Analysis of Responses to Valerian Root Extract in the Feline Pulmonary Vascular Bed

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ABSTRACT

Objectives: This study was undertaken to investigate pulmonary vascular response to valerian (Valeriana officinalis) in the feline pulmonary vasculature under constant flow conditions.

Design: In separate experiments, the effects of NG-l-nitro-l-arginine methyl ester (L-NIO), a nitric oxide synthase inhibitor, glibenclamide, an adenosine triphosphate (ATP)-sensitive potassium (K⁺) channel blocker, meclofenamate, a nonselective cyclooxygenase (COX) inhibitor, bicuculline, a GABA_A receptor antagonist, and saclofen, a GABA_B antagonist, were investigated on pulmonary arterial responses to various agonists in the feline pulmonary vascular bed. These agonists included valerian, muscimol, a GABA_A agonist, SKF-97541 a GABA_B agonist, acetylcholine (ACh), and bradykinin, both inducers of nitric oxide synthase, arachidonic acid, a COX substrate, and pinacidil, an ATP-sensitive K⁺ channel activator, during increased tone conditions induced by the thromboxane A₂ mimic, U46619.

Settings/location: Laboratory investigation.

Subjects: Mongrel cats of either gender.

Interventions: Injections of the abovementioned agonists and antagonists were given.

Outcome measures: Baseline pulmonary tone, responses to the agonists, and responses to the agonists after injections of antagonists were all measured via a pulmonary catheter transducer and recorded.

Results: Valerian root extract is a potent smooth muscle dilator in the feline pulmonary vascular bed. The vasodilatory effects of valerian root extract were unchanged after the administration of L-NIO, glibenclamide, and meclofenamate. These effects were ablated, however, by both saclofen and bicuculline. The ability of saclofen and bicuculline to modulate the dilatory effects of valerian root extract was not statistically different.

Conclusions: The vasodilatory effects of valerian root extract are mediated by a nonselective GABA mechanism.

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INTRODUCTION

The use of herbal medicine is widespread and growing, with as many as 3 in 10 Americans using botanical remedies (Barrett et al., 1999). In a recent hospital survey of patients scheduled to undergo elective surgery or pain procedures, 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. (Kaye et al., 2000)

Valerian (Valeriana officinalis) is commonly used for treating restlessness and as a sleeping aid. Recent studies have demonstrated that valerian was as effective as benzodiazepines without withdrawal effects (Schmitz and Jackel, 1998). It is also used as a sedative and for treating epilepsy and infantile convulsions (Pinn, 2001). The goal of this investigation was to establish the chemical and pharmacologic basis of valerian’s activity. Its efficacy has been demonstrated in a number of animal and clinical studies; however, the constituents, efficacy, and adverse effects have yet to be studied (Houghton, 1999). A randomized, double-blinded, clinical study comparing valerian and oxazepam on treatment of nonorganic causes of insomnia demonstrated no differences in the efficacy of these two drugs (Dorn, 2000). Another study utilizing quantitative topographic electroencephalogram (EEG) showed slight but clear visible effects on the central nervous system, especially after intake of a high-dose valerian-hops mixture in healthy young adults compared to placebo (Vonderheid-Guth et al., 2000). Use of valerian, along with barbiturates/benzodiazepines, can result in potential drug–herb interactions including additive adverse effects (Miller, 1999). Valerian interacts with GABA and/or benzodiazepine sites. At low concentrations, valerian extracts enhanced binding of a radiolabeled benzodiazepine, [3H] flunitrazepam. However, higher concentrations resulted in inhibition of binding (Ortiz, 1999). Valerian extracts also potentiated potassium ($K^+$) or veratridine-stimulated release of radioactivity from hippocampal slices preloaded with [3H] GABA (Ortiz et al., 1999). This confirms that valerian extracts have an effect on GABA$_A$ receptors and can also interact at other presynaptic components of GABA-releasing neurons (Ortiz et al., 1999). Valerenic acid (volatile oil) has been shown to inhibit enzyme-induced breakdown of GABA in the brain, resulting in sedation (Houghton, 1999). A recent finding is the presence of a lignan, hydroxypinoresinol, and its ability to bind to benzodiazepine receptors (Houghton, 1999).

Although valerian has been studied in neuronal settings, little, if anything, is known about the effects of valerian in the pulmonary vascular bed. The present study was, therefore, undertaken to investigate pulmonary vascular response to valerian 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, 34 adult mongrel cats of either gender weighing 3.0–4.7 kg were sedated with intramuscular ketamine hydrochloride (10–15 mg/kg) and were 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 and the animals spontaneously breathed a 40% oxygen mixture. Arterial pressure was measured via an aortic catheter and intravenous injections were given into a femoral vein catheter.

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. After the animal had been given heparin (1000 U/kg intravenously), the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter. 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 perfu-
sion 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, and in some experiments, left atrial pressure was measured with a radiopaque 6F single-lumen catheter passed transseptally into the left atrium from an external jugular vein. 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).

Blood pH was maintained between 7.35 and 7.45 by the addition of small amounts of

FIG. 1. Valerian (*Valeriana officinalis*) dilates pulmonary vascular tone in a dose-dependent manner.

FIG. 2. Influence of glibenclamide on valerian (*Valeriana officinalis*), acetylcholine (ACh), bradykinin, and pinacidil. Responses were compared before and beginning 20–30 minutes after administration of glibenclamide, given intravenously.
NaHCO₃ solution. 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 is constant, any changes in lobar pressure would represent changes in pulmonary arterial vascular resistance. All vascular pressures are expressed in absolute units (mm Hg) as means ± standard error (SE). The data were analyzed with a paired and unpaired t test and Scheffé’s F test (Excel 2002, Microsoft, Redmond, WA). A p value < 0.05 was used as the criterion for significance.

The antagonists, L-NIO, glibenclamide, bicuculline, saclofen, and meclofenamate were dissolved in normal saline immediately before use. The agonists acetylcholine (ACh; 100–1000 µg) and bradykinin (0.1–0.5 mg), muscimol (1.25–12.5 mg), SKF-97541 (1.25–12.5 mg), arachidonic acid (30 µg), and pinacidil (0.3–3.0 µg) were also dissolved in normal saline immediately before use. Stock solutions of U46619 (Upjohn, Kalamazoo, MI) were prepared in 100% ethanol at concentrations of 5–10 mg/mL and were stored at 20°C. Valerian extract was purchased in a preprepared, 99.9% pure extract solution from Herbalist and Alchemist (Washington, NJ). Vehicle solutions alone produced no significant effect on lobar arterial pressure. Working solutions were prepared just before use and kept on crushed ice during the experiments. Because the pulmonary vascular bed has little vasoconstrictor tone under resting conditions when the fraction of inspired oxygen (FiO₂) is 0.21, pulmonary arterial pressure must be actively increased so that vasodilator responses can be expressed. In all experiments, tone was raised to an average value of 35 ± 2 mm Hg (32–40) with an intralobar infusion of U46619.

The infusion rate ranged from 40 to 200 ng/min after the animals (n = 8) were treated with L-NIO. Under conditions of increased tone in the control period, pulmonary vascular responses to ACh, muscimol, SKF-97541, bradykinin, arachidonic acid, and pinacidil

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**FIG. 3.** Influence of NG-l-nitro-l-arginine ester on valerian (Valeriana officinalis), acetylcholine (ACh), nitroglycerine, and pinacidil. Responses were compared before and beginning 20–30 minutes after administration of L-NIO, given intravenously.
were obtained. The agonists were injected in a random sequence during the control period.

In the first through fifth set of experiments, the responses to intralobar agonists were measured after administration of glibenclamide (5 mg/kg intravenously, \( n = 5 \)), meclofenamate (2.5 mg/kg intravenously, \( n = 5 \)), L-NIO (100 mg/kg intravenously, \( n = 5 \)), saclofen (0.1 mg/kg intravenously, \( n = 5 \)), and bicuculline (0.1 mg/kg, \( n = 4 \)).

Finally, in the sixth set of experiments (\( n = 6 \)), responses to intralobar agonists were evaluated after administration of both saclofen (0.1 mg/kg intravenously) and bicuculline (0.1 mg/kg intravenously).

RESULTS

Influence of valerian on vascular tone

The effects of valerian on the pulmonary vascular bed of the intact chest cat are illustrated in Figure 1. At high-steady arterial pressure, in doses of 0.1 to 3.0 \( \mu \text{L} \), valerian extract caused significant dose-related decreases in lobar and arterial pressure. Decreases in lobar arterial pressure in response to valerian were rapid in onset, and pressure returned to control value over a 3- to 5-minute period, depending on the dose injected.

Influence of glibenclamide on responses to valerian, pinacidil, acetylcholine, and bradykinin

The effects of glibenclamide in response to valerian, pinacidil, ACh, and bradykinin are illustrated in Figure 2. In a dose that significantly attenuated the vasodilatory effects of pinacidil, the vasodepressor effects of valerian extract, ACh, and bradykinin were not significantly ablated after administration of glibenclamide (5 mg/kg).

Influence of L-NIO on responses to valerian, acetylcholine, and nitroglycerin

The effects of L-NIO on responses to valerian, ACh, nitroglycerine, and pinacidil are illustrated in Figure 3. In a dose that significantly blocked the effects of ACh, the vasodilatory effects of valerian extract, nitroglycerine, and pinacidil were not significantly attenuated by administration of L-NIO (100 mg/kg intravenously).

Influence of meclofenamate on responses to valerian, acetylcholine, and arachidonic acid

The effects of meclofenamate on responses to valerian, ACh, and arachidonic acid are illustrated in Figure 4. In a dose that significantly decreased the vasoconstrictor effects of arachidonic acid, the vasodilatory effects of valerian and ACh were not significantly attenuated by the administration of meclofenamate (2.5 mg/kg).

Influence of saclofen on responses to valerian, muscimol, SKF-97541, and pinacidil

The effects of saclofen on responses to valerian, muscimol, SKF-97541, and pinacidil are
illustrated in Figure 5. In a dose that significantly attenuated the dilatory effects of the selective GABAB agonist, SKF-97541, the vasodepressor effects of muscimol and pinacidil were not significantly attenuated by the administration of saclofen (0.1 mg/kg intravenously). The decrease in pulmonary vascular tone induced by valerian extract was ablated to a significant degree by the GABAB selective antagonist, saclofen.

**Influence of bicuculline on responses to valerian, muscimol, SKF-97541, and pinacidil**

The effects of bicuculline on responses to valerian, muscimol, SKF-97541, and pinacidil are illustrated in Figure 6. In a dose that significantly attenuated the dilatory effects of the selective GABA A agonist, muscimol, the vasodepressor effects of SKF-97541 and pinacidil were not significantly attenuated by the administration of bicuculline (0.1 mg/kg intravenously). The decrease in pulmonary vascular tone induced by valerian extract was ablated to a significant degree by the GABA A selective antagonist, bicuculline.

**Influence of bicuculline and saclofen on responses to valerian, muscimol, SKF-97541, and pinacidil**

The effects of bicuculline and saclofen on responses to valerian, muscimol, SKF-97541, and pinacidil are illustrated in Figure 7. When both selective antagonists, bicuculline (0.1 mg/kg intravenously) and saclofen (0.1 mg/kg intravenously) were given 15 minutes apart the vasodilatory effects of pinacidil were unchanged. However, the vasodilatory effects of valerian, muscimol, and SKF-97541 were all significantly ablated.

**Influence of bicuculline compared to the influence of saclofen on responses to valerian**

The effects of bicuculline compared to the influence of saclofen on responses to valerian are illustrated in Figure 8. There was no significant difference in the ability of bicuculline and the
DISCUSSION

Results of the present study show that valerian root extract decreases lobar arterial pressure when tone in the pulmonary vascular bed was increased to a high steady-state level with U46619. When pulmonary blood flow was constant and left arterial pressure was unchanged, the decreases in lobar arterial pressure were dose-dependent and were reduced by saclofen and bicuculline but not by meclofenamate, L-NIO, or glibenclamide. These results suggest that decreases in pulmonary vascular resistance in response to valerian extract appear to be dependent on the binding of either GABA\(_A\) or GABA\(_B\) receptors. The valerian root extract induced dilation of the vascular bed, after GABA\(_A\) antagonism, was attenuated. In addition, GABA\(_B\) blockade decreased the ability of valerian to lower the pulmonary vascular tone. Therefore, the present data suggest that valerian is a non selective GABA agonist. Furthermore, the differences in the ability of saclofen and bicuculline to ablate the vasodepressor response were not statistically significant, suggesting that valerian has equal affinity for both receptor subtypes.

GABA subtype receptor agonists, SKF-97541 and muscimol, both induced pulmonary vascular dilation. Each of their effects was blocked by the selective receptor antagonists to a significant degree: SKF-9731 by saclofen and muscimol by bicuculline.

Although the exact mechanism by which valerian extract dilates the pulmonary vascular bed is unknown, this herbal has significant pulmonary vasodilatory activity. Valerian root extract produced dose-related decreases in the canine pulmonary vascular bed that were not significantly changed in the presence of meclofenamate, suggesting that valerian extract dilates the pulmonary vascular bed independent of formation of cyclooxygenase products. Furthermore, these vasodilatory effects of valerian were not ablated in the presence of L-NIO, suggesting that valerian extract dilates...
the pulmonary vascular bed independent of de novo formation of nitric oxide. Finally, the vasodepressor effects of the extract were not attenuated by glibenclamide, which suggests that these effects are caused by a mechanism other than the opening of potassium channels. Previous studies have shown that valerian has several effects in vitro. It has been shown to increase the release and decrease the reuptake of radiolabeled GABA in presynaptic neurons (Santos et al., 1994). In addition, in vivo experiments have demonstrated that aqueous extracts of valerian have the ability to displace radiolabeled agonists from the GABA receptor (Cavadas et al., 1995). This suggests that valerian has both direct and indirect GABA agonist properties. Virtually no studies have been done on the ability of valerian to bind GABA subtype receptors (Dunaev et al., 1987).

Recently, it has been proven in the rat that GABA receptors can be found in a wide range of organs, including the lungs (Castelli et al., 1999). GABA subtype receptors have been shown to mediate smooth muscle contraction (Ong and Kerr, 1990). In addition, it has been shown that GABA subtype receptors play an important role in many lung functions (Chapman, et al., 1993). In previous experiments of human and guinea pig lung vascular smooth
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muscle, there was no evidence to support the presence of peripheral benzodiazepine receptors (PBR) (Mak and Barnes, 1990). However, recent studies using more sensitive techniques revealed that renal vascular smooth muscle cells are PBR-positive (Bribes et al., 2002). Studies in the rabbit artery support the concept of two GABA receptors being responsible for the vasodilatation of vascular smooth muscle in response to GABA agonists. (Anwar and Mason, 1982).

In vivo experiments have shown that the vasodilatory effects of benzodiazepines could be partially attributed to its ability to open a chloride channel (French et al., 1989). It has long been known that the GABA ion channel is associated with the benzodiazepine receptor (Collins et al., 2002). Finally, it has been shown that GABA induces chloride ion channel opening (Yokota et al., 2002). Therefore, it is clear that the peripheral effects of GABA could induce vascular smooth muscle dilatation.

In summary, valerian has significant vasodilator activity in the feline pulmonary vascular bed when tone is increased experimentally. The results of the present investigation suggest that vasodilator responses to valerian are, in part, mediated by a GABA receptor sensitive pathway.

Future studies are warranted to better define the role of valerian in complex pathophysiological states associated with pulmonary hypertension.

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