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Comparison of responses to siguazodan, rolipram, and zaprinast in the feline pulmonary vascular bed

Bracken J. De Witt, James R. Marrone, Philip J. Kadowitz *

Department of Pharmacology SL83, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA

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Abstract

The present study was undertaken to investigate and compare responses to the cyclic nucleotide phosphodiesterase inhibitors siguazodan (type III, guanosine 3′,5′-cyclic monophosphate (cGMP)-inhibited adenosine 3′,5′-cyclic monophosphate (cAMP)), rolipram (type IV, cAMP-specific), and zaprinast (type V, cGMP-specific) in the feline pulmonary vascular bed. When tone in the pulmonary vascular bed was raised to a high steady level with a constant infusion of the thromboxane mimic U46619 (9,11-dideoxy-11,α9α-epoxymethano prostaglandin $F_{2\alpha}$), intralobar injections of the three phosphodiesterase inhibitors caused dose-related decreases in lobar arterial pressure. In terms of relative vasodilator activity, rolipram was more potent at higher doses than siguazodan, which was more potent than zaprinast. The duration of the pulmonary vasodilator response to zaprinast was shorter than for siguazodan or rolipram. Furthermore, siguazodan and rolipram, but not zaprinast, decreased systemic arterial pressure when injected into the perfused lobar artery in the range of doses studied. The present data demonstrate that the three phosphodiesterase inhibitors have potent, long-lasting vasodilator activity in the pulmonary vascular bed of the cat. These data suggest that there is rapid turnover of cAMP and cGMP in the pulmonary circulation and indicate that phosphodiesterase enzyme types III, IV, and V may play an important role in the regulation of vasomotor tone in the feline lung.

Keywords: Phosphodiesterase; Pulmonary circulation; (Pharmacology); cAMP; cGMP; (Cat)

1. Introduction

Mammalian tissue has been shown to contain at least seven families of cyclic nucleotide phosphodiesterase isozymes (Polson and Strada, 1996). Most tissues contain several different phosphodiesterase enzymes in varying amounts, proportions, and cytologic locations (Polson and Strada, 1996). These phosphodiesterase isozymes convert guanosine 3′,5′-cyclic monophosphate (cGMP) and adenosine 3′,5′-cyclic monophosphate (cAMP) into their corresponding lower energy 5′-nucleotide inactive forms. Phosphodiesterase isozymes represent the only known means whereby cells can inactivate cyclic nucleotides (MacLean et al., 1997).

The role of phosphodiesterase inhibitors has been studied in numerous physiologic models. Responses to phosphodiesterase inhibitors have been studied in airway smooth muscle, platelets and peripheral blood mononuclear cell lines, erectile and systemic vascular smooth muscle using both selective and nonselective phosphodiesterase inhibitors (Heaslip et al., 1991; Peachell et al., 1992; Torphy et al., 1993; Champion et al., 1997; Murray et al., 1990). Clinically, phosphodiesterase III (cGMP-inhibited cAMP) inhibitors have been used in the treatment for congestive heart failure and as positive inotropic agents in post-operative cardiac surgery patients (Bailey et al., 1999; Heinz et al., 1999). Recently, there has been widespread clinical use of a type V (cGMP-specific) phosphodiesterase inhibitor for the treatment of male erectile dysfunction (Goldstein et al., 1998), and there is continued interest in phosphodiesterase IV (cAMP-specific) inhibitors for treatment of asthma and chronic obstructive coronary disease (Schudt et al., 1995). Studies have been undertaken both in vitro and in vivo to investigate the role of individual phosphodiesterase isozymes in the pulmonary circulation (Haynes et al., 1991; McMahon et al., 1993; De Witt et al., 1994; Thusu et al., 1995; Wagner et al., 1997; Ichinose et al., 1998; Jeffery and Wanstall, 1998). Moreover, the analysis and comparison of vascular responses to inhibition of phosphodiesterase III, IV, and V inhibitors should improve

* Corresponding author. Tel.: +1-504-588-5444; fax: +1-504-588-5283.

E-mail address: champion@mailhost.tcs.tulane.edu (P.J. Kadowitz).

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our knowledge of the role of phosphodiesterase isozymes in the regulation of the pulmonary vascular function. The present study was, therefore, undertaken to investigate and compare responses to siguazodan (type III, cGMP-inhibited cAMP), rolipram (type IV, cAMP-specific), and zaprinast (type V, cGMP-specific) in the pulmonary vascular bed of the intact-chest cat under controlled flow conditions.

2. Methods

Adult cats of either sex weighing 2.6–3.4 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). 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 95% O_2 and 5% CO_2. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from the femoral artery, and intravenous injections were made into a catheter positioned in the inferior vena cava from a femoral vein. For perfusion of the left lower lung lobe, a triple-lumen 28 cm, 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 animals had been heparinized (1000 U/kg i.v.), the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter. The lobe was perfused with

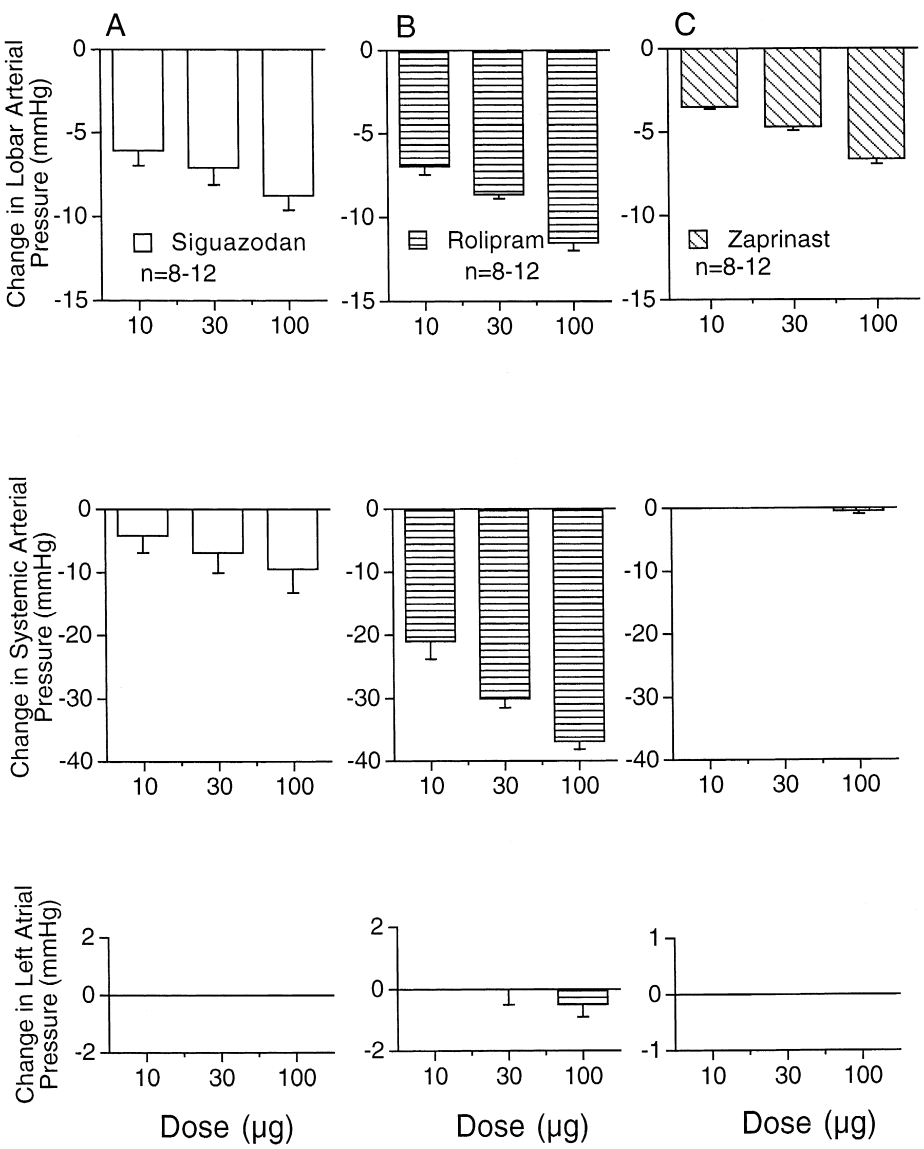

Fig. 1. Bar graph comparing changes in lobar arterial pressure, systemic arterial pressure, and left atrial pressure in response to siguazodan (A), rolipram (B), and zaprinast (C). The phosphodiesterase inhibitors were injected into the perfused lobar artery when baseline pressure in the perfused lobar artery was raised to a high steady value (32–38 mm Hg) with an intralobar infusion of U46619. *n* indicates number of experiments.

a perfusion pump (model 1210, Harvard Instruments) by way of the catheter lumen beyond the cuff with blood withdrawn from a femoral artery. Lobar arterial pressure was measured from a second catheter port 5 mm beyond the cuff on the perfusion catheter. The perfusion rate was adjusted so that lobar arterial perfusion pressure approximated mean pressure in the main pulmonary artery and was not changed thereafter. The flow rate ranged from 30 to 45 ml/min, and left atrial pressure was measured with a radiopaque 6F double-lumen catheter or a 6F radiopaque polyethylene catheter passed transseptally into the left atrium. Mean vascular pressures, measured with Spectromed DTX Plus transducers zeroed at the right atrial level, were recorded on a Grass model 7 recorder after characteristic waveforms had been confirmed. These procedures have been described previously (McMahon et al., 1993; De Witt et al., 1994).

Siguazodan (SK&F 94836, (*R,S*)-2-cyano-1-methyl-3-[4-(methyl-6-oxo-1,4,5,6 tetrahydrophyridazine-3-yl)]-guanidine; SmithKline Beecham, Sussex, UK) and rolipram (ZK 62771, 4-(3-cyclopentyloxy-4-methoxyphenyl)2-pyrrolidone; SmithKline Beecham) were dissolved in 20% dimethylsulfoxide (DMSO) and diluted with normal saline; and zaprinast (M&B 22,948, 2-*o*-propoxyphenyl-8-azapurin-6-one; Rhône-Poulec, Dagenham, Essex, UK) was dissolved in 0.15 N NaOH in normal saline at a concentration of 3 mg/ml with subsequent dilutions made in normal saline. These compounds were injected into the perfused lobar artery in fixed small volumes, and injections were randomized. Responses were allowed to return to control values and, after approximately 15 min, a subsequent injection was administered. All solvents used produced no significant effect on lobar arterial pressure. The thromboxane A_2 mimic U46619 (9,11-dideoxy-11,α9α-epoxymethano prostaglandin $F_{2\alpha}$; Upjohn, Kalamazoo, MI) was dissolved in 100% ethanol at a concentration of 10 mg/ml and was diluted in 0.9% saline. The thromboxane A_2 mimic was then infused into the perfused lobar artery with a Harvard infusion pump at rates (50–320 ng/min) required to raise lobar arterial pressures to values of 32–38 mm Hg.

Arterial blood gases and pH were measured with a Corning model 178 analyzer and were in the normal range. All hemodynamic data are expressed in absolute units and are presented as mean $\pm$ SEM. Responses represent peak changes, and data were analyzed using a one-way analysis of variance and Scheffe's *F*-test or a paired *t*-test (Snedecor and Cochran, 1980). A P value of less than 0.05 was used as the criterion for statistical significance.

3. Results

Responses to siguazodan, rolipram, and zaprinast were compared in the pulmonary vascular bed of the cat, and these results are summarized in Fig. 1. Under conditions of controlled blood flow when lobar arterial pressure was at normal resting conditions, injections of siguazodan, rolipram, and zaprinast produced no significant change in lobar arterial pressure (data not shown). When tone in the pulmonary vascular bed was raised to a high steady level with U46619, injections of siguazodan, rolipram, and zaprinast into the perfused lobar artery in doses of 10–100 μg produced significant dose-related decreases in lobar arterial pressure without altering left atrial pressure (Fig. 1). Siguazodan and rolipram decreased systemic arterial pressure when injected into the perfused lobar artery in

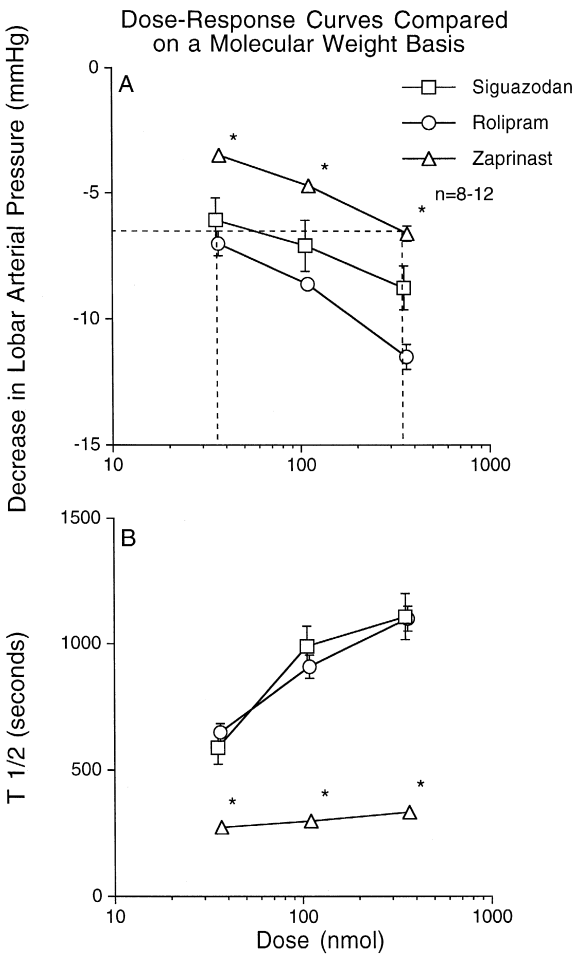

Fig. 2. (A) Dose–response curves comparing decreases in lobar arterial pressure in response to siguazodan, rolipram, and zaprinast. The dashed vertical lines represent the estimated dose required to decrease lobar arterial pressure 6.5 mm Hg ($ED_{6.5}$ mm Hg). The asterisk indicates that the responses to all three doses of siguazodan and rolipram were significantly greater ($P < 0.05$) than the responses to similar doses of zaprinast. In addition, the responses to the 30- and 100-μg doses of rolipram were significantly greater than the response to the same doses of siguazodan. (B) Dose–response curves showing the recovery half-life ($T_{1/2}$) of the decrease in lobar arterial pressure in response to siguazodan, rolipram, and zaprinast at doses of 10, 30, and 100 μg. The phosphodiesterase inhibitors were injected into the perfused lobar artery in small volumes in a randomized sequence, while baseline pressure in the perfused lobar artery was raised to a high steady value (32–38 mm Hg) with an infusion of U46619. *n* indicates the number of experiments.

doses of 10–100 μg, whereas zaprinast had no effect on systemic arterial pressure when injected in the same doses (Fig. 1). In terms of relative vasodilator activity in the pulmonary vascular bed, when doses of the compounds required to decrease lobar arterial pressure 6.5 mm Hg ($ED_{6.5}$ mm Hg) were compared, siguazodan and rolipram were significantly more potent than zaprinast (siguazodan 37 nmol, rolipram 37 nmol, zaprinast 350 nmol) (Fig. 2A).

The recovery half-lives ($T_{1/2}$), as measured by 50% response recovery time, of the decreases in lobar arterial pressure in response to siguazodan, rolipram, and zaprinast are compared in Fig. 2B. The $T_{1/2}$ of the vasodilator responses to siguazodan and rolipram are similar and responses to both agents had a significantly longer $T_{1/2}$ than zaprinast (Fig. 2B). The time-course of the decrease in lobar arterial pressure in response to siguazodan, rolipram, and zaprinast is shown in Fig. 3. The onset of the vasodilator response and the decrease in lobar arterial pressure in response to these compounds were significantly different. The maximal decrease in lobar arterial pressure for rolipram and zaprinast at all doses occurred within 200 s following administration. However, siguazodan required greater than 225 s at the lowest dose for the maximal decrease in lobar arterial pressure to occur (Fig. 3). Siguazodan had a similar duration of vasodilatory action when compared with rolipram at equivalent doses, while the

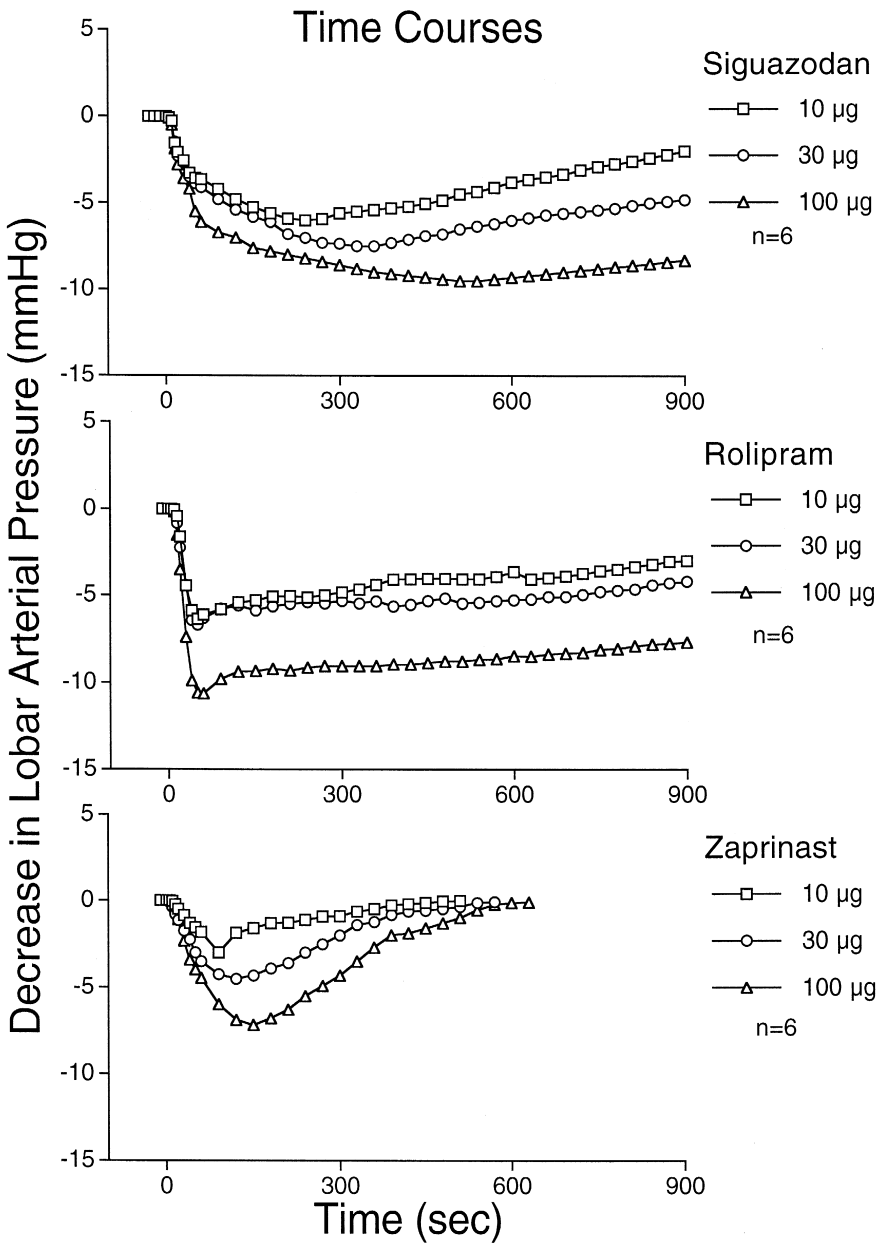

Fig. 3. Line graphs showing the time-course of the decreases in lobar arterial pressure in response to intralobar injections of siguazodan, rolipram, and zaprinast (10–100 μg). *n* indicates the number of experiments.

duration of action of zaprinast was significantly shorter (Fig. 3).

4. Discussion

Results of the present investigation demonstrate that the phosphodiesterase inhibitors siguazodan, rolipram, and zaprinast cause dose-related decreases in lobar arterial pressure when tone in the pulmonary vascular bed was raised to a high steady level. In as much as blood flow and left atrial pressure were unchanged, the decreases in lobar arterial pressure reflect decreases in pulmonary lobar vascular resistance. In terms of relative vasodilator activity in the pulmonary vascular bed, the dose that decreased lobar arterial pressure 6.5 mm Hg ($ED_{6.5}$ mm Hg) was similar for siguazodan and rolipram, and both agents had a smaller $ED_{6.5}$ mm Hg than did zaprinast.

Phosphodiesterase III inhibitors have been shown to produce concentration-dependent relaxations in numerous vascular preparations, including isolated guinea pig pulmonary artery, human pulmonary artery, and isolated rat lung (Braner et al., 1993; Polson and Strada, 1996; MacLean et al., 1997). In the present study, siguazodan produced significant decreases in pulmonary, as well as systemic arterial pressure. The results of the present study extend the hypothesis that inhibition of cGMP-inhibited cAMP phosphodiesterase produces vasodilation in the pulmonary vascular bed of the cat.

In contrast to the actions of phosphodiesterase III inhibitors, phosphodiesterase IV inhibitors are generally thought to have weak vascular relaxant activity. Rolipram produced only small relaxations in rat aorta, human pulmonary artery, and isolated rat lung (Polson and Strada, 1996; MacLean et al., 1997). However, in the present investigation, rolipram was significantly more potent than either phosphodiesterase III inhibition or phosphodiesterase V inhibition in decreasing lobar arterial pressure when doses of inhibitor were compared on a nanomolar basis. Furthermore, intralobar injections of rolipram produced significant dose-dependent decreases in systemic arterial pressure. Recently, a subthreshold dose of rolipram in combination with aerosolized PGI_2 was described to have selective pulmonary vasodilatory action without affecting systemic pressure (Schermuly et al., 1999). In the present study, rolipram was also more potent in decreasing systemic arterial pressure when compared to the effects of the phosphodiesterase III inhibitor siguazodan or the phosphodiesterase V inhibitor zaprinast. The reason for the difference between the results of this study and other studies is unknown, but may suggest a fundamental difference in species studied or that inactivation of the phosphodiesterase inhibitors is different in the feline pulmonary vascular bed. The present results also suggest that rolipram or other selective phosphodiesterase IV inhibitors may be useful as antihypertensive agents in the pulmonary or systemic vascular bed.

The actions of phosphodiesterase V inhibitors have been extensively studied in the pulmonary vascular bed in vitro and in vivo. Zaprinast has been shown to decrease pulmonary arterial pressure in a variety of species, including the mouse, rat, cat, and lamb (Braner et al., 1993; Mc Mahon et al., 1993; De Witt et al., 1994; MacLean et al., 1997; Champion et al., 1999). The results of the present study provide support for the hypothesis that inhibition of cGMP-specific phosphodiesterase produces substantial vasodilation of the pulmonary vascular bed of the cat.

In human pulmonary artery segments, phosphodiesterase III, IV, and V have been shown to contribute in different proportions to the total hydrolyzing activity of cAMP and cGMP (Rabe et al., 1994). In this study, in addition to differences in vasodilator potency, there were significant differences in duration of action, as determined by the total time and the half-life ($T_{1/2}$) of the decreases in lobar arterial pressure observed in response to siguazodan, rolipram, and zaprinast. Pulmonary vasodilator responses to siguazodan were slightly longer in duration than were responses to rolipram and both were significantly longer than responses to zaprinast. Furthermore, there were differences in the onset of the vasodilator response. Siguazodan was very slow in onset, while rolipram was very rapid. The onset of the vasodilator response of zaprinast was between that of siguazodan and rolipram. Responses to siguazodan and rolipram were relatively long-lived in the pulmonary vascular bed, lasting greater than 900 s in the larger doses, while zaprinast lasted approximately 500–600 s. Differences in hydrolyzing activity of the phosphodiesterase isozymes may explain the differences in onset of action, potency, and duration of pulmonary vasodilatory responses measured in this study.

In conclusion, the results of the present investigation demonstrate that under elevated tone conditions the phosphodiesterase inhibitors siguazodan, rolipram, and zaprinast have potent, long-lasting vasodilator activity in the pulmonary vascular bed of the cat. Furthermore, intralobar injections of siguazodan and rolipram produced dose-dependent decreases in systemic arterial pressure. These data suggest that the turnover of cAMP and cGMP is rapid and that the cyclic nucleotide phosphodiesterase isozyme types III, IV, and V may play an important role in the regulation of vasomotor tone in the feline vascular bed. Furthermore, these data suggest that inhibitors of type IV phosphodiesterase may be useful in the treatment of pulmonary hypertensive disorders.

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