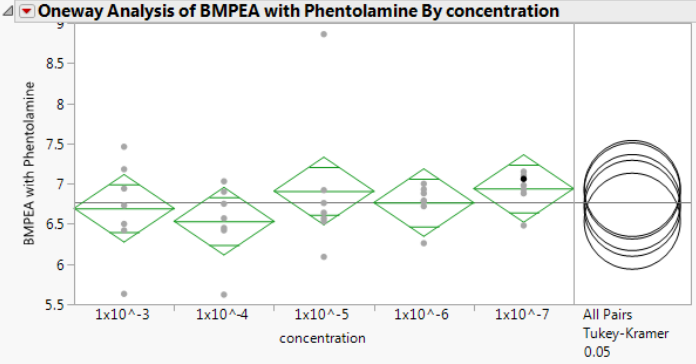
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10-3	1x10-7	1.395000	2.865738	-6.74786	9.537858	0.9882
1x10-3	1x10-6	1.221000	2.865738	-6.92186	9.363858	0.9929
1x10-3	1x10-5	1.163000	2.865738	-6.97986	9.305858	0.9941
1x10-3	1x10-4	0.817000	2.865738	-7.32586	8.959858	0.9985
1x10-4	1x10-7	0.578000	2.865738	-7.56486	8.720858	0.9996
1x10-4	1x10-6	0.404000	2.865738	-7.73886	8.546858	0.9999
1x10-4	1x10-5	0.346000	2.865738	-7.79686	8.488858	1.0000
1x10-5	1x10-7	0.232000	2.865738	-7.91086	8.374858	1.0000
1x10-6	1x10-7	0.174000	2.865738	-7.96886	8.316858	1.0000
1x10-5	1x10-6	0.058000	2.865738	-8.08486	8.200858	1.0000



BMPEA with phentolamine one way ANOVA:

Untitled 2 - Fit Y by X of BMPEA with Phentolamine by concentration - JMP Pro

Oneway Analysis of BMPEA with Phentolamine By concentration



Missing Rows 5

Oneway Anova

Summary of Fit

Rsquare	0.079665
Adj Rsquare	-0.04305
Root Mean Square Error	0.544657
Mean of Response	6.769
Observations (or Sum Wgts)	35

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
concentration	4	0.7703543	0.192589	0.6492	0.6319
Error	30	8.8995357	0.296651		
C. Total	34	9.6698900			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10^-3	7	6.69429	0.20586	6.2739	7.1147
1x10^-4	7	6.53429	0.20586	6.1139	6.9547
1x10^-5	7	6.91000	0.20586	6.4896	7.3304
1x10^-6	7	6.76571	0.20586	6.3453	7.1861
1x10^-7	7	6.94071	0.20586	6.5203	7.3611

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.90061	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10^-7	1x10^-5	1x10^-6	1x10^-3	1x10^-4
1x10^-7	-0.84446	-0.81374	-0.66946	-0.59803	-0.43803
1x10^-5	-0.81374	-0.84446	-0.70017	-0.62874	-0.46874
1x10^-6	-0.66946	-0.70017	-0.84446	-0.77303	-0.61303
1x10^-3	-0.59803	-0.62874	-0.77303	-0.84446	-0.68446
1x10^-4	-0.43803	-0.46874	-0.61303	-0.68446	-0.84446

Positive values show pairs of means that are significantly different.

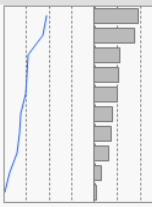
Connecting Letters Report

Level		Mean
1x10^-7	A	6.9407143
1x10^-5	A	6.9100000
1x10^-6	A	6.7657143
1x10^-3	A	6.6942857
1x10^-4	A	6.5342857

Levels not connected by same letter are significantly different.

Ordered Differences Report

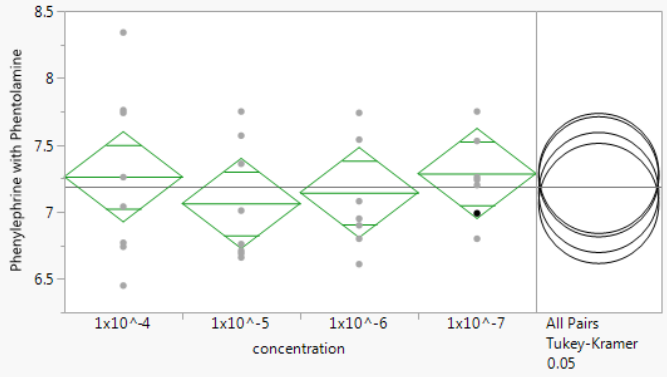
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10^-7	1x10^-4	0.4064286	0.2911314	-0.438030	1.250887	0.6348
1x10^-5	1x10^-4	0.3757143	0.2911314	-0.468744	1.220173	0.6989
1x10^-7	1x10^-3	0.2464286	0.2911314	-0.598030	1.090887	0.9137
1x10^-6	1x10^-4	0.2314286	0.2911314	-0.613030	1.075887	0.9301
1x10^-5	1x10^-3	0.2157143	0.2911314	-0.628744	1.060173	0.9450
1x10^-7	1x10^-6	0.1750000	0.2911314	-0.669459	1.019459	0.9738
1x10^-3	1x10^-4	0.1600000	0.2911314	-0.684459	1.004459	0.9812
1x10^-5	1x10^-6	0.1442857	0.2911314	-0.700173	0.988744	0.9872
1x10^-6	1x10^-3	0.0714286	0.2911314	-0.773030	0.915887	0.9992
1x10^-7	1x10^-5	0.0307143	0.2911314	-0.813744	0.875173	1.0000



Phenylephrine with phentolamine one way ANOVA:

Untitled 2 - Fit Y by X of Phenylephrine with Phentolamine by concentration - JMP Pro

Oneway Analysis of Phenylephrine with Phentolamine By concentration



Missing Rows 8

Oneway Anova

Summary of Fit

Rsquare	0.041534
Adj Rsquare	-0.06116
Root Mean Square Error	0.464515
Mean of Response	7.189688
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
concentration	3	0.2618094	0.087270	0.4044	0.7509
Error	28	6.0416875	0.215775		
C. Total	31	6.3034969			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10^-4	8	7.26250	0.16423	6.9261	7.5989
1x10^-5	8	7.06375	0.16423	6.7273	7.4002
1x10^-6	8	7.14500	0.16423	6.8086	7.4814
1x10^-7	8	7.28750	0.16423	6.9511	7.6239

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.73031	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10^-7	1x10^-4	1x10^-6	1x10^-5
1x10^-7	-0.63414	-0.60914	-0.49164	-0.41039
1x10^-4	-0.60914	-0.63414	-0.51664	-0.43539
1x10^-6	-0.49164	-0.51664	-0.63414	-0.55289
1x10^-5	-0.41039	-0.43539	-0.55289	-0.63414

Positive values show pairs of means that are significantly different.

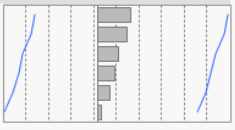
Connecting Letters Report

Level		Mean
1x10^-7	A	7.2875000
1x10^-4	A	7.2625000
1x10^-6	A	7.1450000
1x10^-5	A	7.0637500

Levels not connected by same letter are significantly different.

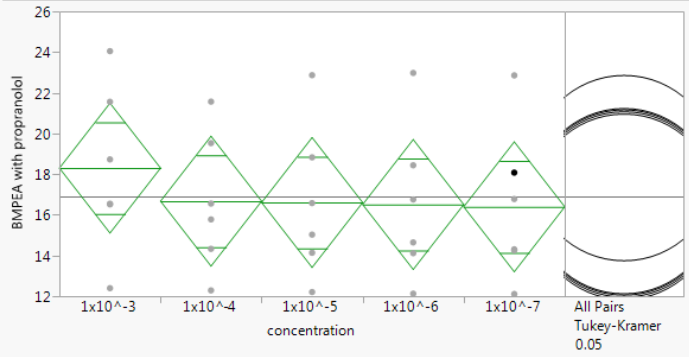
Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10^-7	1x10^-5	0.2237500	0.2322577	-0.410386	0.8578862	0.7710
1x10^-4	1x10^-5	0.1987500	0.2322577	-0.435386	0.8328862	0.8273
1x10^-7	1x10^-6	0.1425000	0.2322577	-0.491636	0.7766362	0.9269
1x10^-4	1x10^-6	0.1175000	0.2322577	-0.516636	0.7516362	0.9570
1x10^-6	1x10^-5	0.0812500	0.2322577	-0.552886	0.7153862	0.9850
1x10^-7	1x10^-4	0.0250000	0.2322577	-0.609136	0.6591362	0.9995



BMPEA with propranolol one-way ANOVA:

Oneway Analysis of BMPEA with propranolol By concentration



Missing Rows 10

Oneway Anova

Summary of Fit

Rsquare	0.039897
Adj Rsquare	-0.11372
Root Mean Square Error	3.798411
Mean of Response	16.90033
Observations (or Sum Wgts)	30

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
concentration	4	14.98895	3.7472	0.2597	0.9009
Error	25	360.69815	14.4279		
C. Total	29	375.68710			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10^-3	6	18.3017	1.5507	15.108	21.495
1x10^-4	6	16.6767	1.5507	13.483	19.870
1x10^-5	6	16.6100	1.5507	13.416	19.804
1x10^-6	6	16.5133	1.5507	13.320	19.707
1x10^-7	6	16.4000	1.5507	13.206	19.594

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.93687	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10^-3	1x10^-4	1x10^-5	1x10^-6	1x10^-7
1x10^-3	-6.4406	-4.8156	-4.7489	-4.6523	-4.5389
1x10^-4	-4.8156	-6.4406	-6.3739	-6.2773	-6.1639
1x10^-5	-4.7489	-6.3739	-6.4406	-6.3439	-6.2306
1x10^-6	-4.6523	-6.2773	-6.3439	-6.4406	-6.3273
1x10^-7	-4.5389	-6.1639	-6.2306	-6.3273	-6.4406

Positive values show pairs of means that are significantly different.

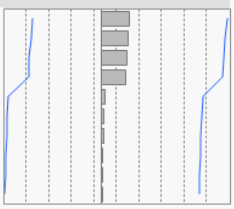
Connecting Letters Report

Level		Mean
1x10^-3	A	18.301667
1x10^-4	A	16.676667
1x10^-5	A	16.610000
1x10^-6	A	16.513333
1x10^-7	A	16.400000

Levels not connected by same letter are significantly different.

Ordered Differences Report

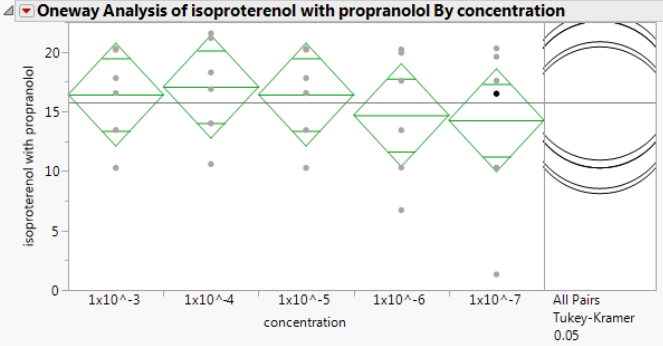
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10^-3	1x10^-7	1.901667	2.193014	-4.53894	8.342270	0.9062
1x10^-3	1x10^-6	1.788333	2.193014	-4.65227	8.228937	0.9234
1x10^-3	1x10^-5	1.691667	2.193014	-4.74894	8.132270	0.9365
1x10^-3	1x10^-4	1.625000	2.193014	-4.81560	8.065603	0.9446
1x10^-4	1x10^-7	0.276667	2.193014	-6.16394	6.717270	0.9999
1x10^-5	1x10^-7	0.210000	2.193014	-6.23060	6.650603	1.0000
1x10^-4	1x10^-6	0.163333	2.193014	-6.27727	6.603937	1.0000
1x10^-6	1x10^-7	0.113333	2.193014	-6.32727	6.553937	1.0000
1x10^-5	1x10^-6	0.096667	2.193014	-6.34394	6.537270	1.0000
1x10^-4	1x10^-5	0.066667	2.193014	-6.37394	6.507270	1.0000



Isoproterenol with propranolol one way ANOVA:

Untitled 2 - Fit Y by X of isoproterenol with propranolol by concentration - JMP Pro

Oneway Analysis of isoproterenol with propranolol By concentration



Missing Rows 10

Oneway Anova

Summary of Fit

Rsquare	0.051539
Adj Rsquare	-0.10022
Root Mean Square Error	5.137745
Mean of Response	15.77167
Observations (or Sum Wgts)	30

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
concentration	4	35.85893	8.9647	0.3396	0.8486
Error	25	659.91068	26.3964		
C. Total	29	695.76962			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1x10^-3	6	16.4167	2.0975	12.097	20.736
1x10^-4	6	17.0733	2.0975	12.754	21.393
1x10^-5	6	16.4167	2.0975	12.097	20.736
1x10^-6	6	14.6900	2.0975	10.370	19.010
1x10^-7	6	14.2617	2.0975	9.942	18.581

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.93687	0.05

HSD Threshold Matrix

Abs(Dif)-HSD

	1x10^-4	1x10^-3	1x10^-5	1x10^-6	1x10^-7
1x10^-4	-8.7116	-8.0549	-8.0549	-6.3283	-5.8999
1x10^-3	-8.0549	-8.7116	-8.7116	-6.9849	-6.5566
1x10^-5	-8.0549	-8.7116	-8.7116	-6.9849	-6.5566
1x10^-6	-6.3283	-6.9849	-6.9849	-8.7116	-8.2833
1x10^-7	-5.8999	-6.5566	-6.5566	-8.2833	-8.7116

Positive values show pairs of means that are significantly different.

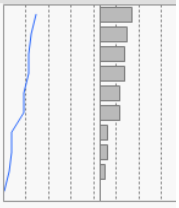
Connecting Letters Report

Level		Mean
1x10^-4	A	17.073333
1x10^-3	A	16.416667
1x10^-5	A	16.416667
1x10^-6	A	14.690000
1x10^-7	A	14.261667

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1x10^-4	1x10^-7	2.811667	2.966279	-5.89992	11.52325	0.8753
1x10^-4	1x10^-6	2.383333	2.966279	-6.32825	11.09492	0.9271
1x10^-3	1x10^-7	2.155000	2.966279	-6.55658	10.86658	0.9483
1x10^-5	1x10^-7	2.155000	2.966279	-6.55658	10.86658	0.9483
1x10^-3	1x10^-6	1.726667	2.966279	-6.98492	10.43825	0.9765
1x10^-5	1x10^-6	1.726667	2.966279	-6.98492	10.43825	0.9765
1x10^-4	1x10^-3	0.656667	2.966279	-8.05492	9.36825	0.9994
1x10^-4	1x10^-5	0.656667	2.966279	-8.05492	9.36825	0.9994
1x10^-6	1x10^-7	0.428333	2.966279	-8.28325	9.13992	0.9999
1x10^-5	1x10^-3	0.000000	2.966279	-8.71158	8.71158	1.0000



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