Potassium channels modulate canine pulmonary vasoreactivity to protein kinase C activation

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Barman, Scott A. Potassium channels modulate canine pulmonary vasoreactivity to protein kinase C activation. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L558-L565, 1999.—The role of Ca$^{2+}$-activated K$^+$-channel, ATP-sensitive K$^+$-channel, and delayed rectifier K$^+$-channel modulation in the canine pulmonary vascular response to protein kinase C (PKC) activation was determined in the isolated blood-perfused dog lung. Pulmonary vascular resistances and compliances were measured with vascular occlusion techniques. The PKC activators phorbol 12-myristate 13-acetate (PMA; 10$^{-7}$ M) and thymeleatoxin (THX; 10$^{-7}$ M) significantly increased pulmonary arterial and pulmonary venous resistances and pulmonary capillary pressure and decreased total vascular compliance by decreasing both microvascular and large-vessel compliances. The Ca$^{2+}$-activated K$^+$-channel blocker tetraethylammonium ions (1 mM), the ATP-sensitive K$^+$-channel inhibitor glibenclamide (10$^{-5}$ M), and the delayed rectifier K$^+$-channel blocker 4-aminoypyridine (10$^{-4}$ M) potentiated the pressor response to both PMA and THX on the arterial and venous segments and also further decreased pulmonary vascular compliance. In contrast, the ATP-sensitive K$^+$-channel opener cromakalim (10$^{-5}$ M) attenuated the vasoconstrictor effect of PMA and THX on both the arterial and venous vessels. In addition, membrane depolarization by 30 mM KCl elicited an increase in the pressor response to PMA. These results indicate that pharmacological activation of PKC elicits pulmonary vasoconstriction. Closure of the Ca$^{2+}$-activated K$^+$ channels, ATP-sensitive K$^+$ channels, and delayed rectifier K$^+$ channels as well as direct membrane depolarization by KCl potentiated the response to PMA and THX, indicating that K$^+$ channels modulate the canine pulmonary vasoconstrictor response to PKC activation.

pulmonary vascular resistance; pulmonary vascular compliance; thymeleatoxin

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 phorbol esters. Phorbol esters appear to exert their effect through the activation of the enzyme PKC by substituting for diacylglycerol (DAG) (10, 31). DAG is thought to be one of the endogenous lipids that activates PKC by increasing the affinity of the enzyme for Ca$^{2+}$ and phosphatidylserine at normal Ca$^{2+}$ levels (32). By activating PKC, phorbols can potentiate vascular contraction by increasing the influx of Ca$^{2+}$ through Ca$^{2+}$ channels into vascular smooth muscle cells (11, 44). Activation of PKC by phorbols induces a slow-developing vascular smooth muscle contraction (20). Phorbol 12-myristate 13-acetate (PMA), an ester derivative of croton oil, has been used to study PKC-induced pulmonary vasoconstriction (3, 24, 25, 29). PMA enhances the vasoconstrictor response produced by vasoactive agents such as acetylcholine, serotonin, and K$^+$ (5, 11). Also, Orton et al. (35) observed that PMA potentiation of hypoxic and K$^+$ vasoconstriction in isolated rat lungs was mediated through the activation of PKC.

Ion channels, including K$^+$ channels, have been identified in vascular smooth muscle cells (12, 34, 35, 40) and are reported to be involved in the regulation of vascular tone (14, 22). Several different K$^+$ channels, including ATP-sensitive, Ca$^{2+}$-activated, and nonspecific voltage-gated K$^+$ channels, are present on vascular smooth muscle. Activation of these channels causes an increase in K$^+$ efflux, membrane hyperpolarization, inhibition of Ca$^{2+}$ influx, and subsequent vascular smooth muscle relaxation. In the pulmonary circulation, vascular smooth muscle tone is an important determinant of pulmonary vascular resistance, pulmonary vascular compliance, and lung blood flow, and the membrane potential of vascular smooth muscle is regulated by K$^+$ channels, which, in turn, modulate vascular smooth muscle tone and vasoconstriction. The importance in identifying the specific vascular segments in the pulmonary circulation that constrict reflects the net effect on pulmonary capillary pressure (Pc) that is determined by the distribution of vascular resistance in the pulmonary arteries and veins.

Although relatively scant, evidence points to a relationship between PKC and K$^+$ channels in vascular smooth muscle cells. Minami et al. (30) observed that PKC inhibited the Ca$^{2+}$-activated K$^+$ channel in coronary arterial smooth muscle, and Aiello et al. (2) showed that PKC activation by phorbol esters blocks the delayed rectifier channel. Most recently, a study by Shimoda et al. (39) reported that endothelin-1 depolarized intrapulmonary arterial smooth muscle cells by inhibiting the delayed rectifier K$^+$ current, an effect dependent on activation of the phospholipase C/PKC signal transduction pathway.

In light of these previous investigations that appear to establish a relationship between K$^+$ channels and PKC activation, it was hypothesized that closing K$^+$ channels would potentiate PKC-mediated canine pulmonary vasoconstriction. Therefore, the present study was done to determine the role of K$^+$-channel modulation on the effect of PKC on pulmonary vascular resistance and compliance in isolated blood-perfused dog lungs. 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. In the present study, the compartmental model of pulmonary vascular resistance and compliance by Audi et al. (4) was used to determine the effect of PKC on segmental vascular resistance and compliance. These vascular occlusion techniques have previously been used to measure the pulmonary vascular resistance-compliance profile in normal lungs and in lungs challenged with vasoactive agents (6–9).

Specific to this study, the role of ATP-sensitive K⁺ channels was investigated with cromakalim (Crom; an activator of ATP-sensitive K⁺ channels) and glibenclamide (Glib; a blocker of ATP-sensitive K⁺ channels) to determine whether ATP-sensitive K⁺ channels modulate the vasoconstrictor response to PKC. In addition, the Ca²⁺-activated K⁺ channel blocker tetraethylammonium ions (TEA) and the delayed rectifier K⁺ channel inhibitor 4-aminopyridine (4-AP) were used to determine whether these specific K⁺ channels also potentiate the pulmonary vascular response to PKC.

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 with 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 (6, 7, 9, 18, 36, 42). Briefly, the lung was perfused at a constant flow with a roller pump (Master Flex-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 remaining lung tissue. The blood was continuously bubbled with a gas mixture of 95% O₂–5% CO₂ to maintain blood gases (arterial Po₂ 100–110 Torr and arterial Pco₂ 30–40 Torr) and pH in normal ranges. After initial hyperinflation, airway pressure (Paw) was set at 3 cmH₂O.

The perfused lobe was placed on a weighing pan that was counterbalanced by a strain gauge transducer (Grass FT-10). Pulmonary arterial (Ppa) and venous (Ppv) pressures were measured using catheters into the lobar artery and vein and connecting them to pressure transducers (Statham 23B) positioned at the openings of the inflow and outflow cannulas. Pressures were zeroed at the level of the lung hilus. Blood flow (Q) was measured with an electromagnetic flow probe (Carolina Medical SF 300A) positioned in the venous outflow line connected to a digital flowmeter (Carolina Medical 701D). Ppa, Ppv, and lung weight were recorded on a Grass polygraph (model 7F). Ppa and Ppv were initially adjusted so that the lung lobe became isovolumetric, i.e., neither gaining or losing weight in zone III conditions (Ppa > Ppv > Paw).

Ppc, Ppv was determined with the double-occlusion technique (42). When both the arterial and venous cannulas are simultaneously occluded, Ppa and Ppv quickly equilibrate 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 cmH₂O of each other, and it has been shown that the double-occlusion pressure is an excellent estimate of Ppc (42).

Pulmonary vascular resistance. Total pulmonary vascular resistance (R₁) was calculated by dividing the measured hydrostatic pressure difference across the isolated lung by the existing Q.

\[
R₁ = (P_{pa} - P_{pv})/Q 
\]

(1)

The pulmonary circulation can be represented by a simple linear model whereby Ppa is separated from Ppv by a precapillary resistance (R₁) and Ppc is separated from Ppv by a postcapillary resistance (R₂). R₁ and R₂ were calculated with the following equations

\[
R₁ = (P_{pa} - P_{pc})/Q 
\]

(2)

\[
R₂ = (P_{pc} - P_{pv})/Q 
\]

(3)

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

Determination of segmental vascular compliance. Total pulmonary vascular compliance (C₁) was calculated with Eq. 4 and the slope of the venous pressure-time transient (ΔP/Δt) measured after venous occlusion with the existing Q at the time of occlusion.

\[
C₁ = Q/(ΔP/Δt) 
\]

(4)

Middle-compartment compliance (C₂) was calculated with the equation derived by Audi et al. (4)

\[
C₂ = C₁ - (R₁C₁/R₂) 
\]

(5)

Arterial compliance (C₁) was determined by the following equation (2)

\[
R₁C₁ = A₂/(P_{pa} - P_{dv}) 
\]

(6)

where A₂ is the area bounded by the arterial pressure curve after arterial occlusion and Pdv is the double-occlusion pressure calculated by numerical integration. Venous compliance (C₃) was then calculated by the following relationship with C₁, C₂, and C₃ obtained from Eqs. 4–6

\[
C₃ = C₁ - C₂ 
\]

(7)

Experimental protocols. Initially, for all isolated lobes, Ppv was set at 4–5 cmH₂O to provide zone III blood flow conditions. Ppa was adjusted (ranging from 15 to 20 cmH₂O) until the isolated lobe attained an isogravimetric state. Q through the lobe was between 500 and 800 ml·min⁻¹·100 g wet wt⁻¹, and during the control period, the lung was allowed to stabilize for ~30 min. 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 13 treatment groups.

Group 1 (PMA control lungs) consisted of isolated lung lobes (n = 5) treated with 10⁻⁷ M PMA (Sigma) for 60 min to achieve a peak pressor response. To study the effect that Ca²⁺-activated K⁺ channel modulation has on the vasoactive
response to PMA, lobes (group 2; n = 5) were pretreated with 1 mM TEA (Sigma) for 30 min before the addition of PMA. For group 3 (n = 5 lobes), 10^{-5} M Glib (Sigma) was used as a pretreatment for 15 min before the addition of PMA. In group 4, the lobes (n = 5) were pretreated with 10^{-5} M Crom (an ATP-sensitive K^+ channel opener) for 15 min before the addition of PMA. In group 5, the lobes (n = 5) were pretreated with 10^{-4} M 4-AP (an inhibitor of delayed rectifier K^+ channels; Sigma) for 15 min before the addition of PMA. For groups 6–10 (n = 5 lobes/group), the PKC activator thymeleatoxin (THX; 10^{-7} M; Calbiochem) was substituted for PMA, with the experimental protocols being identical to groups 1–5. In group 11, the Ca^{2+}-dependent PKC isoform (a and b) inhibitor Go 6976 (10^{-7} M) was used as a pretreatment for 15 min before the addition of PMA. Group 12 lobes were pretreated with norepinephrine (NE; 10^{-7} M; Sigma) for 15 min before the addition of PMA to determine whether a vasoactive agent that increases pulmonary vascular tone independent of K^+ blockade would have a similar effect on PMA. In group 13, KCl (30 mM; Sigma) was used to determine the effect of membrane depolarization on the vasoactive response to PMA. All drugs were dissolved in DMSO except TEA, KCl, and NE that were dissolved in saline. The volume of DMSO or saline used to dissolve the drugs had no significant effect alone (vehicle control) on lung hemodynamics relative to baseline control measurements (data not shown). 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 analysis of variance 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

Figure 1 shows the effect of Ca^{2+}-activated K^+ channel, ATP-sensitive K^+ channel, and delayed rectifier K^+ channel modulation on the Ppa response. Under control conditions, TEA, Glib, and 4-AP elicited a small but significant increase in Ppa. As shown in Fig. 2, PMA caused a significant increase in pulmonary arterial resistance, an effect potentiated when the Ca^{2+}-activated K^+ channels were blocked with TEA, the ATP-sensitive K^+ channels were blocked with Glib, and the delayed rectifier K^+ channels were inhibited with 4-AP. In contrast, opening the ATP-sensitive K^+ channels with Crom attenuated the pressor response to PMA. Figure 3 shows the effects of PMA on pulmonary venous resistance. PMA significantly increased pulmonary venous resistance, an effect potentiated by TEA, Glib, and 4-AP. In contrast, Crom attenuated the increase in pulmonary venous resistance by PMA. Figures 4 and 5 show the effect of the PKC activator THX on pulmonary vascular resistance. THX increased...
both pulmonary arterial resistance (Fig. 4) and pulmonary venous resistance (Fig. 5) similarly to PMA. In addition, TEA, Glib, and 4-AP potentiated the response to THX on both the precapillary and postcapillary vessels, and Crom inhibited the response in the precapillary vessels and partially blocked the pressor response to THX in the postcapillary vessels, with the remaining partial pressor response being insignificant.

The effect of PMA and THX on P_{pc}, which was determined by distribution of the precapillary and postcapillary resistances, is presented in Figs. 6 and 7, respectively. PMA and THX significantly increased P_{pc}, a phenomenon that was decreased by Crom. Closing the Ca^{2+}-activated K^{+} channels with TEA, blocking the ATP-sensitive K^{+} channels with Glib, or inhibiting the delayed rectifier K^{+} current with 4-AP significantly increased P_{pc} relative to the initial increase in P_{pc} observed with PMA or THX, which was reflective of the initial increase in pulmonary venous resistance with PMA (Fig. 3) and THX (Fig. 5). Figure 8 shows that Gö 6976 inhibited the pressor response to PMA, indicating that PMA activates specific Ca^{2+}-dependent PKC isoforms to induce pulmonary vasoconstriction. The possibility also existed that other vasoactive agents as well as membrane depolarization could potentiate the pressor response to PMA, so experiments were done with the pulmonary vasoconstrictors NE (6) and KCl to compare the effect of the K^{+}-channel blockers on the
response to PMA. Figure 9 shows that $10^{-7}$ M NE, which is a concentration that mimics the increase in $P_{pa}$ with the $K^+$-channel inhibitors (Fig. 1) in this experimental model, had no significant effect on the pressor response to PMA. In contrast, 30 mM KCl, which also elicits an increase in $P_{pa}$ similar to that observed with the $K^+$-channel blockers (Fig. 1), did increase the pressor response to PMA (Fig. 10). These data indicate that membrane depolarization as well as direct pharmacological $K^+$-channel blockade by specific inhibitors may be mechanisms of $K^+$-channel modulation that potentiate the vasoconstrictor effect of PMA.

Tables 1 and 2 summarize the effects of PMA and THX, respectively, on pulmonary segmental vascular compliance. PMA and THX significantly decreased total vascular compliance by lowering both middle-compartment and large-vessel (arterial and venous) compliances, effects potentiated by TEA, Glib, or 4-AP but subsequently reversed by Crom. Specifically, TEA, Glib, and 4-AP significantly potentiated the decrease in middle-compartment compliance, arterial compliance, and venous compliance by PMA and THX. In contrast, Crom inhibited the effect of PMA and THX on the arterial, venous, and middle-compartment compliances.

Table 1. Effect of PMA on compartmental pulmonary vascular compliances in isolated dog lung

<table>
<thead>
<tr>
<th></th>
<th>$C_1$</th>
<th>$C_2$</th>
<th>$C_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>$1.51 \pm 0.18$</td>
<td>$0.19 \pm 0.04$</td>
<td>$1.15 \pm 0.15$</td>
</tr>
<tr>
<td>PMA</td>
<td>$0.85 \pm 0.10^*$</td>
<td>$0.20 \pm 0.02^*$</td>
<td>$0.66 \pm 0.05^*$</td>
</tr>
<tr>
<td>Cont</td>
<td>$1.44 \pm 0.20$</td>
<td>$0.27 \pm 0.08$</td>
<td>$0.96 \pm 0.18$</td>
</tr>
<tr>
<td>TEA + PMA</td>
<td>$0.50 \pm 0.11^+$</td>
<td>$0.04 \pm 0.02^+$</td>
<td>$0.42 \pm 0.07^+$</td>
</tr>
<tr>
<td>Cont</td>
<td>$1.39 \pm 0.19$</td>
<td>$0.19 \pm 0.06$</td>
<td>$1.00 \pm 0.13$</td>
</tr>
<tr>
<td>Glib + PMA</td>
<td>$0.53 \pm 0.06^+$</td>
<td>$0.05 \pm 0.01^+$</td>
<td>$0.45 \pm 0.05^+$</td>
</tr>
<tr>
<td>Cont</td>
<td>$1.48 \pm 0.16$</td>
<td>$0.22 \pm 0.07$</td>
<td>$1.11 \pm 0.13$</td>
</tr>
<tr>
<td>4-AP + PMA</td>
<td>$0.48 \pm 0.08^+$</td>
<td>$0.04 \pm 0.02^+$</td>
<td>$0.41 \pm 0.07^+$</td>
</tr>
<tr>
<td>Cont</td>
<td>$1.41 \pm 0.16$</td>
<td>$0.18 \pm 0.03$</td>
<td>$1.07 \pm 0.20$</td>
</tr>
<tr>
<td>Crom + PMA</td>
<td>$1.40 \pm 0.10$</td>
<td>$0.17 \pm 0.01$</td>
<td>$1.10 \pm 0.05$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. $C_1$, total pulmonary vascular compliance; $C_2$, arterial compliance; $C_3$, middle-compartment compliance; $C_4$, venous compliance. Cont, control; PMA, phorbol 12-myristate 13-acetate; TEA, tetraethylammonium ions; Glib, glibenclamide; 4-AP, 4-aminopyridine; Crom, cromakalim. Significantly different ($P < 0.05$) from: *respective control group; †PMA alone.

Fig. 8. Effect of Go 6976 on total pulmonary vascular resistance response to PMA. Values are means $\pm$ SE; $n = 5$ lobes. There was no effect of vehicle alone or inhibitor alone on pulmonary vascular resistance. *Significantly different from respective control group, $P < 0.05$.

Fig. 9. Effect of norepinephrine (NE) on total pulmonary vascular resistance response to PMA. Control groups represent no treatment. Values are means $\pm$ SE; $n = 5$ lobes. *Significantly different from respective control group, $P < 0.05$.

Fig. 10. Effect of KCl on total pulmonary vascular resistance response to PMA. Values are means $\pm$ SE; $n = 5$ lobes. Significantly different ($P < 0.05$) from: *respective control group; **KCl alone.
Table 2. Effect of THX on compartmental pulmonary vascular compliances in isolated dog lung

<table>
<thead>
<tr>
<th>Compliance, ml·cmH2O·100 g⁻¹</th>
<th>C₁</th>
<th>C₂</th>
<th>C₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>1.45⁻⁻⁻⁻</td>
<td>0.16⁻⁻⁻⁻</td>
<td>0.12⁻⁻⁻⁻</td>
</tr>
<tr>
<td>THX</td>
<td>0.84⁻⁻⁻⁻</td>
<td>0.09⁻⁻⁻⁻</td>
<td>0.02⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Cont</td>
<td>1.54⁻⁻⁻⁻</td>
<td>0.21⁻⁻⁻⁻</td>
<td>0.30⁻⁻⁻⁻</td>
</tr>
<tr>
<td>TEA + THX</td>
<td>0.50⁻⁻⁻⁻</td>
<td>0.11⁻⁻⁻⁻</td>
<td>0.03⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Cont</td>
<td>1.41⁻⁻⁻⁻</td>
<td>0.19⁻⁻⁻⁻</td>
<td>0.22⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Glib + THX</td>
<td>0.55⁻⁻⁻⁻</td>
<td>0.07⁻⁻⁻⁻</td>
<td>0.04⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Cont</td>
<td>1.38⁻⁻⁻⁻</td>
<td>0.15⁻⁻⁻⁻</td>
<td>0.20⁻⁻⁻⁻</td>
</tr>
<tr>
<td>4-AP + THX</td>
<td>0.51⁻⁻⁻⁻</td>
<td>0.07⁻⁻⁻⁻</td>
<td>0.03⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Cont</td>
<td>1.48⁻⁻⁻⁻</td>
<td>0.15⁻⁻⁻⁻</td>
<td>0.19⁻⁻⁻⁻</td>
</tr>
<tr>
<td>Crom + THX</td>
<td>1.42⁻⁻⁻⁻</td>
<td>0.11⁻⁻⁻⁻</td>
<td>0.16⁻⁻⁻⁻</td>
</tr>
</tbody>
</table>

Values are means ± SE. THX, thymeleatoxin. Significantly different (P < 0.05) from: * respective control group; ¶ THX alone.

DISCUSSION

In this study, pharmacological activators of PKC (PMA and THX) increased precapillary and postcapillary resistances and Ppc and decreased microvascular and large-vessel compliances. The effect of PMA 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. The greater vasoconstrictor response to PMA and THX observed in the veins in this study may relate to the degree of upregulation and/or translocation of PKC from the cytosol to the membrane in pulmonary venous smooth muscle compared with that in pulmonary arterial smooth muscle. By activating PKC, phorbols can potentiate vascular contraction by increasing the influx of Ca²⁺ through Ca²⁺ channels into vascular smooth muscle cells (11, 44). Previous studies (3, 20) have shown that activation of PKC by phorbols induces a slow-developing vascular smooth muscle contraction, which was a phenomenon also observed in the present study. In isolated perfused canine lungs, Allison et al. (3) reported that PMA increased both precapillary and postcapillary resistances, but they did not measure the effect of PMA on pulmonary vascular compliance. In the present study, the decrease in pulmonary vascular compliance that occurred when vascular pressure was increased by either PMA or THX reflected the relative indistensibility (lack of compliance in the vessels) of the pulmonary vasculature in response to PKC activation. Although both PMA and THX decreased all compartmental compliances (arterial, microvascular, and venous), the percentage of large-vessel compliance (C₁ + C₂) to microvascular compliance (C₂) was similar to that measured under control conditions (C₁ + C₂ = 24% for control lobes, 22% for PMA-treated lobes, and 23% for THX-treated lobes).

PKC represents a family of at least 11 isoforms that are presently classified into four groups: group 1 comprises the classic PKC isoforms (α, β, γ, and δ) that are calcium dependent, group 2 consists of the novel PKC isoforms (ε, μ, η, and ζ) that are calcium independent, group 3 are the atypical isoforms (ι/λ and ι/λ) that are calcium independent and DAG insensitive, and group 4 (PKC-μ) is an isoform that is similar to the isoforms in group 3 but contains a unique signal peptide and transmembrane domain (43). Numerous PKC isoforms are expressed in vascular smooth muscle (α, β, ε, η, and ζ) that may be dependent on species, type of vessel, and age of the vessel (21, 26, 27, 33). A recent study by Damron et al. (15) reported the expression of at least six PKC isoforms including PKC-α, PKC-δ, and PKC-ε in cultured canine pulmonary vascular smooth cells. PMA is widely accepted as an activator of PKC, and THX has recently been shown to cause translocation and downregulation of multiple PKC isoforms (38). Thus, based on the above identification of specific PKC isoforms present in canine pulmonary vascular smooth muscle, the data obtained with PMA and THX suggest that the PKC isoforms α, δ, and ε may be among those activated by these agents in canine pulmonary vascular smooth muscle to elicit vasoactivity. In addition, inhibition of the PMA response by G6 6976 (calmodulin-dependent PKC-α and PKC-β isoform inhibitor) strengthens the possibility that PKC-α may be activated to induce pulmonary vasoactivity.

It appears that Ca²⁺-activated K⁺ channels, ATP-sensitive K⁺ channels, and delayed rectifier K⁺ channels play a role in maintaining normal pulmonary vascular tone. TEA, Glib, and 4-AP caused a small but significant increase in pulmonary vascular pressure under control conditions. Hasanuma et al. (22) observed that 4-AP and TEA caused vasoconstriction in perfused rat lungs, and Pinheiro and Malik (37) reported that activation of ATP-sensitive K⁺ channels had a small but minimal role in maintaining pulmonary vascular tone in piglet lungs. In contrast, ATP-sensitive K⁺-channel blockade appears to have no effect on basal tone in the pulmonary vascular bed of the cat (17) or lamb (41). Thus K⁺-channel modulation of basal pulmonary vascular tone appears to be K⁺-channel selective and species dependent. Under conditions of PKC activation, TEA, Glib, and 4-AP potentiated the response to PMA and THX on the arterial and venous segments and also further decreased pulmonary vascular compliance. Croman significantly attenuated the vasoconstrictor effect of PMA and THX on both the arterial and venous vessels. It is worth noting that the potentiation in arterial and venous resistances by K⁺-channel inhibition during PKC activation was greater in magnitude than by either K⁺-channel blockade or PKC activation separately, indicating a synergistic response rather than an additive effect on precapillary and postcapillary resistances. The increase in postcapillary resistance on K⁺-channel blockade had the net effect of reducing the distribution of pulmonary vascular resistance (1, 13, 16).

The possibility existed that other vasoactive agents as well as membrane depolarization could potentiate the pressor response to PMA independent of K⁺-channel blockade, so experiments were done with the pulmonary vasoconstrictors NE (6) and KCl to compare the effect of the K⁺-channel blockers on the response to
PMA. NE, at a concentration that mimicked the increase in $p_{\text{Pa}}$ by the $K^+$-channel inhibitors under baseline conditions in this experimental model, had no significant effect on the pressor response to PMA. In contrast, KCl, at a concentration that also elicited an increase in $p_{\text{Pa}}$ similar to that observed with the $K^+$-channel blockers did increase the pressor response to PMA. These results suggest that membrane depolarization has a similar effect as direct pharmacological $K^+$-channel blockade on the vasoconstrictor response to PMA.

In the pulmonary circulation, vascular smooth muscle tone is an important determinant of pulmonary vascular resistance, pulmonary vascular compliance, and lung blood flow. The membrane potential of vascular smooth muscle is regulated by $K^+$ channels, which, in turn, modulate vascular smooth muscle tone and vasoconstriction. The physiological significance of $K^+$-channel modulation on the pulmonary vasoactive response to PKC activation may be related to vascular hyperreactivity. It has been shown that PKC inhibits the delayed rectifier $K^+$ current in rabbit vascular smooth muscle cells (2), and PKC activators such as PMA also block the $Ca^{2+}$-dependent $K^+$ channel in coronary arterial smooth muscle cells (30). Shimoda et al. (39) recently reported that inhibition of the voltage-gated $K^+$ current in intrapulmonary arterial myocytes by endothelin-1 was markedly attenuated by staurosporine and GF-109203X, inhibitors of PKC. In addition, it has been hypothesized that $K^+$ channels are secondarily activated in response to increased vasoreactivity to regulate the pulmonary vascular bed under pathophysiological conditions involving pulmonary hypertension (28). Collectively, these results suggest that $K^+$ channels play an important role as a safety mechanism to regulate pulmonary vasoreactivity and prevent hyperreactivity. $K^+$ channels may also modulate endothelium-derived hyperpolarizing factor in vascular smooth muscle through the activation of $Ca^{2+}$-dependent $K^+$ channels (23), and it has recently been suggested that endothelium-derived hyperpolarizing factor may be $K^+$ that effluxes through charybdotoxin- and apamin-sensitive $K^+$ channels (19). Activation of these hyperpolarizing $K^+$ channels would attenuate pulmonary vasoconstriction and lower capillary pressure, a major determinant of lung fluid balance.

In summary, the results of this study showed that agonist activation of PKC by PMA and THX increased pulmonary arterial resistance, pulmonary venous resistance, and $p_{\text{Pa}}$ and decreased pulmonary vascular compliance. TEA, Glib, and 4-AP potentiated the response to PMA and THX on the arterial and venous segments and also further decreased pulmonary vascular compliance. In contrast, the ATP-sensitive $K^+$-channel opener Crom attenuated the vasoconstrictor effect of PKC activation on both the arterial and venous vessels. Although pharmacological agents were used to activate PKC and inhibit $K^+$ channels, these results indicate that physiological closure of the $Ca^{2+}$-activated $K^+$ channels, ATP-sensitive $K^+$ channels, and delayed rectifier $K^+$ channels potentiate the canine pulmonary arterial response during PKC activation and that $K^+$-channel modulation may be a key physiological response toward regulating pulmonary vasoactivity.

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