Effects of PKC isozyme inhibitors on constrictor responses in the feline pulmonary vascular bed

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Effects of PKC isozyme inhibitors on constrictor responses in the feline pulmonary vascular bed. Am J Physiol Lung Cell Mol Physiol 280: L50–L57, 2001.—The effects of Gö-6976, a Ca$^{2+}$-dependent protein kinase C (PKC) isozyme inhibitor, and rottlerin, a PKC-δ isozyme/calmodulin (CaM)-dependent kinase III inhibitor, on responses to vasopressor agents were investigated in the feline pulmonary vascular bed. Injections of angiotensin II, norepinephrine (NE), serotonin, BAY K 8644, and U-46619 into the lobar arterial constant blood flow perfusion circuit caused increases in pressure. Gö-6976 reduced responses to angiotensin II; however, it did not alter responses to serotonin, NE, or U-46619, whereas Gö-6976 produced decreased responses to angiotensin II and NE, did not alter responses to serotonin or U-46619, and enhanced responses to BAY K 8644. Immunohistochemistry of feline pulmonary arterial smooth muscle cells demonstrated localization of PKC-α and -δ isozymes in response to phorbol 12-myristate 13-acetate and angiotensin II. Localization of PKC-α and -δ isozymes decreased with administration of Gö-6976 and rottlerin, respectively. These data suggest that activation of Ca$^{2+}$-dependent PKC isozymes and Ca$^{2+}$-independent PKC-δ isozyme/CaM-dependent kinase III mediate angiotensin II responses. These data further suggest that Ca$^{2+}$-independent PKC-δ isozyme/CaM-dependent kinase III mediate responses to NE. A rottlerin- or Gö-6976-sensitive mechanism is not involved in mediating responses to serotonin and U-46619, but these PKC isozyme inhibitors enhanced BAY K 8644 responses in the feline pulmonary vascular bed.

angiotensin; norepinephrine; calmodulin-dependent kinase III; calcium; BAY K 8644

**PROTEIN KINASE C** is an enzyme involved in the regulation of various cellular processes such as growth, differentiation, metabolism, and smooth muscle contraction (23, 29, 31). Protein kinase C is known to exist in virtually all mammalian tissues and is thought to play an important role in transducing extracellular signals such as hormones, growth factors, neurotransmitters, and drugs by phosphorylation of specific cellular target proteins (15, 22). It has been reported that angiotensin II initiates the process of hydrolysis of a specific class of membrane phosphoinositides and subsequently activates protein kinase C. Angiotensin II has been shown to induce membrane phosphoinositide hydrolysis and the subsequent generation of diacylglycerol (1, 16, 20). Protein kinase C is then activated by diacylglycerol and phosphorylates intracellular substrates, which leads to cellular responses. Since 1986, numerous different isozymic forms of protein kinase C have been described (7, 19). These protein kinase C isozymes are divided into three categories. The classic isozyme group is composed of α-, β1-, β2-, and γ-isozymes. This group is activated by phosphatidylinositol with sn-1,2-diacylglycerol in a Ca$^{2+}$-dependent manner. The novel isozyme group is composed of η-, ε-, δ-, and θ-isozymes. It is also activated by phosphatidylinositol with sn-1,2-diacylglycerol but in a Ca$^{2+}$-independent manner. The atypical group is composed of μ- and ζ-isozymes. It is activated by phosphatidylinositol in a sn-1,2-diacylglycerol- and Ca$^{2+}$-independent manner. The alkaloid staurosporine, a product of the *Streptomyces* species, has been described as the most potent inhibitor of protein kinase C (30). Alteration of this natural compound has improved the selectivity with little decrease in potency in the preferential discrimination between protein kinase C isozymes (18). In particular, Gö-6976, an indolocarbazole, has been shown to inhibit the classic Ca$^{2+}$-dependent isozyme group in nanomolar concentrations (18). Rottlerin (mallotoxin), a compound purified from *Mallotus philippinensis*, is a potent inhibitor of protein kinase C-δ isozyme, a Ca$^{2+}$-independent protein kinase C isozyme, and calmodulin (CaM)-dependent kinase III (6, 7). Topical administration of rottlerin has been shown to suppress 12-O-tetradecanoylphorbol 13-acetate-induced mouse ear edema (5). Although the effects of certain protein kinase C isozymes have been investigated in in vitro experiments, little if anything is known about the role of protein kinase C isozymes in...
mediating responses in the pulmonary circulation. The present study was therefore undertaken to investigate the effects of G6-6976 and rottlerin on vasoconstrictor responses to angiotensin II and other pressor agents that act by various mechanisms in the pulmonary vascular bed of the intact-chest cat.

**METHODS AND MATERIALS**

Twenty-four adult mongrel cats of either sex weighing 3.0–4.5 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 fluorescent 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. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from a femoral artery, and intravenously 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 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 heparinized (1,000 U/kg iv), the lobar artery was vascularly isolated by distension of the balloon cuff on the perfusion catheter, and 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, and in four experiments, left atrial pressure was measured from a catheter inserted into the aorta from a femoral vein.

Working solutions were prepared just before use, stored in a freezer at −10°C for 5 min. To quench endogenous peroxidase activity, the fixed cells were incubated in a 3% solution of hydrogen peroxide in PBS for 10 min. The cells were then incubated in serum block for 20 min, and immediately after this incubation, the cells were incubated with a rabbit primary antibody for protein kinase C-α isoform (1:200) and protein kinase Cδ isoform (1:200; Santa Cruz Biotechnology) for 2 h. The cells were then treated with a biotinylated secondary antibody for 30 min followed by treatment with horseradish peroxidase (HRP)-streptavidin complex and exposed to HRP-substrate mixture for 10 min. The slides were mounted and observed under light microscopy.

**RESULTS**

Influence of G6-6976 on responses to angiotensin II. Under conditions of controlled blood flow in the pulmonary vascular bed of the intact-chest cat, injections of angiotensin II (0.1–1 µg) into the lobar arterial perfusion circuit produced dose-related increases in lobar
arterial perfusion pressure, and the pressor responses to the angiotensin peptide were reproducible with respect to time (Fig. 1). Responses to angiotensin II were compared both before and 30 min after infusion of Gö-6976 at 10, 15 (data not shown), and 30 μg·kg⁻¹·min⁻¹ for 10 min into the lobar arterial perfusion circuit. The increases in lobar arterial pressure in response to angiotensin II were significantly reduced after administration of Gö-6976 (Fig. 1). Higher doses of Gö-6976 (15 and 30 μg·kg⁻¹·min⁻¹ ia) produced a further reduction in the pressor response to angiotensin II (Fig. 1). Administration of Gö-6976 into the lobar artery did not significantly alter baseline arterial pressure (Table 1). The response to angiotensin II returned to ~75% of control value within 60 min, and the response was not significantly different from the control value 120 min after administration of the Ca²⁺-dependent protein kinase C isozyme inhibitor (data not shown).

Influence of Gö-6976 on responses to norepinephrine, U-46619, serotonin, and BAY K 8644. Injections of norepinephrine, U-46619 (the thromboxane A₂ mimic), serotonin, and BAY K 8644 into the lobar arterial perfusion circuit produced dose-related increases in lobar arterial pressure (Figs. 1 and 2). Responses to the agents were compared before and after infusion of Gö-6976 at 10, 15 (data not shown), and 30 μg·kg⁻¹·min⁻¹ for 10 min into the lobar arterial perfusion circuit. The increases in lobar arterial pressure in response to serotonin, U-46619, and norepinephrine were not altered after administration of Gö-6976. How-

![Fig. 1. Effect of Gö-6976 on lobar arterial pressure responses to administration of angiotensin II (A and C) and the thromboxane mimic U-46619 (B and D). A: Gö-6976 at 10 μg·kg⁻¹·min⁻¹ for 10 min intra-arterially (ia). B: Gö-6976 at 10 μg·kg⁻¹·min⁻¹ for 10 min ia. C: Gö-6976 at 30 μg·kg⁻¹·min⁻¹ for 10 min ia. D: Gö-6976 at 30 μg·kg⁻¹·min⁻¹ for 10 min ia. Vasoconstrictor agents were injected directly into the lobar arterial perfusion circuit on a straight weight basis. n, No. of experiments. *Significantly different from control value.](http://ajplung.physiology.org/)

![Fig. 2. Effect of Gö-6976 (10 μg·kg⁻¹·min⁻¹ for 10 min ia) on lobar arterial pressure responses to norepinephrine (A), serotonin (B), and BAY K 8644 (C). Agents were injected directly into the lobar arterial perfusion circuit on a straight weight basis. n, No. of experiments. *Significantly different from control value.](http://ajplung.physiology.org/)
ever, responses to BAY K 8644 were significantly enhanced after treatment with Gö-6976 (Fig. 2).

**Influence of rottlerin on responses to angiotensin II.** Responses to angiotensin II were compared before and 30 min after infusion of rottlerin at 15 and 25 μg·kg⁻¹·min⁻¹ for 10 min into the lobar arterial perfusion circuit. The increases in lobar arterial pressure in response to angiotensin II were significantly reduced after administration of rottlerin (Fig. 3). A higher dose of rottlerin (25 μg·kg⁻¹·min⁻¹ ia) produced further reductions in the response to angiotensin II (Fig. 3). Administration of rottlerin into the lobar artery did not significantly alter baseline arterial pressure (Table 1). The response to angiotensin II returned to ~75% of control value within 75 min, and the response was not significantly different from the control value 135 min after administration of the protein kinase C blocker (data not shown).

**Influence of rottlerin on responses to norepinephrine, U-46619, serotonin, and BAY K 8644.** Responses to norepinephrine, U-46619, serotonin, and BAY K 8644 were compared before and 30 min after infusion of rottlerin at 15 μg·kg⁻¹·min⁻¹ for 10 min into the lobar arterial perfusion circuit. The increases in lobar arterial pressure in response to serotonin and U-46619 were not altered after administration of rottlerin (Figs. 3 and 4). However, the pressor response to norepinephrine was significantly reduced by treatment with rottlerin. Responses to BAY K 8644 were enhanced after treatment with rottlerin in the pulmonary vascular bed of the intact-chest cat (Fig. 4).

**Influence of Gö-6976 and rottlerin on responses to angiotensin II, norepinephrine, U-46619, serotonin, and BAY K 8644.** Responses to angiotensin II, norepinephrine, U-46619, serotonin, and BAY K 8644 were supposed to be investigated before and after administration of Gö-6976 (30 μg/kg over 10 min ia) and rottlerin (25 μg/kg over 10 min ia); however, on administration of the agents, the experimental animals expired. The protocol was repeated with lower doses of the agents Gö-6976 (10 μg/kg over 10 min ia) and rottlerin (15 μg/kg over 10 min ia), with the same lethal results.

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Fig. 3. Effect of rottlerin on lobar arterial pressure responses to angiotensin II (A and C) and U-46619 (B and D). A: rottlerin at 15 μg·kg⁻¹·min⁻¹ for 10 min ia. B: rottlerin at 15 μg·kg⁻¹·min⁻¹ for 10 min ia. C: rottlerin at 25 μg·kg⁻¹·min⁻¹ for 10 min ia. D: rottlerin at 25 μg·kg⁻¹·min⁻¹ for 10 min ia. Vasoconstrictor agents were injected directly into the lobar arterial perfusion circuit on a straight weight basis. n, No. of experiments. *Significantly different from control value.

Fig. 4. Effect of rottlerin (15 μg·kg⁻¹·min⁻¹ for 10 min ia) on lobar arterial pressure responses to norepinephrine (A), serotonin (B), and BAY K 8644 (C). Agents were injected directly into the arterial perfusion circuit on a straight weight basis. n, No. of experiments. *Significantly different from control value.
Immunohistochemistry experiments. Immunohistochemistry of cultured feline pulmonary arterial smooth muscle cells demonstrated localization of protein kinase C-α and -δ isozymes in response to phorbol 12-myristate 13-acetate (data not shown) and angiotensin II (Fig. 5). Simultaneous administration of angiotensin II with Gö-6976 decreased localization of protein kinase C-α isozyme (a Ca²⁺-dependent protein kinase C isozyme; Fig. 5). Also, simultaneous administration of angiotensin II with rottlerin decreased localization of protein kinase C-δ isozyme (Fig. 5).

DISCUSSION

Recent studies (10–12) in the pulmonary vascular bed of the cat have suggested that the protein kinase C pathway plays an important role in mediating responses to angiotensin and norepinephrine. Results of the present study extend the results of the previous studies by showing that protein kinase C isozyme inhibitors can block responses to various constrictor agents. Pressor responses to angiotensin II were inhibited after infusion of Gö-6976 (10–30 μg·kg⁻¹·min⁻¹) and rottlerin (15–25 μg·kg⁻¹·min⁻¹). Neither inhibitor altered pressor responses to serotonin or U-46619, but rottlerin inhibited responses to norepinephrine. The pressor response to BAY K 8644 was enhanced after administration of Gö-6976 and rottlerin. When Gö-6976 and rottlerin were administered simultaneously, there were lethal results. Immunocytochemistry of cultured pulmonary arterial smooth muscle cells demonstrated protein kinase C-α and -δ isozyme localization in response to angiotensin II. Protein kinase C-α isozyme and protein kinase C-δ isozyme localization were decreased after inhibition with Gö-6976 and rottlerin, respectively. These data provide support for the hypothesis that responses to angiotensin II are mediated, in part, by the protein kinase C pathway and that responses to serotonin and U-46619, the thromboxane A₂ analog, appear to be mediated by some other mechanism. Furthermore, these data support the hypothesis that the pressor response to norepinephrine is mediated through a rottlerin-sensitive mechanism in the pulmonary vascular bed of the cat. Responses to these various pressor agents were reproducible with respect to time so that tachyphylaxis to the agents did not play a role in the present studies with the protein kinase C isozyme inhibitors. The inhibitory effects of Gö-6976 and rottlerin were long in duration because pressor responses returned to approximately control values 120–135 min after infusion of the protein kinase C isozyme inhibitors.

Many agonists including norepinephrine, acetylcholine, vasopressin, cholecystokinin, and angiotensin II exert some of their biological effects by increasing the

Fig. 5. Photomicrographs of cellular localization of the α- (A) and δ-isozymes (B) of protein kinase C in feline pulmonary vascular smooth muscle cells treated with angiotensin II (0.5 μM). C: photomicrograph of localization of the α-isozyme after treatment with angiotensin II and Gö-6976 (2 μM), a Ca²⁺-dependent protein kinase C isozyme inhibitor. D: photomicrograph of localization of δ-isozyme after treatment with angiotensin II and rottlerin (1 μM), a protein kinase C-δ isozyme/CaM-dependent kinase III inhibitor. Arrows, localization of protein kinase C isoforms. Original magnification, ×300.
levels of Ca$^{2+}$ and sn-1,2-diacylglycerol in their target cells. These responses appear to involve an initial mobilization of Ca$^{2+}$ from the endoplasmic/sarcoplasmic reticulum and perhaps other intracellular Ca$^{2+}$ stores, followed by alterations in the flux of Ca$^{2+}$ across the plasma membrane. The Ca$^{2+}$ changes are consistently associated with increased turnover of cellular phosphoinositides. The most rapid response is breakdown of phosphatidylinositol 4,5-bisphosphate in the plasma membrane. myo-Inositol 1,4,5-trisphosphate produced by phosphatidylinositol 4,5-bisphosphate breakdown rapidly releases Ca$^{2+}$ from the endoplasmic and sarcoplasmic reticulum. sn-1,2-Diacylglycerol, the other product of phosphatidylinositol 4,5-bisphosphate breakdown, also acts as a second messenger in that it activates protein kinase C, a Ca$^{2+}$-phospholipid-dependent protein kinase, by lowering its requirement for Ca$^{2+}$. The cellular substrates for protein kinase C and its role in the different physiological responses to the Ca$^{2+}$-mediated agonists are under investigation. The major intracellular target for Ca$^{2+}$ is the Ca$^{2+}$-dependent regulatory protein CaM. This binds Ca$^{2+}$ with high affinity, and the resulting complex interacts with a variety of enzymes and other cellular proteins, modifying their activities. A major target is the multifunctional CaM-dependent protein kinase that phosphorylates and alters the activities of many proteins including myosin light chain kinase. Myosin light chain kinase, by phosphorylation of myosin light chain, is thought to be the primary mechanism for activating smooth muscle contraction (23).

Increases in membrane phosphoinositide hydrolysis and intracellular Ca$^{2+}$ have been shown after α1-adrenergic, serotonin, and angiotensin II activation (2, 25). Norepinephrine-, serotonin-, and angiotensin II-induced vasoconstriction have been demonstrated (10, 11) to be dependent on phospholipase C and protein kinase C activation in the rat and cat. Previous studies (10, 21) involving serotonin demonstrated species differences in the effects of protein kinase C inhibitors in the rat basilar artery and in the pulmonary vascular bed. The results of the present study support the hypothesis that responses to serotonin are not mediated by a protein kinase C mechanism in the pulmonary vascular bed of the cat.

Protein kinase C is involved in the contraction of vascular smooth muscle (26, 29, 31). Stauroporine and other nonselective protein kinase C inhibitors have been reported to inhibit 12-O-tetradecanoylphorbol 13-acetate-induced contraction of rat aortic smooth muscle (27). In smooth muscle cell preparations, protein kinase C isoforms have been implicated in contractile responses (13, 14). Recently, protein kinase C-α and -δ isoforms were demonstrated to be involved in rat mesenteric arterial contraction in response to phorbol esters (24). However, specific protein kinase C isoforms involved in smooth muscle contractions in the pulmonary vascular bed have not been described. These data support the finding that protein kinase C isoforms play a role in mediating angiotensin II and norepinephrine responses in vivo.

The roles of protein kinase C isoforms in mediating responses in the pulmonary circulation in the intact animal are difficult to investigate because little is known about the pharmacokinetic and pharmacodynamic properties of these agents in this system. The specificity profiles for Gö-6976 and rottlerin have been described in in vitro experiments. Recent kinetic studies suggest that Gö-6976, along with other staurosporine-related protein kinase C inhibitors, interferes with binding of ATP to the kinase and that rottlerin also at least partially interacts with the ATP binding site of protein kinase C (7, 18). Responses to angiotensin II were attenuated to a greater degree after administration of rottlerin than after Gö-6976. However, complete inhibition of pulmonary pressor responses was not seen in our study, suggesting that mechanisms other than protein kinase C isoform activation may be involved. Previous studies (11) have demonstrated that binding of angiotensin II to angiotensin type I receptors results in the activation of phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate. The data with Gö-6976 and rottlerin suggest that protein kinase C isoform/CaM-dependent kinase III activation plays a role in mediating vasoconstrictor responses to angiotensin II and norepinephrine. However, additional studies are needed to provide an exact definition of the role of protein kinase C isoforms and other mechanisms that may be involved in the mediation of responses to angiotensin II and norepinephrine in the pulmonary vascular bed of the cat.

Previous results (12) have shown that the nonselective protein kinase C inhibitors stauroporine and calphostin C reduced pulmonary vasoconstriction of angiotensin II and norepinephrine and that pulmonary vasoconstrictor responses to the thromboxane A$_2$ analog U-46619 were not altered in the cat. Furthermore, these data suggested that protein kinase C plays a role in mediating vasoconstrictor responses in the pulmonary vascular bed of the cat and that there are species differences in certain vasoconstrictor responses (12). It has also been demonstrated that protein kinase C plays a role in mediating norepinephrine-induced contraction in isolated vascular smooth muscle preparations (3, 31). There are data (4, 14) to suggest that norepinephrine uses Ca$^{2+}$-independent isoforms of protein kinase C for signal transduction. In ferret aortic cells with constant Ca$^{2+}$, phenylephrine-induced contraction was observed and inhibited by an antagonist of protein kinase C (4, 14). These data further inferred that the protein kinase C-ε isoform (a Ca$^{2+}$-independent protein kinase C isoform) was responsible for this contraction. Furthermore, in human tracheal epithelial cells, rottlerin, the protein kinase C-δ isoform/Gö-6976 dependent kinase III inhibitor, blocked norepinephrine-induced α$_1$-adrenergic activation of the Na$^{+}$-K$^{+}$-2Cl$^{-}$ cotransport (17). However, in rat mesenteric artery, it has been suggested that the protein kinase C-δ isoform does not mediate norepinephrine responses (24). The reason for the difference in re-
response to norepinephrine between the isolated rat mesenteric artery and the pulmonary vascular bed of the cat is unknown but may be related to species, sex, the vascular bed studied, or in vivo versus in vitro preparation. The present results demonstrate that responses to norepinephrine were not altered by Gö-6976, whereas norepinephrine responses were attenuated after administration of rottlerin; this suggests a protein kinase C-δ isozyme/CaM-dependent kinase III-mediated mechanism in the pulmonary vascular bed of the cat.

Results of the present study demonstrate that responses to angiotensin II are inhibited by both Gö-6976 and rottlerin, whereas norepinephrine responses are inhibited by rottlerin. The reason for the difference between angiotensin II, norepinephrine, and the other vasoconstrictor substances studied is unknown. However, it may be related to the signal transduction mechanisms involved in mediating the various responses. Angiotensin II is well known to be a smooth muscle cell growth promoter or mitogen. This effect of angiotensin II, as opposed to the other vasoconstrictor agents, may be partially responsible for the stimulation of multiple protein kinase C isozyme stimulation. The results also demonstrated that simultaneous administration of Gö-6976 and rottlerin produced lethal results. The reason for this is unknown and requires further study, but it supports the concept of the necessary and ubiquitous nature of protein kinase C.

The importance of Ca$^{2+}$ in mediating smooth muscle contraction is well known. However, the interaction between protein kinase C and Ca$^{2+}$ in smooth muscle contraction is less well understood. It has been reported that angiotensin II amplifies norepinephrine-induced contraction without an associated augmentation of $^{45}$Ca$^{2+}$ influx or net Ca$^{2+}$ uptake. This amplification is prevented by the protein kinase C inhibitor staurosporine (8). Furthermore, staurosporine reduced norepinephrine-induced tone in rabbit aortic rings (9). However, previous results (11) demonstrated that Ca$^{2+}$ uptake or influx is critical for angiotensin II-induced vasoconstriction. In the present study, responses to BAY K 8644, the Ca$^{2+}$ channel agonist, were significantly enhanced after administration of Gö-6976 and rottlerin, suggesting that inhibition of these protein kinase C isozymes enhances the vascular smooth muscle response to increases in intracellular free Ca$^{2+}$. The explanation for the enhanced response requires further study but may be related to an increased sensitivity to intracellular free Ca$^{2+}$ or an alteration in the Ca$^{2+}$ gradient after inhibition of protein kinase C isozymes in the pulmonary vascular bed of the cat.

In summary, the results of the present study show that the protein kinase C isozyme inhibitors Gö-6976 and rottlerin reduced pulmonary vasoconstriction by angiotensin II. Both protein kinase C isozyme inhibitors did not alter pressor responses to serotonin or U-46619, whereas only rottlerin inhibited responses to norepinephrine. The pressor response to BAY K 8644 was enhanced after administration of the protein kinase C isozyme inhibitors. These data suggest that the pulmonary arterial pressor responses to angiotensin II are mediated, in part, by the activation of Ca$^{2+}$-dependent protein kinase C isozymes and the Ca$^{2+}$-independent protein kinase C-δ isozyme/CaM-dependent kinase III. These data further suggest that the norepinephrine pressor response is mediated, in part, by the Ca$^{2+}$-independent protein kinase C-δ isozyme/CaM-dependent kinase III. A rottlerin- or Gö-6976-sensitive mechanism is not involved in the mediation of pressor responses to serotonin and U-46619 in the pulmonary vascular bed of the cat.

**REFERENCES**


