

Pulmonary Vascular Responses to Ma Huang Extract

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ABSTRACT

Objective: To test the hypothesis that *ma huang* induces a pressor response in the pulmonary vascular bed of the cat by activating α_1 -adrenergic receptors

Design: Prospective vehicle-controlled study.

Setting: Research laboratory at Texas Tech University School of Medicine, Lubbock, TX.

Subjects: Intact chest preparation; adult mongrel cats.

Interventions: The effects of phentolamine, a nonselective α receptor blocker, and prazosin, an α_1 selective antagonist, were investigated on pulmonary arterial responses to *ma huang*, phenylephrine, norepinephrine, and U-46619, a thromboxane A_2 mimic.

Measurements and main results: Lobar arterial perfusion pressure was continuously monitored, electronically averaged, and recorded with constant flow in the isolated left lower lobe vascular bed of the cat. Phentolamine and prazosin significantly reduced vasoconstrictor pulmonary perfusion pressure increases induced by *ma huang*.

Conclusions: *Ma huang* has significant vasopressor activity in the pulmonary vascular bed of the cat mediated predominantly by α_1 -adrenergic receptor activation.

INTRODUCTION

M*a huang* consists of dried, young branchlets of *Ephedra sinica*, *Ephedra shennungiana*, or other equivalent *Ephedra* species (Nam, 2003). The predominant pharmacologically active component is ephedra, an α and β adrenergic agonist that also enhances norepinephrine release from sympathetic neurons. The various species are collectively known as *ma huang* (Namba, 1976). In Traditional Chinese Medicine, ephedra was used as an antipyretic and antitussive agent, and extracts of ephedra were also used in the treatment of joint pain (Ling, 1995). Currently, ephedra formulations are found in over-the-counter cold and cough med-

icines, nasal decongestants, central nervous system stimulants, weight-reduction aids, and for induction of euphoric states (Cupp, 1999). Ephedra is also an effective bronchodilator in asthma, although long-term use leads to down-regulation of β receptors and decreased bronchial responsiveness (Neve, 1986; Ziment, 2000).

Different *Ephedra* species vary in their ephedra content (Karch, 2000). Noncommercial varieties of *Ephedra* may also contain no ephedra (Zhang, 1989). Recently, the toxicities of various extracts were assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) calorimetry on a battery of cell lines, and composition was measured by high-performance liquid chromatography (HPLC).

It was concluded that cytotoxicity of all *ma huang* extracts is not related completely to their ephedra content, and other toxins may be present and active (Lee, 2000). HPLC studies comparing the main content of three ephedra alkaloids, namely ephedrine, pseudoephedrine, and norephedrine, in 12 species of Chinese *Ephedra* collected in 24 regions, showed that some samples contain little alkaloid (<0.1%) (Karch, 2000). However, because the main component of *ma huang* is ephedra, they are at times used synonymously. Mass spectroscopic identification of major alkaloid ephedra components have allowed for determination of the nature of the origin of some over-the-counter preparations (Hansen, 2001). More than half of herbal dietary supplements containing *ma huang* exhibit discrepancies between label claims and actual alkaloid content (Gurley, 2000). Ingestion of ephedra in excess amounts leads to a rise in blood pressure, heart rate, and cardiac output along with variable increases in peripheral resistance (Webb, 1998). In this regard, the cardiovascular properties of *ma huang* in normotensive, healthy adults demonstrate effects on blood pressure and heart rate (White, 1997).

In clinical practice, patients often do not inform their health care providers about herbal medicine use. In a recent hospital survey of patients scheduled to undergo elective surgery, more than 70% of participants did not disclose their use of herbal agents. More than 30% were found to be taking one or more herbal supplements; of these, 18% were using compounds with ephedra alkaloids (Kaye, 2000a). Recently, the Food and Drug Administration (FDA) has proposed a dosage limit of 8 mg every 6 hours (24 mg/d) for ephedra alkaloids, but life-threatening adverse reactions have been reported to occur with doses of 1–5 mg (dosages of 4 to 20 mg/d) (Ling, 1995). As a result of these adverse outcomes, the 2002 Ephedra Alkaloid Consumer Protection Act now requires standardized health warning labels on ephedra containing products, with sales prohibited to those less than 18 years of age.

Although *ma huang*-containing compounds have been studied in systemic circulation, little is known about the response to it in the pulmonary vascular bed. The present study

was, therefore, undertaken to investigate pulmonary vascular response to *ma huang* in the pulmonary vascular bed of the intact cat chest under constant flow conditions.

MATERIALS AND METHODS

After approval by the Institutional Review Board for the care of animal subjects, and while maintaining standards of care and handling of the animals in accordance with National Institutes of Health guidelines, 49 adult mongrel cats of either gender weighing 3.0–4.7 kg were sedated with intramuscular ketamine hydrochloride (10–15 mg/kg) then anesthetized with intravenous pentobarbital sodium (30 mg/kg). The animals were restrained in the supine position on a fluoroscopic table, and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 100% O₂ at a rate of 0.5 to 1.5 L/min. Room air was allowed to freely flow into the tube. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from a femoral artery, and intravenous injections were via a catheter positioned in the inferior vena cava from a femoral vein.

For perfusion of the left lower lung lobe, a triple-lumen 6F balloon perfusion catheter was passed under fluoroscopic guidance from an external jugular vein into the artery to the left lower lung lobe. The animal was heparinized (1000 U/kg intravenously) and the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter. Then the lobe was perfused with a Harvard model 1210 perfusion pump (Harvard Apparatus, South Natick, MA) by way of the catheter lumen beyond the balloon cuff with blood withdrawn from a femoral artery. The perfusion rate was adjusted so that lobar arterial perfusion pressure approximated the mean pressure in the main pulmonary artery and was not changed thereafter. The flow rate ranged from 30 to 41 mL/min. All vascular pressures were measured with SpectroMed DTX Plus (Viggo-Spectromed, Oxnard, CA) transducers zeroed at the

right atrial level and were recorded on a Grass model 7D recorder (Grass Instruments, Quincy, MA).

The present experiments were divided into five groups. In the first set of experiments, serial administration of *ma huang* extract in two doses (0.5 mL and 1.0 mL) every 15 minutes for 3 hours was given to evaluate the possibility of tachyphylaxis. In the second series of experiments, the influence of catecholamine depletion by reserpine (1.5 mg/kg intramuscularly) pretreatment, 24 hours prior to the administration of *ma huang*, phenylephrine, norepinephrine (NE), and U-46619, was investigated. In the third series of experiments, the influence of the non selective α -adrenergic antagonist, phentolamine (1 mg \cdot kg⁻¹ intravenously), on responses to *ma huang* extract, phenylephrine, an α_1 -receptor agonist, NE, and U-46619 were investigated. While U-46619, a thromboxane A₂ mimic, has no α -receptor activity, it was given as a control. In the fourth series of experiments, the influence of the α_1 receptor antagonist, prazosin (0.2 mg/kg intravenously), on responses to *ma huang*, phenylephrine, NE, and U-46619 were studied. In the fifth set of experiments, the influence of a higher dose of prazosin (1.0 mg/kg intravenously) on responses to *ma huang*, phenylephrine, NE, and U-46619 were studied.

Ma huang was purchased in a preprepared, 99.9% pure extract solution from Herbalist and Alchemist (Washington, NJ). HPLC was run on this *ma huang* preparation, which revealed the concentration of ephedrine to be 3.3 mg/mL. Stock solutions of U-46619 (Upjohn, Kalamazoo, MI) were prepared in 100% ethanol at concentrations of 5–10 mg/mL and stored in a freezer at -20°C . Working solutions were prepared on a frequent basis by diluting the stock solutions in 0.9% NaCl solution. phenylephrine (10 $\mu\text{g}/\text{mL}$) and NE (100 μg to 1 mg/mL) were dissolved in 0.9% NaCl. The antagonists prazosin (1 mg/mL) and phentolamine (1 mg/mL) were also dissolved in 0.9% NaCl. Vehicle solutions used produced no significant effect on lobar arterial pressure. Working solutions were prepared just before use, stored in brown-stoppered bottles, and kept on crushed ice during the experiments.

All agonists were injected directly into the lobar arterial perfusion circuit in small volumes

in a random sequence, and sufficient time was permitted between injections for pressure to return to baseline values. Because lobar arterial perfusion flow was constant, changes in lobar pressure would reflect changes in pulmonary arterial vascular resistance. All vascular pressures are expressed in absolute units (mm Hg) as means \pm standard error of the mean (SEM). Responses represent peak changes, unless otherwise noted. The data were analyzed with a paired *t* test or ANOVA and Scheffé's *F* test (StatView 5.0, SAS Institute, Berkeley, CA) (Snedecor, 1980). A *p* value < 0.05 was used as the criterion for significance.

RESULTS

Influence of ma huang on vascular tone

Under low tone conditions, intra-arterial injections of *ma huang* extract produced significant dose-dependent increases in lobar arterial pressure (Fig. 1). In a separate series of experiments, serial administration of two doses of *ma huang* extract (0.5 mL and 1.0 mL) every 15 minutes for 3 hours resulted in continued vasoconstriction with return to baseline pressures between doses, demonstrating tachyphylaxis to this pressor response does not occur. Pretreatment with intramuscular reserpine (1.5 mg/kg) 24 hours prior to the experiment did not alter the vasoconstrictor responses to *ma*

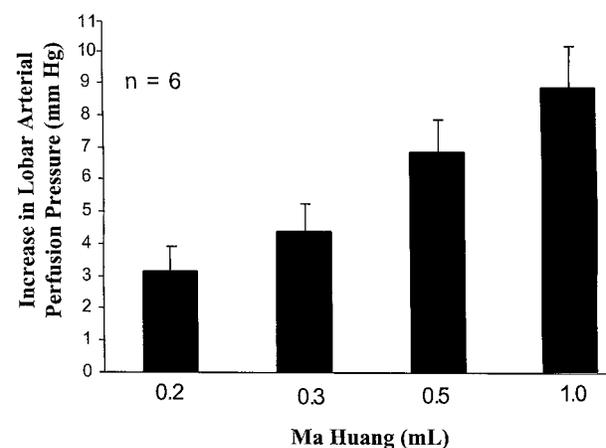


FIG. 1. Influence of Ma huang (mL) on lobar arterial perfusion pressure in the intact chest cat. Data are expressed as mean \pm standard error of the mean.

huang extract. This demonstrates that these effects are the result of the direct effects of *ma huang* extract and not an increased release of endogenous agonists.

Influence of phentolamine on responses to *ma huang*, phenylephrine, NE and U-46619

Responses to *ma huang* (0.2–0.5 mL), phenylephrine (0.1–1.0 μg), NE (0.3–1.0 μg), and U-46619 (0.3–3.0 ng), the thromboxane A_2 mimic, were compared before and 30 minutes after infusion of the nonselective α -antagonist, phentolamine at 1 mg/kg intravenously (Fig. 2). The increases in lobar arterial pressure in response to *ma huang* and phenylephrine were significantly reduced after administration of phentolamine. (Fig. 2).

After intralobar injections of NE and U-46619, the increased lobar vascular tone 30 minutes after the infusion of phentolamine at 1 mg/kg intravenously were not significantly different from the values obtained when NE

and U-46619 were injected during the control period (Fig. 2).

Influence of low-dose prazosin on responses to *ma huang*, phenylephrine, NE, and U-46619

Injections of *ma huang* and phenylephrine into the lobar arterial perfusion circuit produced dose-related and reproducible increases in lobar arterial perfusion pressure that were significantly reduced 30 minutes after administration of the α_1 selective antagonist, prazosin at 0.2 mg \cdot kg $^{-1}$ (Fig. 3). However, vasopressor responses to injections of U-46619 (0.3–3.0 ng) and NE (0.3–1.0 μg) into the lobar arterial perfusion circuit were not altered after administration of this dose of prazosin (Fig. 3).

Influence of high-dose prazosin on responses to *ma huang*, phenylephrine, NE, and U-46619

Responses to *ma huang*, NE, phenylephrine, and U-46619 were compared both before and

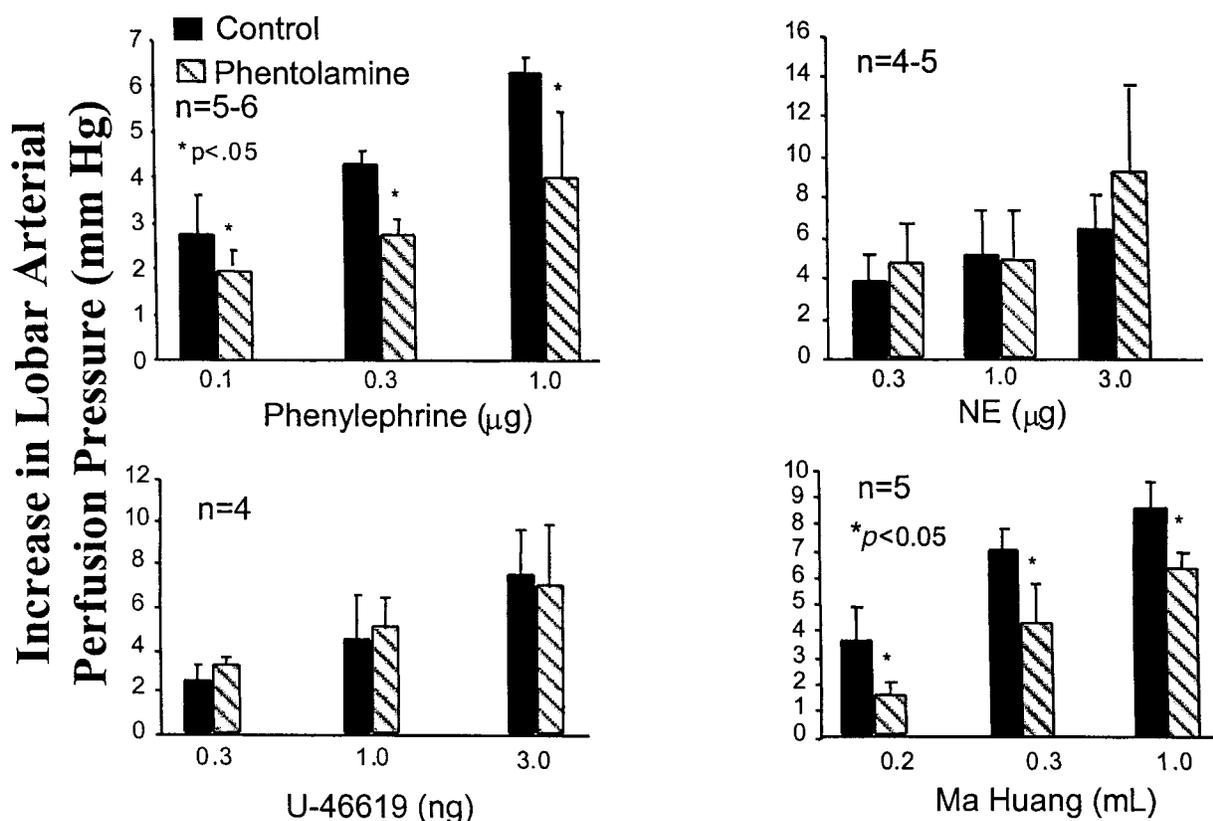


FIG. 2. Influence of phentolamine (hatched bars, $n = 5-6$) on vasoconstrictor responses to phenylephrine (top left), norepinephrine (NE; top right), U-46619 (bottom left), and Ma huang (bottom right). * $p < 0.05$. Solid bars, control. Data are expressed as mean \pm standard error of the mean.

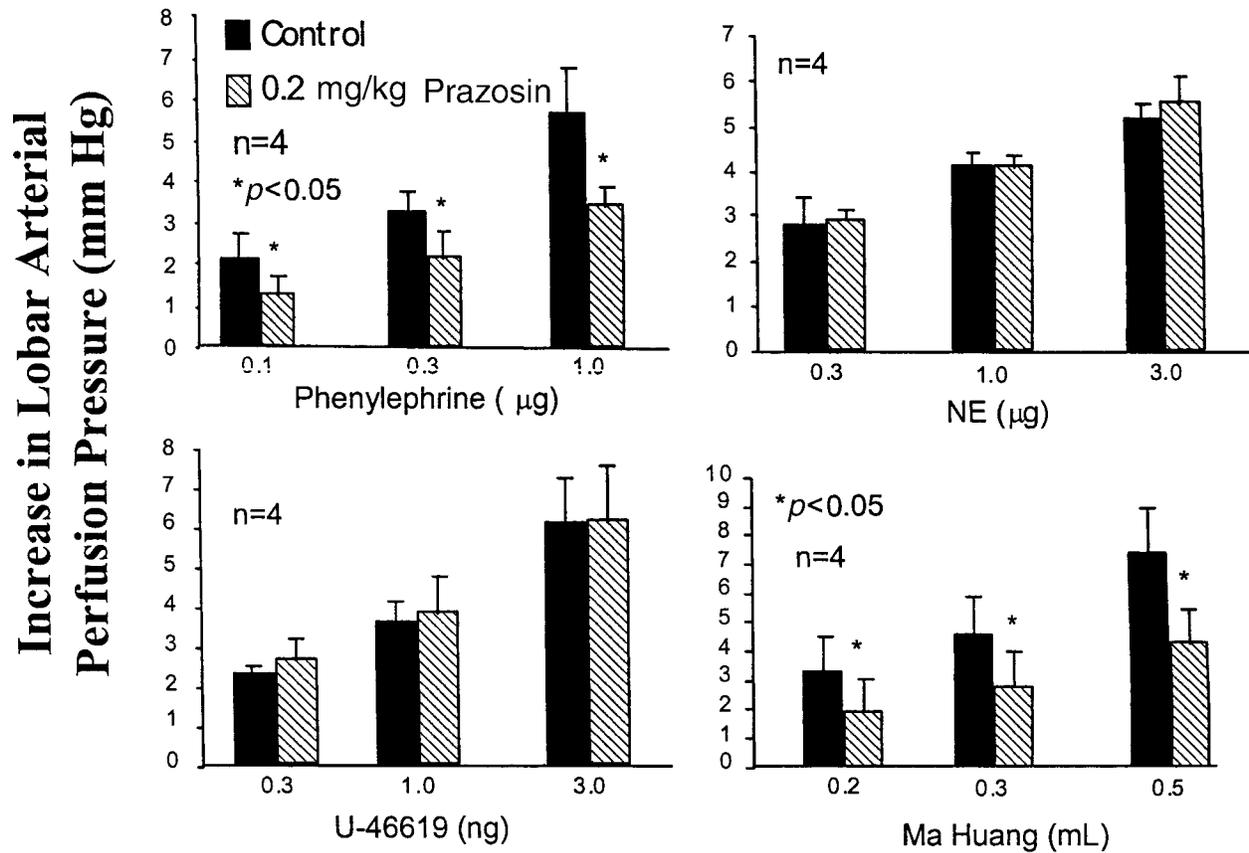


FIG. 3. Influence of prazosin 0.2 mg/kg (hatched bars, $n = 5-6$) on vasoconstrictor responses to phenylephrine (top left), norepinephrine (NE; top right), U-46619 (bottom left), and Ma huang (bottom right). * $p < 0.05$. Solid bars, control. Data are expressed as mean \pm standard error of the mean.

30 minutes after infusion of prazosin in a dose of 1.0 mg/kg intravenously (Fig. 4) The increases in lobar arterial pressure in response to *ma huang*, phenylephrine, and NE were significantly reduced after administration of prazosin. However, the increases in lobar arterial pressure in response to U-46619 were not altered after administration of high-dose prazosin.

DISCUSSION

The primary find of this study is *ma huang* extract increases lobar arterial pressure in the pulmonary vascular bed of the intact chest cat. The increases in lobar arterial pressure were dose-dependent and were partially attenuated by the nonselective α -blocker, phentolamine, and the α_1 -selective antagonist, prazosin. Nearly

complete attenuation of vasopressor responses to *ma huang* was achieved after a higher dose of prazosin. Furthermore, these increases in vascular tone were resistant to tachyphylaxis and were demonstrated in the presence of endogenous catecholamine depletion by pretreatment with reserpine. These results suggest that increases in pulmonary vascular resistance in response to *ma huang* appear to be mediated through an α receptor pathway and in particular via an α_1 mediated mechanism in the pulmonary vascular bed of the cat.

The presence of both postjunctional α_1 - and α_2 -adrenoceptors mediating vasoconstriction in the pulmonary vascular bed has been established. Previous studies demonstrate vasoconstrictor responses to injected norepinephrine in the cat pulmonary vascular bed are due because of activation of α_1 -adrenoceptors (Hyman, 1986). In our model, phentolamine and

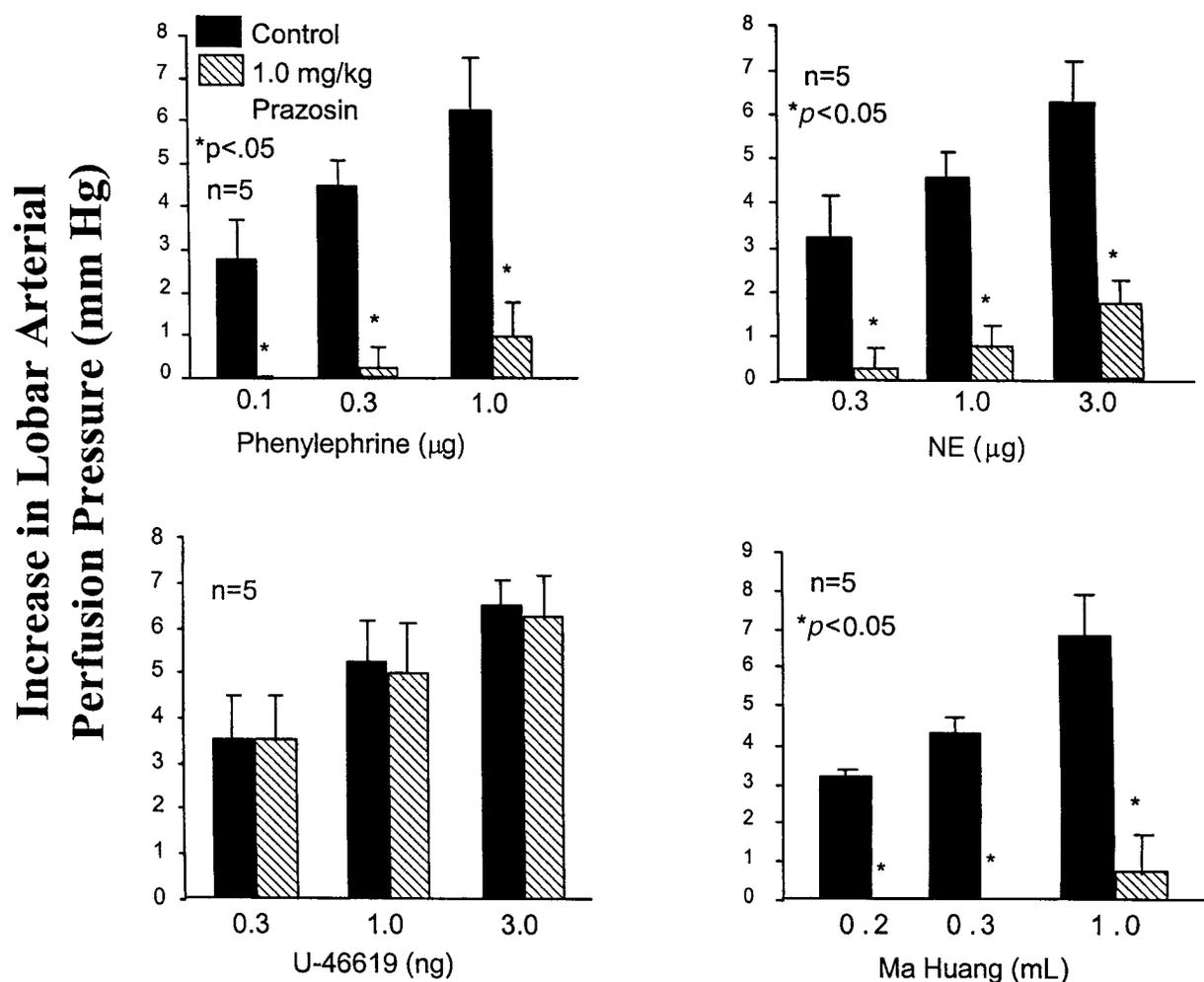


FIG. 4. Influence of prazosin 1.0 mg/kg (hatched bars, $n = 5-6$) on vasoconstrictor responses to phenylephrine (top left), norepinephrine (NE; top right), U-46619 (bottom left), and Ma huang (bottom right). $*p < 0.05$. Solid bars, control. Data are expressed as mean \pm standard error of the mean.

low-dose prazosin failed to block the constrictor effects of norepinephrine; however, high-dose prazosin did. This is possibly explained by a higher receptor affinity of norepinephrine than prazosin or phentolamine. This was overcome by high-dose prazosin.

A review of 140 reports of adverse events related to the use of dietary supplements containing ephedra alkaloids that were submitted to the FDA has shown hypertension to be the single most frequent adverse effect (17 reports), followed by palpitations, tachycardia, or both (13); stroke (10), and seizures (7). Ten (10) events resulted in death, and 13 events produced permanent disability, representing 26% of the definite, probable, and possible cases (Haller, 2000). Other reported adverse reactions

include insomnia, nervousness, tremor, headaches, hypertension, seizures, arrhythmias, heart attack, stroke, and death (Kaye, 2000b; Ling, 1995).

Results of the present study show that the herb, *ma huang*, has significant vasoconstrictor activity in the pulmonary vascular bed of the cat. Furthermore, the results of the present investigation suggest that the vasoconstrictor response to *ma huang* is mediated by an α_1 -receptor and its subsequent effector pathway. The present study suggests this herbal supplement may contribute to pulmonary hypertensive pathophysiologic states. *Ma huang* must be used with caution due to its capacity to elevate systemic and pulmonary vascular pressures. Further studies are warranted to more clearly

elucidate the specific α_1 -adrenergic receptor subtype controlling *ma huang*-mediated responses in the pulmonary vascular bed of the cat.

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