Effect of protein kinase C inhibition on hypoxic pulmonary vasoconstriction

SCOTT A. BARMAN
Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia 30912

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Barman, Scott A. Effect of protein kinase C inhibition on hypoxic pulmonary vasoconstriction. Am J Physiol Lung Cell Mol Physiol 280: L888–L895, 2001.—The current study was done to test the hypothesis that protein kinase C (PKC) inhibitors prevent the increase in pulmonary vascular resistance and compliance that occurs in isolated, blood-perfused dog lungs during hypoxia. Pulmonary vascular resistances and compliances were measured with vascular occlusion techniques. Hypoxia significantly increased pulmonary arterial resistance, pulmonary venous resistance, and pulmonary capillary pressure and decreased total vascular compliance by decreasing both microvascular and large-vessel compliances. The nonspecific PKC inhibitor staurosporine (10−7 M), the specific PKC blocker calphostin C (10−7 M), and the specific PKC isozyme blocker Go−6976 (10−7 M) inhibited the effect of hypoxia on pulmonary vascular resistance and compliance. In addition, the PKC activator thymeleatoxin (THX; 10−7 M) increased pulmonary vascular resistance and compliance in a manner similar to that in hypoxia, and the L-type voltage-dependent Ca2+ channel blocker nifedipine (10−6 M) inhibited the response to both THX and hypoxia. These results suggest that PKC inhibition blocks the hypoxic pressor response and that the pharmacological activation of PKC by THX mimics the hypoxic pulmonary vasoconstrictor response. In addition, L-type voltage-dependent Ca2+ channel blockade may prevent the onset of the hypoxia- and PKC-induced vasoconstrictor response in the canine pulmonary vasculature.

THE MECHANISMS OF HYPOXIC pulmonary vasoconstriction, first observed by von Euler and Liljestrand (49a), remain unclear. Although it has been established that hypoxia induces a rise in pulmonary vascular resistance in hypoxic regions of the lung, controversy exists regarding which vascular segments constrict to hypoxia. Hakim (19) reported that hypoxia primarily constricted the arterial segments, with a lesser effect on the venous segments, whereas Hillier et al. (22) observed that hypoxic vasoconstriction occurred in the small postcapillary venules. Shirai and colleagues (46) concluded that alveolar hypoxia induced vasoconstriction in small pulmonary arteries and pulmonary veins, with the larger effect occurring in the arteries, and Nagasaka et al. (36) suggested that hypoxia elicited constriction in small-artery segments. Most recently, studies (33, 48, 54) have reported that hypoxia primarily affects the arterial segments of the pulmonary circulation. The importance in the identification of the specific vascular segments that respond to hypoxia relates to I) the effect on pulmonary capillary pressure, which is determined by the distribution of vascular resistance in the pulmonary arteries and veins, and 2) the maintenance of ventilation-perfusion matching by the constriction of the pulmonary arteries that direct blood flow away from hypoxic regions.

Protein kinase C (PKC) represents an important component of a signal transduction pathway that regulates vascular smooth muscle contraction. The role of PKC in vascular smooth muscle contraction has been investigated with the use of phorbol esters and phorbol derivatives. Phorbols appear to exert their effect through the activation of the enzyme PKC by substituting for diaclyglycerol (11, 35). Diacylglycerol is thought to be one of the endogenous lipids that activates PKC by increasing the affinity of the enzyme for Ca2+ and phosphatidylserine at normal Ca2+ levels (37). By activating PKC, phorbols can potentiate vascular contraction by increasing the influx of Ca2+ through Ca2+ channels into vascular smooth muscle cells (12, 52). Activation of PKC by phorbols induces a slow-developing vascular smooth muscle contraction (18). Phorbol 12-myristate 13-acetate (PMA), an ester derivative of croton oil, and thymeleatoxin (THX), a phorbol derivative (44), have been used to study PKC-induced pulmonary vasoconstriction (1, 8, 25, 26, 32).

Although relatively scant, evidence points to a relationship between PKC signaling and hypoxic vasoconstriction in the pulmonary circulation. Dempsey et al. (14) showed that PKC is activated in pulmonary vascular smooth muscle cells during hypoxia, and Weismann and colleagues (53) recently provided evidence for the activation of PKC as a signal transducer in the pulmonary vasoconstrictor response to alveolar hypoxia. Specifically, these investigators showed that bisindolylmaleimide (BIM), a selective BIM PKC inhibitor, suppressed hypoxic pulmonary constriction in intact rabbit lungs. An earlier study (40) reported that H-7, a nonspecific PKC inhibitor, attenuated hypoxic pulmonary vasoconstriction in perfused rat lungs.

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In light of these previous investigations that appear to establish a relationship between hypoxic vasoconstriction and PKC activation in other experimental models, it was hypothesized that inhibition of PKC may also block canine hypoxic pulmonary vasoconstriction. Therefore, the current study was done to determine the effect of PKC inhibition on pulmonary vascular resistance and compliance in isolated blood-perfused dog lungs during hypoxia. The vascular occlusion technique was used to partition the pulmonary circulation into segmental resistances and compliances. Measurements generated by these occlusion techniques are based on the theory that the pulmonary circulation is represented by a resistance-compliance circuit (4, 20, 29, 30). In the present study, the compartmental model of pulmonary vascular resistance and compliance by Audi et al. (3) was used to determine the effect of PKC activation and hypoxia on segmental vascular resistance and compliance. These vascular occlusion techniques have been used previously (5–7, 9, 10, 20) to measure the pulmonary vascular resistance-compliance profile in normal lungs and in lungs challenged with vasoactive agents.

Specific to this study, the role of PKC in canine hypoxic pulmonary vasoconstriction was investigated with the use of the nonspecific PKC inhibitor staurosporine, the specific PKC blocker calphostin C, and the specific PKC isozyme inhibitor Gö-6976, which at the concentration used in this study, primarily blocks the Ca$^{2+}$-dependent PKC isozymes PKC-α and PKC-β as well as PKC-μ. The PKC activator THX was used to pharmacologically induce pulmonary vasoconstriction to compare to the hypoxic pressor response, and the L-type voltage-dependent Ca$^{2+}$ channel blocker nifedipine was used to inhibit both THX- and hypoxia-induced pulmonary vasoconstriction because reported mechanisms of action of PKC- and hypoxia-induced vascular smooth muscle contraction include the activation of Ca$^{2+}$ channels.

**METHODS**

Adult heartworm-negative mongrel dogs of either sex (15–19 kg) were anesthetized with pentobarbital sodium (30 mg/kg), intubated, and ventilated with a Harvard respirator that used room air at a tidal volume of 15 ml/kg. A left thoracotomy was performed through the fifth intercostal space. The left upper and middle lobes of the lung were removed, and the lower left lobe was prepared for isolation by placing loose ligatures around the left main pulmonary artery and lower left bronchus. Each animal was then heparinized (10,000 U iv) and, after 5–10 min, was rapidly bled through a carotid arterial cannula. Three hundred milliliters of blood were used to prime the perfusion apparatus. After bleeding was completed, the pulmonary artery was ligated, and, with the heart still beating, the lower left lobe with the attached left atrial appendage was rapidly excised and weighed. Plastic cannulas were secured in the lobar artery, lobar vein, and bronchus, and blood perfusion was started within 30 min of lung excision.

The isolated lung circuit has previously been described in detail (5, 6, 9, 15, 41, 48). Briefly, the lung was perfused at a constant flow with a roller pump (Masterflex, Cole Parmer) that pumped blood from a venous reservoir through a heating coil encased in a water jacket (37.5 ± 0.5°C) to the rest of the closed circuit. The blood was continuously bubbled with a gas mixture of 95% O$_2$-5% CO$_2$ to maintain blood gases (arterial Po$_2$ = 100–110 Torr and arterial Pco$_2$ = 30–40 Torr) and pH in the normal range. After the initial hyperinflation, airway pressure (Paw) was set at 3 cmH$_2$O.

The perfused lobe was placed on a weighing pan that was counterbalanced by a strain gauge transducer (FT10, Grass). Pulmonary arterial (Ppa) and venous (Ppv) pressures were measured by inserting catheters into the lobar artery and vein and connecting them to pressure transducers (23BC, Statham) positioned at the openings of the inflow and outflow cannulas. Pressures were zeroed at the level of the lung hilus. Blood flow was measured by an electromagnetic flow probe (SF300A, Carolina Medical) positioned in the venous outflow line and connected to a digital flowmeter (701D, Carolina Medical). Ppa, Ppv, and lung weight were recorded on a Grass polygraph (model 7F). Ppa and Ppv were initially zeroed so that the lung lobe became isogravimetric, i.e., neither gaining nor losing weight in zone III conditions (Ppa > Ppv > Paw).

### Pulmonary capillary pressure

Pulmonary capillary pressure (Ppc) was determined with the double-occlusion technique (49). When both arterial and venous cannulas were simultaneously occluded, Ppa and Ppv quickly equilibrated to the same pressure (Ppc). If Ppa and Ppv did not exactly equilibrate to the same pressure on double occlusion, then the mean of both pressures was determined and defined as Ppc. The occlusion pressures were consistently within 1 cmH$_2$O of each other, and it has been shown (49) that the double-occlusion pressure is an excellent estimate of Ppc.

### Pulmonary vascular resistance

Total pulmonary vascular resistance (Rt) was calculated by dividing the measured hydrostatic pressure difference across the isolated lung by the existing blood flow (Q)

\[
R_t = \frac{(P_{pa} - P_{pv})}{Q} \tag{1}
\]

The pulmonary circulation can be represented by a simple linear model whereby Ppa is separated from Ppc by a pre-capillary resistance ($R_1$), and Ppc is separated from Ppv by a post-capillary resistance ($R_2$). The precapillary and postcapillary resistances were calculated with the following equations

\[
R_1 = \frac{(P_{pa} - P_{pc})}{Q} \tag{2}
\]

\[
R_2 = \frac{(P_{pc} - P_{pv})}{Q} \tag{3}
\]

All pulmonary vascular resistances are reported in units of centimeters of water per liter per minute per 100 g.

### Determination of segmental vascular compliance

Total pulmonary vascular compliance (Ct) was calculated with Eq. 4 (4, 29, 30), and the slope of the Ppv time transient ($\Delta P/\Delta t$) was measured after venous occlusion with the existing Q at the time of occlusion

\[
C_t = \frac{Q}{(\Delta P/\Delta t)} \tag{4}
\]

Middle-compartment compliance ($C_2$) was calculated with the equation derived by Audi et al. (3)

\[
C_2 = C_t - \left(\frac{R_t C_1}{R_2}\right) \tag{5}
\]

Arterial compliance ($C_1$) was determined by the following equation as determined by Audi et al. (3)

\[
R_1C_1 = \frac{A_j}{(P_{pa} - P_{do})} \tag{6}
\]
where $A_2$ is the area bounded by the arterial pressure curve $[P(a)]$ after arterial occlusion ($P_{oa}$) and double-occlusion ($P_{odo}$) pressures were calculated by numerical integration.

Venous compliance ($C_v$) was then calculated by the following relationship with $C_t$, $C_1$, and $C_2$ values obtained from Eqs. 4–6:

$$C_v = C_t - C_1 - C_2$$  

(7)

**Experimental protocols.** Initially, for all isolated lobes, Ppv was set at 4–5 cmH$_2$O to provide zone III blood flow conditions. Ppa was adjusted (ranging from 15 to 20 cmH$_2$O) until the lower left lobe attained an isogravimetric state. Blood flow through the lobe was between 500 and 800 ml·min$^{-1}$·100 g wet weight$^{-1}$, and during the control period, the lung was allowed to stabilize for ~30 min. In all experiments, $10^{-5}$ M indomethacin (cyclooxygenase inhibitor; Sigma) was added during the stabilization period to prevent the production of vasodilatory prostanoids because a previous study (23) showed that cyclooxygenase inhibition of the pulmonary vasodilator prostacyclin during hypoxia induced a hypoxic pulmonary pressor response in the dog. After this stabilization period, all vascular occlusions were done and repeated at least three times to obtain average control values. After control measurements were done, the lobes were divided into nine treatment groups.

**Group 1** ($n = 5$), the hypoxic control group, consisted of isolated lung lobes ventilated with 95% N$_2$-5% CO$_2$ for 30 min to achieve a $P_{o2} < 50$ mmHg (23). To study the effect of PKC inhibition on the vasoactive response to hypoxia, lobes (group 2; $n = 5$) were pretreated with the nonselective PKC inhibitor staurosporine ($10^{-7}$ M; Sigma) or, in group 3 ($n = 5$), the specific PKC inhibitor calphostin C ($10^{-7}$ M; Calbiochem) for 15 min before the hypoxic exposure. Group 4 lobes ($n = 5$) were pretreated with $10^{-7}$ M G6-6976 (Calbiochem) for 15 min before hypoxic exposure to determine if specific PKC isoforms mediate the hypoxic pressor response. In group 5 ($n = 5$), the voltage-dependent L-type Ca$^{2+}$ channel blocker nifedipine ($10^{-7}$ M; Sigma) was used as a pretreatment for 15 min before hypoxia to determine the effect of blocking this specific Ca$^{2+}$ channel on hypoxic pulmonary vasoconstriction. For group 6 ($n = 5$), the PKC activator THX ($10^{-7}$ M; Calbiochem) was given under normoxic conditions to characterize the pulmonary vasoactive response to PKC activation, and group 7 lobes ($n = 5$) were pretreated with $10^{-7}$ M nifedipine (Sigma) for 15 min before $10^{-7}$ M THX was given to determine if the L-type Ca$^{2+}$ channel modulates THX-induced pulmonary vasoconstriction. Group 8 lobes ($n = 5$) consisted of lobes pretreated with $10^{-7}$ M G6-6976 for 15 min before treatment with $10^{-5}$ M histamine (Sigma) to compare a pharmacological agonist response with hypoxic stimulation (group 4), and group 9 lobes ($n = 3$) were pre-treated with $10^{-7}$ M THX for 15 min before hypoxia for comparison with THX alone (group 6). All drugs were dissolved in DMSO, and the volume of DMSO (<0.5% vol%) used to dissolve the drugs had no significant effect alone (vehicle controls), relative to baseline control measurements, on lung hemodynamics under normoxic or hypoxic conditions (data not shown). The concentrations of the inhibitors chosen were the smallest concentrations found to block the agonist responses in this experimental model and had no effect alone on baseline normoxic hemodynamic values. All drugs were given as a bolus into the venous reservoir, and all drug concentrations were calculated based on the final volume of the perfusion system after the drug(s) was to be given.

**Statistical analysis.** All values are expressed as means ± SE. Significance was determined with an ANOVA for within-group and between-group comparisons. If a significant $F$-ratio was found, then specific statistical comparisons were made with the Bonferroni-Dunn post hoc test. Significance was accepted when $P < 0.05$.

**RESULTS.**

To measure the effect of PKC inhibition on hypoxic pulmonary vasoconstriction, total pulmonary vascular resistance was partitioned into arterial (precapillary) and venous (postcapillary) resistances (see METHODS) as shown in Fig. 1. Figure 1A shows the effect of the PKC inhibitors staurosporine and calphostin C on pulmonary arterial resistance under hypoxic conditions. Hypoxia significantly increased pulmonary arterial resistance, an effect inhibited by staurosporine and calphostin C. The effect of hypoxia on pulmonary venous resistance is shown in Fig. 1B. Hypoxia significantly increased postcapillary resistance, which was also blocked by staurosporine and calphostin C. These results indicate that hypoxic vasoconstriction in both precapillary and postcapillary pulmonary vessels is mediated by PKC activation.

The effect of hypoxia on Ppc, which is determined by the distribution of precapillary and postcapillary resistances, is presented in Fig. 1C. Hypoxia significantly increased Ppc, primarily because of the increase in postcapillary resistance (Fig. 1B), an effect that was blocked by staurosporine and calphostin C. With respect to voltage-dependent Ca$^{2+}$ channel modulation, Fig. 1, A and B, shows that the dihydropyridine Ca$^{2+}$ channel antagonist nifedipine inhibited the hypoxic pressor response on both the arterial and venous segments. These results indicate that hypoxic vasoconstriction in both precapillary and postcapillary pulmonary vessels is mediated by PKC activation.

Figure 2 shows the effect of the specific PKC isoform inhibitor G6-6976 on the hypoxic vasoconstrictor response. G6-6976 inhibited the pressor response to hypoxia in both the pulmonary arteries and pulmonary veins, indicating that specific PKC isoforms may be activated during hypoxia to induce pulmonary vasoconstriction. In contrast, G6-6976 had no effect on the pharmacological pulmonary venoconstrictor response to histamine (Fig. 2), indicating a specific role for PKC isoform mediation in the hypoxic response.

The PKC activator THX was used to determine if PKC activation would elicit vasoconstriction in the canine pulmonary vasculature similar to that observed during hypoxia. Figure 3 shows the effect of THX on pulmonary vascular resistance. THX increased both pulmonary arterial resistance and pulmonary venous resistance in a manner similar to the increases observed with hypoxia, which was inhibited by nifedipine. In addition, hypoxic stimulation after THX did not elicit further vasoconstriction compared with THX alone, which would suggest that THX mimics hypoxia in the mechanism of stimulation. Collectively, these data indicate that THX and hypoxia may both activate PKC to induce vasoconstriction and that L-type voltage-dependent Ca$^{2+}$ channels modulate both pharmacological PKC-induced (Fig. 3) and hypoxia-induced pulmonary vasoconstriction (Fig. 2).

Table 1 summarizes the effect of hypoxia on pulmonary segmental vascular compliance. Hypoxia signifi-
Ssignificantly decreased total vascular compliance by lowering both middle-compartment compliance and large-vessel (arterial and venous) compliance, effects inhibited by staurosporine, calphostin C, and Gö-6976. THX decreased both middle-compartment compliance and large-vessel compliance similar to that which occurred with hypoxia. In addition, nifedipine blocked the effects of hypoxia and THX on arterial and venous compliance and middle-compartment compliance.

**DISCUSSION**

This study showed that PKC inhibition blocked the canine hypoxic pulmonary vasoconstrictor response because the nonspecific PKC inhibitor staurosporine, the specific PKC blocker calphostin C, and the specific PKC isozyme inhibitor Gö-6976 inhibited the precapillary and postcapillary hypoxic pressor responses. Staurosporine has been shown (18) to act at the catalytic site of PKC. The concentration of staurosporine used in this study ($10^{-7}$ M) blocked the pressor response to hypoxia and has been previously shown (9) to have no effect on the vasoconstrictor response to other vasoactive stimuli such as histamine in the same experimental model. Calphostin C is reported (28) to act directly with the regulatory domain of PKC. The concentration of calphostin C used in the present study ($10^{-7}$ M) also has been shown to specifically inhibit all PKC isozymes and completely abolish the contractile response to PKC activation by phorbol esters while having minimal effects on other vasoactive stimuli (45), such as the canine pulmonary vascular response to histamine (9). In addition, Gö-6976 inhibited the response to hypoxia...
but also had no effect on the constrictor response to histamine.

A paucity of evidence points to a relationship between PKC and hypoxic signaling in the pulmonary circulation. Earlier studies have reported that inhibition of PKC abolished hypoxic pulmonary vasoconstriction. Jin et al. (24) observed that the second phase of the contractile response of rat pulmonary arterial muscle to hypoxia (characterized by a slow onset and sustained contraction) was blocked by H-7, suggesting that the contractile response was dependent on PKC activation. In addition, H-7 was reported (40) to attenuate hypoxic pulmonary vasoconstriction in perfused rat lungs. Most recently, Weissmann and colleagues (53) showed that activation of PKC is involved in the signal transduction pathway linking pulmonary vasoconstriction to alveolar hypoxia in intact rabbit lungs. Specifically, these investigators showed that BIM, a selective BIM PKC inhibitor, suppressed hypoxic pulmonary vasoconstriction in intact rabbit lungs. Although it is apparent that PKC is involved in canine hypoxic pulmonary vasoconstriction, it has also been shown that PKC mediates other types of pulmonary vasoreaction. Barman et al. (10) reported that serotoninergic-induced pulmonary vasoconstriction is mediated through PKC activation, and it has been shown (9) that phorbol ester potentiation of the canine pulmonary vascular response to histamine is blocked by both staurosporine and calphostin C.

In addition to involvement in vascular contraction, PKC is also an important signaling mechanism for cell growth (37, 38) and has been implicated in hypoxic mitogenesis in pulmonary vascular smooth muscle (14, 50). Dempsey et al. (14) showed that PKC is activated in pulmonary vascular smooth muscle cells during hypoxia and also provided evidence that activation of selective PKC isozymes is important to the onset of hypoxic growth in pulmonary vascular smooth muscle. Specifically, it was shown that the Ca^{2+}-dependent PKC isozymes (α in particular) contributed to the augmented growth of pulmonary arterial vascular smooth muscle cells from hypoxia-exposed calves (50). PKC represents a family of at least 11 isozymes that are presently classified into four separate groups. Group 1 is composed of the classic PKC isozymes (α, βI, βII, and γ) that are Ca^{2+}-dependent; group 2 consists of the novel PKC isozymes (δ, ε, μ, θ, and η) that are Ca^{2+}-independent; group 3 contains the atypical isozymes (ζ and ζcational and diacylglycerol insensitive; and group 4 is composed of an isozyme (PKC-μ) that is similar to the isozymes in group 3 but contains a unique signal peptide and transmembrane domain (51). Numerous PKC isozymes (α, β, ε, ζ, and δ), which may be dependent on species, type of vessel, and age of the vessel (21, 27, 31, 39), are expressed in vascular smooth muscle. Although specific PKC isozyme identification was not attempted in the current study, recent work by Damron et al. (13) reported the expression of six PKC isozymes (PKC-α, PKC-δ, PKC-ε, PKC-ζ, PKC-η, and PKC-μ) in cultured canine pulmonary vascular smooth muscle. THX is a diterpene derivative of mezerein that has been reported (44) to selectively activate PKC-α, PKC-β, PKC-γ, PKC-δ, and PKC-ε and cause translocation and downregulation of multiple PKC isozymes. Thus based on the above identification of specific PKC isozymes present in canine pulmonary vascular smooth muscle, the data obtained with hypoxia and THX in this study suggest that specific PKC isozymes may be activated by hypoxia and THX in canine pulmonary vascular smooth muscle to elicit vasoreactivity. In particular, inhibition of the hypoxic pressor response by Go-6976 strengthens the possibility that specific PKC isozymes may be activated during hypoxia to induce canine pulmonary vasoreactivity.

Table 1. Effect of protein kinase C inhibition on hypoxic pulmonary vascular compliance in isolated dog lung

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
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<tbody>
<tr>
<td>Con</td>
<td>1.50 ± 0.17</td>
<td>0.23 ± 0.05</td>
<td>1.10 ± 0.14</td>
</tr>
<tr>
<td>HPX</td>
<td>0.88 ± 0.11</td>
<td>0.10 ± 0.02</td>
<td>0.69 ± 0.11</td>
</tr>
<tr>
<td>C + HPX</td>
<td>1.47 ± 0.20</td>
<td>0.28 ± 0.06</td>
<td>0.97 ± 0.19</td>
</tr>
<tr>
<td>ST + HPX</td>
<td>1.51 ± 0.16</td>
<td>0.30 ± 0.08</td>
<td>0.95 ± 0.07</td>
</tr>
<tr>
<td>Con</td>
<td>1.50 ± 0.19</td>
<td>0.26 ± 0.09</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>C + HPX</td>
<td>1.63 ± 0.06</td>
<td>0.25 ± 0.07</td>
<td>1.15 ± 0.15</td>
</tr>
<tr>
<td>Con</td>
<td>1.48 ± 0.13</td>
<td>0.20 ± 0.08</td>
<td>1.11 ± 0.12</td>
</tr>
<tr>
<td>C + HPX</td>
<td>1.52 ± 0.09</td>
<td>0.24 ± 0.07</td>
<td>1.15 ± 0.17</td>
</tr>
<tr>
<td>Con</td>
<td>1.59 ± 0.14</td>
<td>0.24 ± 0.09</td>
<td>1.12 ± 0.12</td>
</tr>
<tr>
<td>THX</td>
<td>0.92 ± 0.08*</td>
<td>0.12 ± 0.05*</td>
<td>0.70 ± 0.11*</td>
</tr>
<tr>
<td>Con</td>
<td>1.55 ± 0.14</td>
<td>0.23 ± 0.07</td>
<td>1.10 ± 0.11</td>
</tr>
<tr>
<td>N + HPX</td>
<td>1.68 ± 0.20</td>
<td>0.27 ± 0.05</td>
<td>1.14 ± 0.17</td>
</tr>
<tr>
<td>Con</td>
<td>1.49 ± 0.14</td>
<td>0.21 ± 0.09</td>
<td>1.10 ± 0.12</td>
</tr>
<tr>
<td>N + THX</td>
<td>1.46 ± 0.08</td>
<td>0.22 ± 0.05</td>
<td>1.11 ± 0.17</td>
</tr>
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Values are means ± SE. C1, total pulmonary vascular compliance; C2, arterial compliance; C3, middle-compartment compliance; C4, venous compliance. Con, control; HPX, hypoxia; ST, staurosporine; C, calphostin C; G, Go-6976; N, nifedipine; THX, thymeleatoxin.

*Significantly different from Con, P < 0.05.
In this study, the L-type voltage-dependent Ca\(^{2+}\) channel blocker nifedipine inhibited both hypoxia- and THX (activator of PKC)-induced pulmonary vasoconstriction. The results obtained with nifedipine and THX in this study are consistent with observations in other studies (1, 18, 34, 40, 42, 43), suggesting that L-type voltage-dependent Ca\(^{2+}\) channels modulate the hypoxic pressor response as well as PKC-induced vascular contraction. Post et al. (43) observed that the dihydropyridine Ca\(^{2+}\) channel blocker nisoldipine prevented hypoxic inhibition of K\(^{+}\) currents in pulmonary arterial smooth muscle cells. In addition, it has been shown (2, 27) that a decrease in oxygen from a normoxic to a hypoxic level causes depolarization of the resting membrane potential in pulmonary vascular smooth muscle and subsequent Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels. In this study, the fact that nifedipine inhibited the hypoxic pressor response indicates that hypoxic signaling in canine pulmonary vascular smooth muscle involves Ca\(^{2+}\) influx through a dihydropyridine-sensitive membrane pathway, probably modulated by voltage-dependent Ca\(^{2+}\) channels. Many studies suggest that PKC elicits vascular contraction through Ca\(^{2+}\)-mediated mechanisms. Forder et al. (18) showed that the dihydropyridine Ca\(^{2+}\) channel antagonist nitrindipine greatly reduced tissue-type plasminogen activator (tPA)-induced vascular contraction, and Michael et al. (34) provided evidence that removing extracellular Ca\(^{2+}\) and inhibiting Ca\(^{2+}\) entry through voltage-dependent channels significantly reduced the vasoconstriction caused by PMA. In isolated perfused dog lungs, Allison et al. (1) reported that verapamil significantly attenuated PMA-induced pulmonary vasoconstriction, and Orton et al. (40) observed that nifedipine attenuated the constrictor response to PMA in isolated perfused rat lungs. In addition, Sperti and Colucci (47) observed that tPA increased the initial rate of dihydropyridine-sensitive Ca\(^{2+}\) flux in monolayers through voltage-dependent Ca\(^{2+}\) channels through activation of PKC, whereas Fish et al. (17) recently reported that phorbol esters increase dihydropyridine-sensitive Ca\(^{2+}\) conductance in a clonal cell line derived from aortic smooth muscle cells. Thus it appears that the effect of nifedipine may occur downstream of PKC activation through inhibition of voltage-dependent Ca\(^{2+}\) channels, although other mechanisms of Ca\(^{2+}\) inhibition to block the pressor response cannot be ruled out.

Specific to this study, it was hypothesized that if PKC signaling was involved in the canine pulmonary pressor response to hypoxia, then PKC activation in the absence of hypoxia would mimic the pressor response that occurs under hypoxic conditions. Therefore, the PKC activator THX was used to determine if PKC activation increased pulmonary vascular resistance and compliance in a manner similar to hypoxia. It was observed that THX significantly increased pulmonary vascular resistance and pulmonary vascular compliance similar to increases seen in hypoxia, which suggests that specific PKC isozymes may be activated by hypoxia to induce pulmonary vasoconstriction. If this does occur, further studies are needed to identify which PKC isozymes are specifically activated by hypoxia to elicit this pressor response.

In the pulmonary circulation, vascular reactivity is an important determinant of pulmonary vascular resistance, pulmonary vascular compliance, and lung blood flow. The importance in the identification of the specific vascular segments that respond to hypoxia and the signaling mechanisms that mediate the pressor response relate to the effect on Ppc, which is determined by the distribution of vascular resistance in the pulmonary arteries and veins and the maintenance of ventilation-perfusion matching by constriction of pulmonary arteries that direct blood flow away from hypoxic regions. In this study, both hypoxia and THX increased precapillary resistance, postcapillary resistance, and Ppc and decreased microvascular compliance and large-vessel compliance. The effect of hypoxia and THX was greater in the veins (postcapillary resistance) than in the arteries (precapillary resistance), which reflects the net increase in Ppc due to the greater constriction in the veins. In addition, the decrease in pulmonary vascular resistance that occurred when vascular pressure was increased by either hypoxia or THX reflected the relative indistensibility (lack of compliance in the vessels) of the pulmonary vasculature in response to either hypoxia or PKC activation.

In summary, the results of this study showed that hypoxia increased pulmonary arterial resistance, pulmonary venous resistance, and Ppc and decreased pulmonary vascular compliance. Staurosporine, calphostin C, and Gö-6976 blocked the effect of hypoxia on the arterial and venous segments and also inhibited the decrease in pulmonary vascular compliance. In addition, the L-type voltage-dependent Ca\(^{2+}\) channel blocker nifedipine inhibited the hypoxia- and THX-induced pulmonary pressor responses. Collectively, these results suggest that PKC may be a signal that links hypoxia to pulmonary vasoconstriction through activation of L-type voltage-dependent Ca\(^{2+}\) channels.

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