

Role of NO pathway, calcium and potassium channels in the peripheral pulmonary vascular tone in dogs

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ABSTRACT: Because hypoxic pulmonary vasoconstriction occurs mainly in the small pulmonary arteries, the authors investigated the effects of drugs acting on the nitric oxide (NO) pathway and the calcium and potassium channels in the peripheral pulmonary circulation, without interference with the overall pulmonary or systemic circulation.

Mixed venous blood was infused in wedged areas to study the pressure/flow relationship and to compute peripheral pulmonary vascular resistance (PPVR). The authors studied the effects of *N*^o-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, sodium nitroprusside (SNP, an NO donor), the calcium channel blockers verapamil, nifedipine and nicardipine, and the potassium channel opener levcromakalim, during normoxia and acute mild normocapnic hypoxia.

In the peripheral pulmonary circulation, L-NAME caused an increase in PPVR during normoxia (+95%; *p*<0.001) and hypoxia (+60%; *p*<0.01). Following the increase by L-NAME, SNP decreased PPVR during normoxia (-24%; *p*<0.05) and hypoxia (-23%; *p*<0.05). Verapamil, nifedipine and nicardipine did not modify PPVR during normoxia but during hypoxia they decreased PPVR (-28%, nonsignificant; -27%, *p*<0.01 and -33%, *p*<0.05, respectively). Levcromakalim did not modify PPVR during normoxia or hypoxia.

In conclusion, the nitric oxide pathway and voltage-dependent calcium channels, and not adenosine triphosphate sensitive potassium channels, play an important role in the control of peripheral pulmonary circulation in dogs.

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Segmental differences in vasomotor reactivity are well documented in the pulmonary vasculature. Hypoxia causes sustained constriction in resistance pulmonary arteries [1] while causing a biphasic response in conduit pulmonary arteries [2]. Investigations on resistance vessels have been performed mainly *in vitro* on small isolated pulmonary artery rings. Some authors investigated the relationship between pressure and flow in the pulmonary circulation *in vivo* for a lobe *in situ* [3], but the preparation included predominantly conduit vessels and the flow changes induced modifications of other variables. Because alveolar hypoxia is an important regulator of pulmonary vascular tone and causes vasoconstriction mainly in small pulmonary arteries, the authors studied, *in vivo*, the pharmacology of the vasomotor response of the peripheral pulmonary circulation, during normoxia and mild acute hypoxia.

Under physiological conditions, the vasodilator nitric oxide (NO) is continually released by endothelial cells and regulates organ and perfusion pressure and flow [4]. Endogenous NO may contribute to the maintenance of normal pulmonary vasomotor tone. Pulmonary vascular tone is also regulated by the activity of calcium and potassium channels, and the links between activation of both calcium and potassium channels and

NO-induced relaxation in the proximal part of the pulmonary artery have been emphasized, *in vitro* [5]. Various inhibitors of NO synthase (NOS) have been shown to increase the normoxic pulmonary vascular tone in some [6, 7] but not in all [8, 9] experiments. The inhibition of NO synthesis increases hypoxic pulmonary vasoconstriction in intact lungs, suggesting an increased NO synthesis in response to hypoxia and/or vasoconstriction [6].

The calcium and potassium channels seem also to play a role in the mechanism of hypoxic pulmonary vasoconstriction (HPV). Potassium channel blockers cause pulmonary vasoconstriction [10] largely through their effects on membrane potential and calcium channels. It has been proposed that hypoxia might inhibit outward potassium current in pulmonary smooth muscle cells, causing membrane depolarization and thus permitting calcium entry through the voltage-dependent calcium channels sensitive to dihydropyridine [11]. Calcium channel blockers like verapamil, a phenylalkylamine, have been shown to inhibit HPV [12], but YOUNG *et al.* [13] did not observe this inhibition with verapamil whereas nifedipine, a dihydropyridine, appeared to be a more effective pulmonary vasodilator.

Studies of the peripheral pulmonary circulation are difficult to perform *in vivo*. The present authors studied the pressure/flow relationship in a small peripheral portion of the pulmonary vascular bed, excluding most of the large conduit arteries, where pressure could be increased by hypoxia or pharmacologically modified without influencing the rest of the circulation [14]. This technique was used to study, during normoxia or acute normocapnic hypoxia, the action of the NO pathway by the administration of sodium nitroprusside (SNP), an NO donor, or by inhibition of NOS by *N*^ω-nitro-L-arginine methyl ester (L-NAME). The role of calcium channels was studied by a blockade with verapamil, nifedipine and nicardipine, and the role of potassium channels by the administration of a potassium channel opener, levcromakalim, in the peripheral pulmonary vasculature.

Material and methods

All experiments were conducted according to the Helsinki convention for the care and use of animals.

Study design

In anaesthetized dogs breathing spontaneously, vasoactive drugs were infused in a distal portion of the lung vascular bed, and the pressure/flow relationship was determined to compute the peripheral pulmonary vascular resistance (PPRV). Isotonic glucose was used as control. The drugs were given while the dogs breathed either room air, or a hypoxic mixture (O₂ 10%, CO₂ 3%, balance N₂).

Peripheral pulmonary vascular resistance model

The system used has been described previously in detail [14]. Anaesthesia was induced with an initial dose of 20 mg·kg⁻¹ thiopental *i.v.*, and maintained by a slow-rate infusion (14 mg·kg⁻¹·h⁻¹), with an electrical syringe (Vial SE 400, Grenoble, France). The dogs were intubated with a cuffed tracheal cannula whilst breathing spontaneously. Under sterile conditions, a femoral artery was cannulated with a catheter, and an external jugular vein with two catheters: a conventional Swan-Ganz thermodilution catheter, with the proximal lumen opening at 20 cm from the tip, and a 7F custom-made balloon catheter with both lumens extending to the tip (part No 600518 model, American Edwards Laboratories, Santa Ana, CA, USA). Both catheters were advanced, under radiograph, pressure and electrocardiograph (ECG) monitoring, into the pulmonary artery. The thermodilution catheter tip was advanced until a wedge pressure could be obtained by balloon inflation (the proximal lumen was then in the right atrium). The double lumen catheter was advanced, in another pulmonary artery, until it wedged itself *i.e.* when the internal diameter of the vessel was equal to the external diameter of the catheter (2.3 mm).

Femoral arterial pressure was continuously monitored. Pulmonary arterial (PAP), pulmonary wedge (PWP), and right atrial (RAP) pressures, as well

as cardiac output (CO), were measured with the Swan-Ganz catheter. Blood samples were withdrawn simultaneously from both femoral and pulmonary arterial catheters to determine arterial and mixed venous blood gases. To determine the pressure/flow curve, one lumen of the double lumen catheter was connected to a pressure transducer, the other one to a peristaltic pump (Ismatec, Zurich, Switzerland). Blood was withdrawn through the pump from the distal lumen of the thermodilution catheter, which was lying free in the pulmonary artery. Blood flow was monitored with a Transonic Systems (Ithaca, NY, USA) flowmeter. The catheters were primed with blood. Drugs or control solution (isotonic glucose 50 g·L⁻¹), were administered with an electrical syringe (Vial SE 400, Grenoble, France) and added to the blood infusion, with a rate of infusion equal to 15% of that of the blood infusion.

To study the pressure/flow curves, blood flow was increased by steps from 0 to ~5, 8 and 10 mL·min⁻¹; each flow was maintained for ≥ 1 min, and the pressure was measured after equilibration at the end of each period. The drugs were used at increasing concentrations.

After the pressure/flow runs had been completed, contrast medium was slowly infused into the wedged catheter, until the draining vein was visualized. The volume infused was taken as a measurement of the volume of the wedged area. The angiogram was always performed after the end of the pharmacological study since a previous study showed that contrast medium increased the PPVR [15].

At the end of the experiment, the special catheter was withdrawn, and blood was sampled from the pulmonary artery *via* the thermodilution catheter, in order to calibrate the flowmeter with a stop-watch and a graduated tube.

Hypoxia

The dogs breathed alternately, at random, room air or a hypoxic mixture (O₂ 10%, CO₂ 3%, N₂ 87%) administered from a bag through a nonrebreathing valve. Haemodynamic data were measured after 10 min hypoxic ventilation. During this time, blood flow was maintained in the wedged area, after which, the pressure/flow curve was determined. Before switching between normoxia and hypoxia, the double lumen catheter was wedged in another site. Different drugs were tested in different areas, except for sodium nitroprusside after L-NAME.

Pharmacological studies

The results were obtained from 96 experiments in 24 dogs, weighing 24.9±0.8 kg (mean±SEM). Seven protocols were performed, during normoxia and hypoxia. Control experiments were performed with isotonic glucose (50 g·L⁻¹) repeated four times consecutively in the same area (protocol 1). In separate experiments, the effects of L-NAME (10⁻⁷, 10⁻⁵ and 10⁻³ M) were studied (protocol 2). The effects of SNP (10⁻⁷, 10⁻⁵ and 10⁻³ M) were investigated following L-NAME (10⁻³ M; protocol

3). To assess the effects of calcium channel inhibitors, the effects of verapamil (10^{-10} , 10^{-8} , 10^{-6} M), nifedipine (10^{-11} , 10^{-9} , 10^{-7} M) and nicardipine (10^{-10} , 10^{-8} , 10^{-6} M) were studied (protocols 4, 5 and 6, respectively). In experiments designed to assess the role of potassium channel opening, the effects of levcromakalim (10^{-8} , 10^{-6} and 10^{-4} M) were investigated (protocol 7).

Drugs

L-NAME and SNP were purchased from Sigma (Saint Quentin-Fallavier, France). Verapamil was obtained from Knoll (Levallois-Perret, France), nifedipine from Bayer (Leverkusen, Germany), and nicardipine from Sandoz (Basel, Switzerland). Levcromakalim was kindly supplied by Smith Klein Beecham (Worthing, UK). Drugs were solubilized in glucose $50 \text{ g}\cdot\text{L}^{-1}$ (L-NAME and SNP), in distilled water (levcromakalim), in alcohol and polyethylene glycol (nifedipine) and in hydrochloric acid (nicardipine). Then an aliquot of these solutions was dissolved in isotonic glucose ($50 \text{ g}\cdot\text{L}^{-1}$) to obtain the various concentrations used. Control solutions were prepared from the solvents in the absence of active principle. The pH (~ 6) and the viscosity of the control and drug solution were the same. All solutions were prepared just prior to use.

Derived variables

The perfusion pressure during the pressure/flow runs was taken as the difference between the actual wedge pressure and the wedge pressure measured at zero flow. Overall pulmonary vascular resistance (PVR, $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$) was computed as $80 \times \text{PAP-PWP}$ (mmHg)/CO ($\text{L}\cdot\text{min}^{-1}$). PPVR ($10^3\text{-dyn}\cdot\text{s}\cdot\text{cm}^{-5}$) was determined applying the same formula to the local pressure/flow curve, with the perfusion pressure taken at $5 \text{ mL}\cdot\text{min}^{-1}$ flow [14].

Statistical analysis

In each protocol, differences between groups were tested by analysis of variance (ANOVA), differences between periods by paired t-tests, and relations between variables by least squares regression analysis [16]. All values are expressed as mean \pm SEM.

Results

The pressure/flow relationship was determined in 49 sites during normoxia and 47 other areas during hypoxia. The volume of the wedged area was $0.65 \pm 0.03 \text{ mL}$ in air, and $0.61 \pm 0.04 \text{ mL}$ during hypoxia. Blood gases during normoxia were within normal limits (table 1). During hypoxia, due to the presence of CO_2 in the inspired mixture, P_{a,CO_2} remained unchanged, P_{a,O_2} fell, and ventilation volume doubled with increased breathing frequency. Haemodynamic data were within normal limits during normoxia. During hypoxia, pulmonary artery pressure and pulmonary vascular resistance increased significantly (+20% and +49%, respectively). During the pressure/flow runs, heart rate, systemic arterial pressure, pulmonary arterial pressure

Table 1. – Blood gases, ventilation and haemodynamic data during normoxia and hypoxia

	Normoxia	Hypoxia	p
Subjects n	49	47	
pH	7.34 ± 0.01	7.35 ± 0.01	NS
P_{a,CO_2} mmHg	37.3 ± 0.5	35.6 ± 0.6	NS
P_{a,O_2} mmHg	84 ± 1	45 ± 1	NA
\dot{V}' $\text{L}\cdot\text{min}^{-1}$	6.3 ± 0.3	14.0 ± 0.6	<0.001
\dot{f}_R $\text{c}\cdot\text{min}^{-1}$	21 ± 1	28 ± 2	<0.01
mSAP mmHg	159 ± 2	165 ± 3	NS
HR min	202 ± 4	200 ± 4	NS
CO $\text{L}\cdot\text{min}^{-1}$	4.9 ± 0.2	4.7 ± 0.2	NS
mPAP mmHg	20 ± 1	24 ± 1	<0.001
mPWP mmHg	9 ± 0.3	9 ± 0.3	NS
PVR $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$	181 ± 10	269 ± 14	<0.001
PPVR $10^3\text{-dyn}\cdot\text{s}\cdot\text{cm}^{-5}$	101 ± 9	126 ± 10	<0.05

Data are presented as mean \pm SEM. n: number of sites; \dot{V}' : minute ventilation; \dot{f}_R : breathing frequency; mSAP: mean systemic arterial pressure; HR: heart rate; CO: cardiac output; mPAP: mean pulmonary artery pressure; mPWP: mean wedge pressure; PVR: pulmonary vascular resistance; PPVR: peripheral pulmonary vascular resistance; NS: non-significant; NA: not applicable.

and right atrial pressure did not change. No significant change in haemodynamic variables, including CO and cardiac filling pressures was induced by the pharmacological studies.

Protocol 1: effects of isotonic glucose

PPVR, computed from four consecutive curves with isotonic glucose, showed no difference with time during normoxia (n=8) or hypoxia (n=4).

Protocols 2–3: effects of NOS inhibition and SNP

L-NAME increased PPVR under normoxia (n=5) and hypoxia (n=10), and with the highest concentration, PPVR reached the same value in the two conditions (fig. 1). The dose-response correlation was

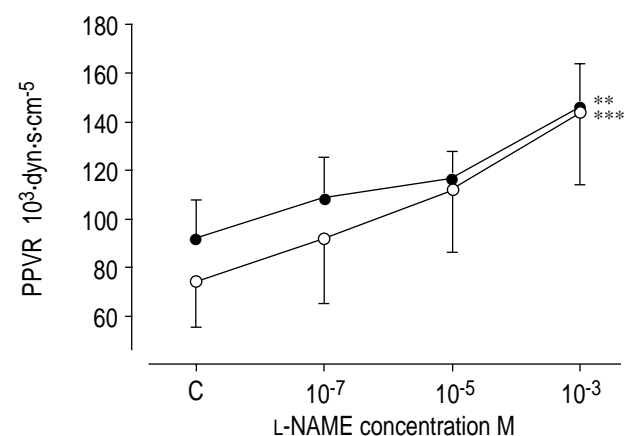


Fig. 1. – Effect of L-NAME on peripheral pulmonary vascular resistance (PPVR) during normoxia (\circ) and during hypoxia (\bullet). Results are expressed as mean \pm SEM. C: control. A statistically significant difference was found by two-way analysis of variance for the responses of PPVR to L-NAME during normoxia (***) ($p < 0.001$) and during hypoxia (**) ($p < 0.01$).

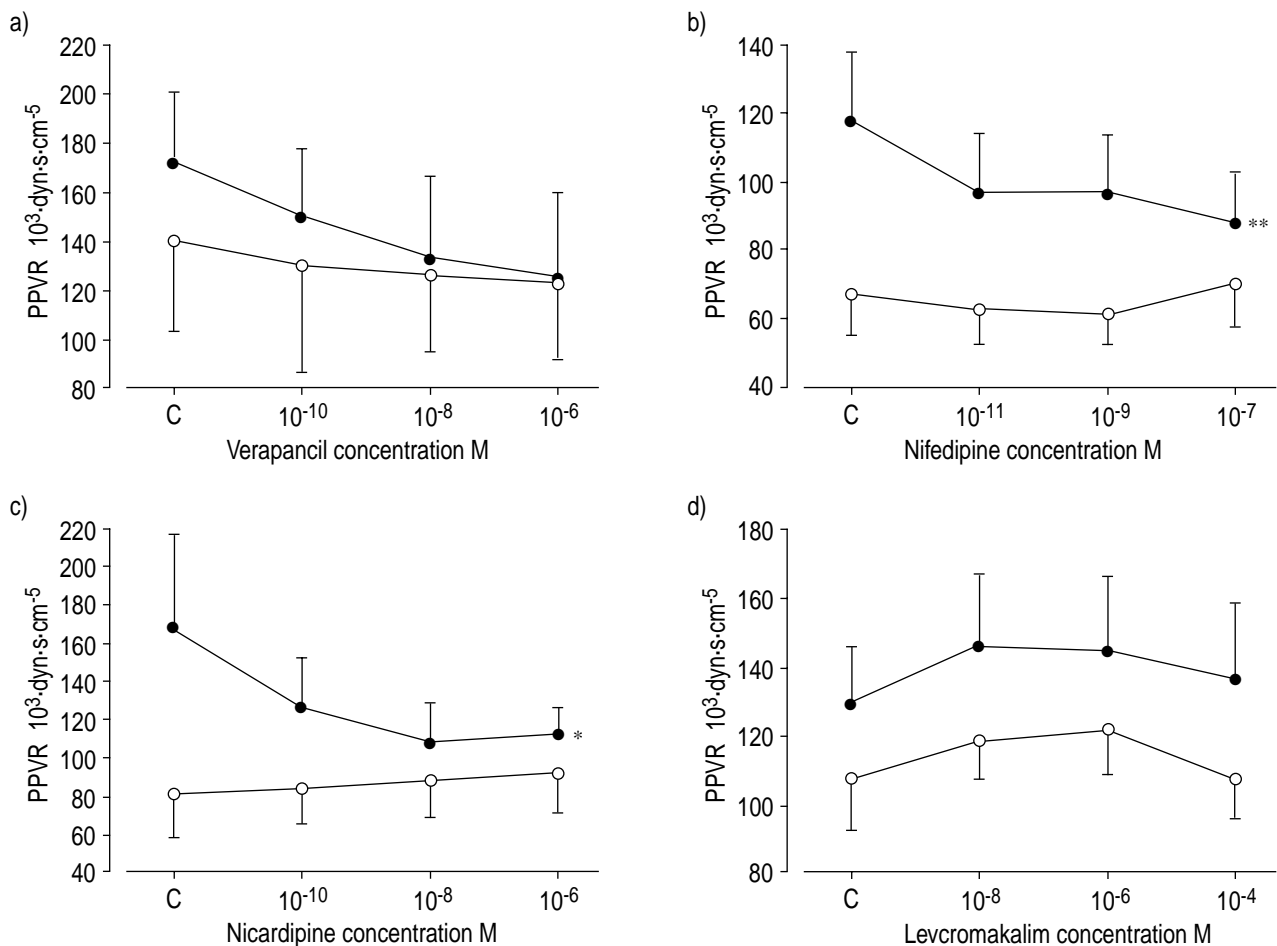


Fig. 2. – Effects of calcium channel blockers and levcromakalim on peripheral pulmonary vascular resistance (PPVR) in air (○) and during acute hypoxia (●). a) verapamil; b) nifedipine; c) nicardipine; d) levcromakalim. Data expressed as mean±SEM. C: control; **: $p < 0.01$ found by two-way analysis of variance (ANOVA) between responses of PPVR from control to nifedipine at 10^{-7} M during hypoxia; *: $p < 0.05$ found by two-way ANOVA between responses from control to nicardipine at 10^{-6} M during hypoxia.

significant in normoxia and in hypoxia ($p < 0.001$ and $p < 0.01$, respectively). After an increase in PPVR with L-NAME (10^{-3} M), perfusion of SNP at 10^{-7} , 10^{-5} and 10^{-3} M, induced a significant decrease in PPVR, during normoxia ($n=5$; $p < 0.05$) and hypoxia ($n=4$; $p < 0.05$).

Protocols 4–6: effects of calcium channel blockers

During normoxia, verapamil ($n=5$), nifedipine ($n=9$), or nicardipine ($n=7$; fig. 2) did not change PPVR. During normocapnic hypoxia, verapamil tended to cause a decrease in PPVR ($n=5$, $p=0.07$) and nifedipine caused a significant decrease in PPVR ($n=5$, $p < 0.01$). The changes with nicardipine were of the same kind as with nifedipine: during hypoxia, PPVR decreased by 33% ($n=9$, $p=0.04$).

Protocol 7: effects of potassium channel opener levcromakalim

As shown in figure 2, levcromakalim, from 10^{-8} to 10^{-4} M, did not induce a significant change in PPVR,

during normoxia ($n=10$) or hypoxia ($n=9$). PPVR baseline values, obtained with control solution before infusion of levcromakalim, were of the same kind as in other protocols, both in air and in hypoxia, and no correlation was observed between the baseline value of PPVR during hypoxia and any change following levcromakalim.

The summary of the effects of the various stimuli is shown in table 2. No significant decrease in the peripheral pulmonary vascular tone was observed with tested vasodilators when the pulmonary vascular tone had not been increased by prior exposure to L-NAME or during hypoxia. After an increase in PPVR, vasodilator agents (NO donor and calcium channel blockers) induced a decrease in PPVR, although PPVR remained elevated compared to baseline.

Discussion

The present results were observed in intact, though anaesthetized, animals, in a small portion of the lung vasculature, peripheral to a pulmonary artery with a 2.3 mm inner diameter. The amount of drug infused

Table 2. – Peripheral pulmonary vascular resistance (PPVR) difference between control and drugs

	Normoxia	Hypoxia
L-NAME	70±18*** (+95%)	55±21** (+60%)
SNP	-35±19* (-24%)	-30±8* (-23%)
Verapamil	-17±16 (-12%)	-48±37 (-28%)
Nifedipine	2±9 (+5%)	-31±10* (-27%)
Nicardipine	10±21 (+12%)	-60±40* (-33%)
Levcromakalim	1±14 (-1%)	+7±14 (+5%)

Mean absolute PPVR difference ($10^3 \cdot \text{dyn} \cdot \text{s} \cdot \text{cm}^{-5}$) between the highest drug concentration, and control (or L-NAME for SNP). The % difference is in relation to the control (or L-NAME) PPVR. SNP: sodium nitroprusside. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

was too low to generate any effect on the systemic or overall pulmonary circulation. PPVR baseline values varied widely from one location to another because of local variations such as differences in the volume of the wedged vascular bed [14] but did not vary with a local infusion of glucose repeated four times in the same area, during normoxia or during hypoxia (protocol 1), showing that this procedure is suitable to study pharmacological interventions in the peripheral pulmonary circulation.

Due to the low pH of the control and drug solutions, minor acidosis of the blood infused during the pressure/flow runs cannot be excluded, although a significant decrease in blood pH is unlikely because of the small rate of infusion (15% of the blood infusion). Acidosis stimulates NO production, both *in vitro* and *in vivo*, leading to changes in vasomotor tone [17, 18]. However, *in vitro* pH measurements in blood sampled from the pulmonary artery and diluted with 15% of a control solution showed a small pH decrease of 0.03 ± 0.01 ($n=4$). During protocol 1, no change in the overall pulmonary or systemic circulation, or in PPVR was observed. These results exclude significant acidosis and NO production along the experiments.

The pharmacological studies in the peripheral circulation *in vivo* were performed during normoxia, and during moderate hypoxia with a mean P_{a,O_2} of 45 mmHg, *i.e.* in the order of magnitude one might see in clinical situations such as pneumonia, acute respiratory distress syndrome or high altitude. As previously described [14], increases in PAP and PPVR during hypoxia have been observed.

The present data show that: 1) L-NAME caused an increase in the peripheral pulmonary vascular tone, potentiated the pulmonary vasoconstrictor response to hypoxia and did not suppress the vasodilation to SNP; 2) the calcium channel blocker dihydropyridines (nifedipine and nicardipine) decreased the PPVR during hypoxia; and 3) a potassium channel opener, levcromakalim, did not modify PPVR, during normoxia or acute mild hypoxia.

L-NAME

Variable effects of NO inhibitors on the normoxic pulmonary circulation have been observed. In adult animals, acute administration of arginine analogues did

not influence pulmonary vascular tone in perfused dog lungs [9], in conscious dogs [8], or in intact anaesthetized dogs [19]. In other studies, however, inhibitors of NO synthesis increased normoxic pulmonary vascular tone [7]. In response to acute hypoxia, NO synthesis inhibitors have been shown to potentiate pulmonary vasoconstriction in intact anaesthetized dogs [7, 19]. In the present study, L-NAME significantly increased normoxic and hypoxic pulmonary vascular tone. These results suggest that the background production of NO is important in the modulation of the peripheral pulmonary vascular tone in the dog.

SNP inhibited the hypoxic pulmonary vasoconstriction in anaesthetized dogs [20]. Moreover, the NO donor was an effective vasodilator of all pulmonary vessels with the exception of the smallest arteries in a model of sheep isolated pulmonary arteries and veins [21]. SNP also showed a dilator action on the pulmonary vascular pressure/flow relationship in conscious dogs after pulmonary vasoconstriction, and was not consistently affected by blockade of endogenous release by an inhibitor of NO synthesis [8]. The present results in the peripheral lung vasculature were consistent with these since, after L-NAME, SNP decreased the PPVR during normoxia and hypoxia, and confirmed that exogenous NO was able to induce a decrease in the peripheral pulmonary vascular tone [21].

Calcium blockers

The effects of verapamil, nifedipine and nicardipine on the PPVR were dependent on vascular tone: these drugs were ineffective in normoxia, and during acute hypoxia the vasodilation they produced was related to the increased resistance value. However, vasodilator action did reach statistical significance for dihydropyridines only. With verapamil during hypoxia, PPVR seemed to decrease, but the changes were not significant because of the dispersion of the results. In the literature, verapamil had no significant effect on the pulmonary vascular tone during normoxia in isolated pulmonary artery rings [22] nor in anaesthetized dogs. Similarly nifedipine had no significant effect on tone during normoxia in anaesthetized dogs [13]. During acute HPV, various studies have reported effective vasodilating actions of calcium channel inhibitors. During hypoxia, verapamil inhibited HPV in isolated rat lungs [12] but not in human pulmonary artery rings [22]. Although verapamil did not induce any significant change in the pulmonary vascular tone in anaesthetized dogs, nifedipine decreased pulmonary vascular resistance [13]. In the peripheral pulmonary vasculature, the present study observed that dihydropyridines decreased the enhanced hypoxic pulmonary vascular tone.

Levcromakalim

The present findings did not confirm a vasodilating action of the potassium (K^+) channel opener levcromakalim in the peripheral pulmonary circulation. Levcromakalim, the active enantiomer of cromakalim, is a K^+ channel opener that activates adenosine

triphosphate (ATP)-sensitive K^+ channels (K_{ATP} channels) in vascular smooth muscle [23]. Several studies suggest the role of K^+ channel inhibition in HPV [10, 24]. Levromakalim has a relaxant activity in K^+ -precontracted pulmonary vessels [25], and cromakalim causes vasorelaxation on HPV [24]. The low basal pulmonary vascular tone could explain the ineffectiveness of levromakalim in our study in normoxia but not in hypoxia, because hypoxia increases PPVR [14]. Moreover, this study's baseline PPVR values, during hypoxia, before levromakalim infusion, were in the same order of magnitude as those observed in other protocols where calcium channel blockers and SNP were effective.

The fact that contrasting data have been observed regarding the effects of various drugs on the pulmonary vascular tone may be due to both the diversity in experimental conditions and the regional vasoreactivity. KEMP *et al.* [21] recently demonstrated in sheep that the reactivity to many vasoconstrictors and vasodilators was not uniform along isolated pulmonary vessels. In conduit arteries, the calcium activated K^+ channels (K_{Ca} channels) are predominant. No K_{ATP} cell was identified in resistance arteries which have a majority of voltage-gated potassium (K_v) channels [26]. Nine families of K_v channels are recognized from cloning studies (K_{v1} – K_{v9}), each with subtypes. The contribution of an individual K_v channel to the whole cell current is difficult to determine pharmacologically because K_v channel inhibitors are nonspecific. Using anti- K_v antibodies to immunolocalize and inhibit K_v channels, ARCHER *et al.* [27] showed that the pulmonary arterial smooth muscle cell contains numerous types of K_v channels among which $K_{v2.1}$ and $K_{v1.5}$ contribute to the initiation of HPV. The present experiments operated on a peripheral portion of the pulmonary vascular bed where the arteries are mainly resistance arteries. As we did not find any activity of K_{ATP} channel openers, these results are consistent with those of ARCHER *et al.* [26].

The comparison of PPVR differences with the vasodilators and vasoconstrictor agents studied here *in vivo*, demonstrated that, in the present model, the peripheral pulmonary vasoreactivity to vasodilator agents was moderate. The low basal pulmonary vascular tone could explain the absence of effects from vasodilator drugs when pulmonary vascular tone had not been previously increased, and their moderate effects despite increased pulmonary vascular tone by mild acute hypoxia and vasoconstrictive drugs. Even during acute hypoxia, the peripheral pulmonary vasculature failed to totally relax in response to calcium channel blockers, as in previous works [21, 28]. The reason for this small response is unclear, but it was also observed with NO and β -adrenoceptor-mediated relaxation in other models [21].

In conclusion, the effects of several vasoactive drugs on the peripheral pulmonary circulation without any effect on the systemic or overall pulmonary circulation, *in vivo*, in anaesthetized intact dogs, during normoxia and during mild acute normocapnic hypoxia have been reported. This study has also demonstrated that the unstressed peripheral pulmonary vasculature displayed little reactivity to vasodilator drugs. These results

underline the role of the nitric oxide pathway, and suggest the lack of functional adenosine triphosphate sensitive potassium channels in peripheral pulmonary circulation during normoxia and during acute hypoxia. The role of calcium channels seems essential during mild acute hypoxia in the peripheral lung vasculature. One of the possible implications in clinical practice would be the pharmacological decrease in peripheral pulmonary vascular resistance during hypoxia, in agreement with recent studies showing beneficial effects of calcium channel blockers during high altitude pulmonary oedema [29]. This model could help to select new drugs such as other potassium channel openers or endothelin receptor antagonists that could be useful in clinical practice [30].

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