Effects of sildenafil on hypoxic pulmonary vascular function in dogs

Pierre Fesler,1 Alberto Pagnamenta,2 Benoît Rondelet,2 François Kerbaul,2 and Robert Naeije2

1Department of Internal Medicine, Hôpital Lapeyronie, Montpellier, France; and
2Laboratory of Physiology, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

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We therefore investigated the hemodynamic effects of sildenafil compared with those of inhaled nitric oxide (NO) and intravenous sodium nitroprusside (SNP) as specific and non-specific pulmonary vasodilating interventions in a canine model of whole lung hypoxic vasoconstriction (23). Pulmonary vascular resistance (PVR) was defined by rapid pulmonary vascular pressure-flow relationships to discriminate between active (tone-dependent) and passive (flow-dependent) changes in pulmonary artery pressures (Ppa) (1). Pulmonary vascular impedance was calculated from a spectral analysis of Ppa and flow waves to differentiate distal (PVR) from proximal (compliance, wave reflection) functional changes in the pulmonary vascular tree (1). The site of action of drugs and interventions was further defined by the partitioning of PVR into arterial and capillary-venous segments by the arterial occlusion method (8). Because an increase in cyclic guanylate monophosphate (cGMP) following PDE5 inhibition might affect myocardial contractility (38), we also investigated the effects of the interventions on RV function and its coupling to the pulmonary circulation (2).

METHODS

All experiments were approved by the Animal Ethics Committee of the Brussels Free University School of Medicine and were done in accordance with the “Guiding Principles in the Care and Use of Animals” of the American Physiological Society.

Preparation. The study included eight anesthetized mongrel dogs (mean weight, 31 kg; range, 22–40 kg). The animals were anesthetized with sufentanil (10 μg/kg iv) and α-chloralose intravenous (80 mg/kg), followed by infusions of sufentanil (1 μg·kg⁻¹·h⁻¹) and α-chloralose (20 mg·kg⁻¹·h⁻¹) (2). They were ventilated and equipped with catheters and flow probes as previously described (1, 2). Once the preparation completed, the inspired fraction of oxygen (FIO₂) was decreased twice to 0.1 for 10 min to stabilize the hypoxic pulmonary vasoconstriction (HPV) (23).

Measurements. Details of the measurements were also reported previously (1, 2). Briefly, PVR evaluation was completed by Ppa minus left atrial pressure (Pla) vs. flow (Q) curves (Ppa − Pla/Q plots) obtained by rapid inflation of a vena cava inferior balloon (1). Pulmonary arterial impedance (PVZ) was calculated from Fourier series expressions of instantaneous pressure and flow (24). The PVZ modulus was computed as the ratio between pressure and flow moduli and the impedance phase as the difference between flow and pressure phases. The impedance at 0 Hz was taken as the total resistance, and characteristic impedance (Zc) was calculated as the average of impedance moduli between 2 and 15 Hz (24). Partial pressure-volume loops were generated from synchronized RV pressure and integrated pulmonary arterial flow, and a single-beat method was used to measure end-systolic elastance (Ees) as the slope of end-systolic pressure-volume relationship, pulmonary artery effective elastance.
Capillary pulmonary pressure (Pc) was computed in triplicate from the Ppa decay curve after inflation of the balloon at the tip of the pulmonary artery catheter. For this measurement, the dogs were disconnected from the ventilator at end expiration for 10 s. The Ppa decay curve was analyzed by a dual-exponential fitting procedure, which includes a rapidly decreasing exponential (filling of the capillary compartment from the arterial one) and a slowly decreasing one (emptying of the capillary compartment into venous one). The resulting compartmental resistance and compliance values were used to generate a capillary pressure decay curve and estimate Pc at the instant of occlusion (8). The arterial component of PVR was calculated as (Ppa − Pc)/Q and expressed as percentage of PVR.

Experimental protocol. After ensuring a stable state, as assessed by unchanged stable heart rate and systemic and pulmonary artery pressures during 15 min, a first hemodynamic evaluation with measurements of systemic and pulmonary vascular pressures, blood gases, cardiac output, arterial occlusion, and rapid (Ppa − Pla)/Q plots, and an acquisition of instantaneous RV pressure, Ppa, and flow signals for Ees and PVZ calculation was performed at the FIO2 of 0.4. The complete set on hemodynamic measurements was then repeated after 15 min at the FIO2 of 0.1. The same sequence of hyperoxic and hypoxic measurements was repeated four times, with addition of either a placebo, inhaled NO at 40 ppm, intravenous SNP at 5 \( \mu \text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \), or intravenous sildenafil at 0.1 mg\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \) after a loading dose of 1 mg\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \) for 5 min.

The dose of inhaled NO at 40 ppm was used because it is about the maximum dose to reverse the HPV without significant systemic effects (30). The dose of SNP at 5 \( \mu \text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \) was chosen because of its maximal effect on HPV without uncontrolled systemic deterioration (25). The dose of sildenafil was derived from a dose-response curve determined in a preliminary study in four dogs equipped with pulmonary and systemic artery catheters and ventilated with FIO2 of 0.1 as indicated above. In this preliminary study, we increased the dose of intravenous sildenafil from 0.003 to 0.3 mg\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \) (half-log steps) and measured PVR and systemic vascular resistance (SVR) after 15 min of stable state at each successive dose. The maximum dose of 0.3 mg\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \) was determined on the basis of a 25% decrease in systemic blood pressure, a tendency to a plateau of the dose-response curve of PVR, and the achievement of a dose of previously reported clinical efficacy (21).

Statistical analysis. Results are expressed as means ± SE. Hemodynamic data and blood-gas results were compared by analysis of variance for repeated measures, with Bonferroni’s correction for seven pairwise comparisons (hyperoxia vs. hypoxia, treatments vs. hypoxia, and between treatments) (44).

RESULTS

Because there were not significant changes in heart rate, PVR, and SVR across the four hyperoxic measurements, only the first series of these measurements is shown in tables and figures. The (Ppa − Pla)/Q relationships were linear in all experimental situations with correlation coefficients higher than 0.98. In the preliminary study to define the optimal pulmonary vasodilating dose of sildenafil, there were proportional dose-dependent decreases in PVR and SVR; and EC50 values were, respectively, 0.021 and 0.022 mg\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \) (Fig. 1).

Effects of hypoxia. As shown in Table 1, hypoxia was associated with a decrease in arterial PO2 from 202 to 28 Torr compared with hyperoxia, whereas arterial pH and Pco2 remained stable. Systemic and pulmonary hemodynamics are summarized in Table 2. Hypoxia increased Ppa as well as heart rate and Q. Hypoxia increased the slope and extrapolated zero flow intercepts of (Ppa − Pla)/Q plots, so that the Ppa − Pla gradient at a standardized mean flow of 3 l\cdot\text{min}^{-1}\cdot\text{m}^{-2} \) was increased (Fig. 2). The partition of PVR was the same as in hyperoxia (Fig. 3). Hypoxia increased 0-Hz impedance but otherwise had no effect on the impedance spectrum (not shown), and Zc remained unchanged (Fig. 4). Pulmonary arterial and end-systolic RV elastances were not affected by hypoxia (Fig. 5) so that ventricular-arterial coupling, represented by Ees/Ea, remained unchanged.

Effects of inhaled NO. Inhaled NO during hypoxia decreased Ppa, without changing Q and systemic arterial pressure (Psa). The slopes and zero flow intercepts of (Ppa − Pla)/Q plots as well as interpolated pressures at 3 l\cdot\text{min}^{-1}\cdot\text{m}^{-2} \) decreased but did not return to control value. Blood gases, partition of PVR, impedance spectrum, and Ees/Ea were unchanged. See Tables 1 and 2 and Figs. 2–5.

Effects of SNP. Continuous infusion of SNP during hypoxia also decreased Ppa, but this reduction tended to be of a lesser magnitude than that observed with inhaled NO. The downward shift of (Ppa − Pla)/Q relationship was also less marked than with inhaled NO. Furthermore, there was a reduction in Psa with an increase in heart rate and without change in Q. Blood gases, partition of PVR, impedance spectrum, and Ees/Ea were not modified. See Tables 1 and 2 and Figs. 2–5.

Effects of sildenafil. The effects of continuous infusion of sildenafil during hypoxia were similar to those of SNP in terms of Ppa, Psa, and (Ppa − Pla)/Q relationships, except for unchanged heart rate. Compared with SNP, blood gases, Q, partition of PVR, impedance spectrum, and Ees/Ea remained unchanged. See Tables 1 and 2 and Figs. 2–5.
Table 1. Blood gases in dogs during hyperoxia (baseline) and hypoxia before and after treatment with nitric oxide, sodium nitroprusside, or sildenafil

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxia</th>
<th>Nitric Oxide</th>
<th>Sodium Nitroprusside</th>
<th>Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pH</td>
<td>7.37±0.01</td>
<td>7.41±0.01*</td>
<td>7.40±0.01</td>
<td>7.39±0.01</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>Arterial PCO₂, Torr</td>
<td>41±1</td>
<td>38±1*</td>
<td>37±1</td>
<td>39±1</td>
<td>40±2</td>
</tr>
<tr>
<td>Arterial PO₂, Torr</td>
<td>199±9</td>
<td>28±1*</td>
<td>27±1</td>
<td>26±2</td>
<td>28±2</td>
</tr>
<tr>
<td>Arterial O₂ saturation, %</td>
<td>99.0±1.0</td>
<td>50.1±3.0*</td>
<td>47.4±2.9</td>
<td>45.1±5.4</td>
<td>47.0±4.3</td>
</tr>
<tr>
<td>Mixed venous O₂ saturation, %</td>
<td>68.7±3.1</td>
<td>26.0±2.9*</td>
<td>23.4±2.5</td>
<td>24.5±4.1</td>
<td>21.1±2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). *P < 0.05 hypoxia vs. baseline. No significant differences between treatments or between treatments and hypoxia.

DISCUSSION

The present results show that inhaled NO inhibits HPV more effectively than sildenafil or SNP, that sildenafil shows no more selectivity for the pulmonary circulation than SNP, and that none of the three interventions has proximal effects on the pulmonary circulation as assessed by pulmonary arterial impedance and elastance, or any consequence on RV systolic function.

In the present experiments, sildenafil partially inhibited HPV, which is in keeping with previous reports of its potent pulmonary-vasodilating properties in various types of acute and chronic pulmonary hypertension (10, 12–14, 29, 35, 37, 45). The magnitude of HPV inhibition by inhaled NO has been previously reported variably, depending on experimental protocol and animal species. Inmurine isolated lungs, sildenafil reduced the pressure response to hypoxia by 65% (45). In rats, sildenafil reduced the hypoxia-induced increase in RV systolic pressure by 37% (27). In two randomized, double-blind, and placebo-controlled crossover trials conducted in healthy human volunteers, pretreatment with sildenafil almost abolished hypoxia-induced increase in Ppa (12, 45). The percentage of inhibition of HPV induced by sildenafil in the present study is in keeping with previous reports.

The magnitude of the inhibition of HPV by inhaled NO or intravenous SNP has also been reported variably. Our laboratory previously observed (19) that, in dogs, inhaled NO inhibited almost completely the increase in Ppa – Pla at several levels of flow, as in the present study. However, another study showed an only partial reversal of HPV in dogs, even at dose of inhaled NO increased to 140 ppm (31). The inhalation of 40 ppm of NO completely reversed HPV in healthy human volunteers (9).

SNP at the dose of 5 μg·kg⁻¹·min⁻¹ has been reported to decrease the hypoxia-induced increase in PVR by 70% in intact dogs (25). In a model of left lower lobe hypoxia in dogs, infusion of SNP prevented the hypoxia-induced decrease in regional plasma blood flow by 62% (36). In the lamb, the efficacy of SNP on HPV was demonstrated, except in newborns, suggesting age-related differences (11).

It is of interest that, in the present study, sildenafil decreased HPV to the same extent as SNP but less so than inhaled NO. The final common pathway of all three interventions is smooth muscle cell cGMP, which is increased as a consequence of guanylate cyclase stimulation by NO (inhaled or released from SNP breakdown) or decreased cGMP degradation due to PDE5 inhibition by sildenafil. Therefore, the increased pulmonary selectivity of inhaled NO can only be explained by increased local NO concentrations, whereas sildenafil and SNP administrations are associated with diffuse increase in systemic and pulmonary vascular smooth muscle cGMP content. Higher doses of inhaled NO up to 80 ppm have been shown to decrease SVR via transfer into the blood of NO equivalents (3). These results are in keeping with the notion that the NO–cGMP-PDE5 pathway is expressed not only in pulmonary vessels (39, 43) but also and with similar functional consequences in systemic vessels (40). It is of importance that in the present experiments all vascular pressure measurements were performed at controlled flow, allowing for correction for any confounding effects of flow-induced activation of endothelial control mechanisms of vascular tone, which could be different in pulmonary and systemic circulation because of different pressure and wall stress regimens.

Hypoxia in the present experiments shifted pulmonary vascular pressure-flow relationships to higher pressures, without

Table 2. Hemodynamic data in dogs during hyperoxia (baseline) and hypoxia before and after treatment with nitric oxide, sodium nitroprusside, or sildenafil

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxia</th>
<th>Nitric Oxide</th>
<th>Sodium Nitroprusside</th>
<th>Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>81±6</td>
<td>122±7*</td>
<td>123±10</td>
<td>180±13‡</td>
<td>153±14‡</td>
</tr>
<tr>
<td>Q, l·min⁻¹·m⁻²</td>
<td>2.5±0.2</td>
<td>3.1±0.2*</td>
<td>3.1±0.2</td>
<td>3.1±0.3</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Mean systolic arterial pressure, mmHg</td>
<td>113±7</td>
<td>115±4</td>
<td>117±4</td>
<td>98±5‡</td>
<td>101±4‡</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>10±1</td>
<td>10±1</td>
<td>9±1</td>
<td>9±1</td>
<td>10±1</td>
</tr>
<tr>
<td>Systemic vascular resistance, dyn·s·cm⁻²·m⁻²</td>
<td>3.33±2.479</td>
<td>2.710±324</td>
<td>2.675±277</td>
<td>2.248±254‡</td>
<td>2.220±252‡</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>16±2</td>
<td>26±2*</td>
<td>19±1‡</td>
<td>22±1</td>
<td>23±1</td>
</tr>
<tr>
<td>Pla, mmHg</td>
<td>12±1</td>
<td>11±1*</td>
<td>11±1</td>
<td>11±1</td>
<td>11±1</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyn·s·cm⁻²·m⁻²</td>
<td>124±16</td>
<td>387±49*</td>
<td>192±18†</td>
<td>265±36†</td>
<td>276±37†</td>
</tr>
<tr>
<td>Slope of (Ppa – Pla)/Q plots, mmHg·l⁻¹·min⁻¹·m⁻²</td>
<td>2.1±0.4</td>
<td>4.4±0.5*</td>
<td>2.3±0.3</td>
<td>3.4±0.4‡</td>
<td>3.3±0.4‡</td>
</tr>
<tr>
<td>Intercept at Q = 0 of (Ppa – Pla)/Q plots, mmHg</td>
<td>0.0±1.0</td>
<td>4.6±1.7*</td>
<td>2.4±1.4</td>
<td>4.2±1.7</td>
<td>4.1±1.3</td>
</tr>
<tr>
<td>Right ventricular-arterial coupling (Ees/Ea)</td>
<td>0.89±0.09</td>
<td>1.07±0.11</td>
<td>1.38±0.20</td>
<td>1.65±0.35</td>
<td>1.27±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). Q, cardiac output; Ppa, mean pulmonary arterial pressure; Pla, left atrial pressure; Ees, end-systolic elastance; Ea, arterial elastance. *P < 0.05 hypoxia vs. baseline; †P < 0.05 vs. hypoxia; ‡P < 0.05 vs. nitric oxide; §P < 0.05 vs. sodium nitroprusside.
Results are presented as means ± SE of cardiac output (Q˙) plots in hyperoxia (base), during hypoxia, and with iNO, SNP, and SIL. Results are presented as means ± SE (n = 8). *P < 0.05 vs. hyperoxia; †P < 0.05 vs. hypoxia; ‡P < 0.05 vs. nitric oxide.

Fig. 2. Pulmonary arterial minus left atrial pressure (Ppa − Pla) interpolated at 3 l·min⁻¹·m⁻² of cardiac output (Q˙) plots in hyperoxia (base), during hypoxia, and with 40 ppm inhaled nitric oxide (iNO), 5 μg·kg⁻¹·min⁻¹ sodium nitroprusside (SNP), and 0.1 mg·kg⁻¹·h⁻¹ sildenafil (SIL). Results are presented as means ± SE (n = 8).

In the present study, the hemodynamic effects of sildenafil differed from those of SNP only by a lesser degree of tachycardia. This has been explained by a lower activation of sympathetic nervous system. Sympathetic activation with SNP is well documented, because it is one of the most widely used drugs to assess baroreflex gain (16). However, sildenafil has also been reported to induce marked increase in sympathetic activation in normal volunteers, as measured by muscle sympathetic nerve activity and plasma catecholamines (26).

Sildenafil has been previously reported to improve arterial oxygenation in hypoxic human volunteers (12, 29). This intriguing observation has been tentatively explained by the preservation of hypoxic regulation of pulmonary perfusion (12), improved pulmonary diffusion because of decreased pulmonary capillary pressure (29), and some yet-undetermined effect on pulmonary alveolocapillary conductance (15). An alternative explanation could be a direct effect of increased mixed venous oxygenation because of increased oxygen transport by increased cardiac output in the absence of increased oxygen consumption (7). In the present experiments, none of the vasodilators had any effect on arterial blood gases, probably because of initially normal ventilation-perfusion relationships and maintained cardiac output. Sildenafil like SNP has previously been reported to increase intrapulmonary shunt and decrease arterial oxygenation (4, 17), which can be explained by an inhibition of HPV also demonstrated in the present study. Inhaled NO would be expected to improve arterial oxygenation in hypoxic human volunteers (12, 29). This intriguing observation has been tentatively explained by the preservation of hypoxic regulation of pulmonary perfusion (12), improved pulmonary diffusion because of decreased pulmonary capillary pressure (29), and some yet-undetermined effect on pulmonary alveolocapillary conductance (15).

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Fig. 4. Characteristic impedance (Zc) in hyperoxia (base), during hypoxia, and with iNO, SNP, and SIL. Results are presented as means ± SE (n = 8). (SNP) pulmonary selectivity using vascular pressure measurements at a controlled cardiac output. The results show that only inhaled NO demonstrated pulmonary-selective effects on the site of hypoxia-induced change in resistance.

Pulmonary selectivity of pulmonary vasodilating interventions is often assessed by the ratio of PVR to SVR (14, 42). Because systemic arterial pressures are five to six times higher than pulmonary arterial pressures, changes in cardiac output affect PVR and SVR calculations differently. In addition, the sensitivities of pulmonary and systemic circulations to baroreflex gain are quite different as well (22) so that the resulting resistance calculations in the presence of vasodilating interventions are differently affected by a baroreflex correction of pressure change. For this reason, we preferred to evaluate the pulmonary selectivity of sildenafil by comparison to interventions of known exclusive (inhaled NO) or completely absent

Fig. 3. Pressure drop across the arterial (open bars) and the venous (shaded bars) segments in hyperoxia (base), during hypoxia, and with 40 ppm inhaled nitric oxide, 5 μg·kg⁻¹·min⁻¹ SNP, and 0.1 mg·kg⁻¹·h⁻¹ sildenafil. Numbers inside the arterial bars are the percentages of the pressure drop across the arterial segment (arterial component of the pulmonary vascular resistance). Results are presented as means ± SE (n = 8). NS, no significant difference between all experimental conditions.

Fig. 5. Right ventricle end-systolic (Ees) and arterial (Ea) elastances in hyperoxia (base), during hypoxia, and with iNO, SNP, and SIL. Results are presented as means ± SE (n = 8).
cause of preferential distribution of pulmonary vasodilation to ventilated lung regions (33). Inhibition of whole lung HPV does not significantly deteriorate ventilation-perfusion relationships when cardiac output is kept constant (20).

An increase in cGMP may be associated with a decreased contractility. In the murine myocardium, cGMP has been shown to mediate the negative inotropic effects of stimulation of muscarinic receptors, by activation of a cGMP-dependent protein kinase I (41). In the same model, inhibition of PDE5 by sildenafil blunted the increase in cardiac contractility with isoproterenol (38). In rabbits, sildenafil decreased the first-time derivative of left ventricular pressure, an afterload-sensitive index of contractility (28). However, in vitro studies on human and dog cardiac muscle strips showed no effect of sildenafil on force of contraction (5, 6). In the present study, there was a (nonsignificant) tendency for Ees, the best possible load-independent measure of contractility in vivo (34), to increase with sildenafil, and even more so with SNP. This is likely explained by reflex sympathetic nervous system activation, which seemed also more important with SNP as assessed by the associated increase in heart rate. Neither sildenafil nor SNP altered the adequacy of RV systolic function adaptation to afterload, as assessed by the Ees/Ea ratio.

Zc is a ratio of inertance to compliance, and as such it is sensitive to pulmonary arterial compliance changes (24). The ratio of 0 Hz to Zc has also been shown to be sensitive to wave reflection. In the present study, both Zc and Ea remained unchanged in hypoxia with or without drugs, indicating unchanged pulmonary arterial compliance and wave reflection. Zc and Ea are important determinants of RV hydraulic load, or dynamic afterload, and are most sensitive to functional changes in the proximal part of the pulmonary arterial tree (24). Our results are compatible with exclusive action of hypoxia and drugs on peripheral smallest resistive arterioles and unchanged site of wave reflection.

In conclusion, sildenafil is a pulmonary vasodilator that acts specifically at peripheral resistive arterioles and has no effect on the proximal pulmonary arterial tree or on RV systolic function and has no particular pulmonary selectivity.

GRANTS

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