Continuous inhalation of carbon monoxide induces right ventricle ischemia and dysfunction in rats with hypoxic pulmonary hypertension

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Gautier M, Antier D, Bonnet P, Le Net J-L, Hanton G, Eder V. Continuous inhalation of carbon monoxide induces right ventricle ischemia and dysfunction in rats with hypoxic pulmonary hypertension. Am J Physiol Heart Circ Physiol 293: H1046–H1052, 2007. First published May 4, 2007; doi:10.1152/ajpheart.01040.2006.—We aimed to investigate the toxicity of carbon monoxide (CO) in rats with right ventricle (RV) remodeling induced by hypoxic pulmonary hypertension (PHT). A group of Wistar rats was exposed to 3-wk hypobaric hypoxia (H). A second group was exposed to 50 ppm CO for 1 wk (CO). A third group was exposed to chronic hypoxia including 50 ppm CO during the third week (H+CO). These groups were compared with controls. RV and left ventricle (LV) functions were assessed by echocardiography and transparietal catheterization. Ventricular perfusion was estimated with the fluorescent microsphere method. Results were confirmed by histology. PHT induced RV hypertrophy and function enhancement. In the H group, RV shortening fraction (RVSF; 71 ± 12% vs. 41 ± 2%) and RV end-systolic pressure (RVESP; 54 ± 6 vs. 19 ± 2 mmHg) were increased compared with controls. Moreover, myocardial perfusion was increased in the RV (36 ± 2% vs. 22 ± 2%) and decreased in the LV (64 ± 3% vs. 78 ± 2%). In the H+CO group, RVSF (45 ± 3% vs. 71 ± 12%) and RVESP (38 ± 3 vs. 54 ± 6 mmHg) were decreased compared with the H group. RV perfusion was decreased in the H+CO group compared with the H group (21 ± 5% vs. 36 ± 2%), and LV perfusion was increased (79 ± 5% vs. 64 ± 3%). PHT and RV hypertrophy were still present in the H+CO group, and fibres localized in the RV were detected. Similar lesions were observed in an additional group exposed simultaneously to hypoxia and 50 ppm CO over 3 wk. We demonstrated that rats with established PHT were more sensitive to CO, which dramatically alters the RV adaptive response to PHT, leading to ischemic lesions.

right ventricle remodeling; right ventricle perfusion

INTERACTION BETWEEN AIR POLLUTION and cardiac diseases has been clearly demonstrated recently (19). Carbon monoxide (CO) is a well-known air pollutant that is present in car and industrial gases as well as in cigarette smoke (13). CO exposure has been shown to support cardiovascular diseases (22). In animal studies, chronic exposures to CO at important doses lead to severe cardiac hypertrophy in both ventricles (16). This cardiotoxic effect of CO has been attributed to increased hematocrit and blood viscosity secondary to chronic exposure. Nevertheless, the cardiotoxicity of CO at low doses is not clearly established (28).

Chronic hypoxia occurs at high altitude and in chronic obstructive pulmonary disease. Alveolar oxygen deprivation induces an increase of pulmonary resistances that leads to pulmonary hypertension (PHT) if hypoxia is sustained (chronic hypoxia). The right ventricle (RV) responds to elevated pulmonary vascular resistances by increasing its contractility. This results in an adaptive response of the RV to chronic hypoxia with morphological changes including hypertrophy and myocardial perfusion enhancement. The increase of perfusion in the hypertrophied RV is thought to be due to expression of VEGF (15). Enhancement of RV perfusion is involved in the mechanism of hypoxic preconditioning, which protects the myocardium against ischemia-reperfusion damage.

On the other hand, people with established chronic respiratory diseases seem more sensitive to air pollution then others (4). Previous studies have demonstrated that cardiac hypertrophy due to CO exposure was increased during a simultaneous exposure to chronic hypoxia (11). In addition, CO inhibits angiogenesis by suppressing the hypoxic induction of VEGF (9). CO is also known to have vasorelaxant properties (27), and it could reduce pulmonary hypertension with a beneficial impact on the RV pressures (3). To our knowledge, little is known about the impact of continuous CO inhalation on the RV response to chronic hypoxia. We hypothesize that CO inhalation could impair the RV physiopathological response to chronic hypoxia and that it could increase the risk of ischemic lesions in remodeled RV.

The aim of our study was to assess the effects of 1-wk exposure to 50 ppm CO on RV function and perfusion of both control rats and rats with hypoxic PHT. Cardiac function was assessed by noninvasive conventional transthoracic echocardiography and intracardiac blood pressure measurements with transparietal catheterization. Myocardial perfusion was evaluated in each ventricle with intracardiac injection of microspheres containing fluorescent dye. Finally, histological examinations were made in order to detect ischemic lesions.

MATERIALS AND METHODS

Animals and housing conditions. All animal experiments were conducted in accordance with the ethical standards of the Ministère Français de l’Agriculture. The protocols were approved by the Comité Régional d’Ethique pour l’Expérimentation Animale (CREAA) (authorization no. 006121). Adult male Wistar rats were obtained from Charles River Laboratories (l’Arbresle, France). They were acclimatized over 1 wk in the animal facilities before exposures. Rats were housed in groups of three in kennels under standard conditions for animal studies (temperature 20–21°C; 12 h artificial lighting/day), were fed ad libitum, and had free access to filtered tap water. For this study, 7-wk-old rats were randomly assigned to five groups (where n represents the number of animals studied). The first group (H; n = 11) was exposed to chronic hypoxia, the second group...
Environmental exposure models. PHT was induced by housing rats in an hypoxic hypobaria chamber as described previously (1). Briefly, 7-wk-old rats were housed for 3 wk in a hypobaria chamber in which atmospheric pressure was kept at 380 mmHg (corresponding to ~5,500-m or ~18,045-ft altitude) with an air vacuum pump (VT4.4 type, Becker). Oxygen pressure was estimated at 75 mmHg.

For the CO group, 7-wk-old rats were housed for 2 wk in a control environment. During the third week, rats were housed in a chamber containing ~50 ppm CO. The CO-polluted environment was obtained as previously described and validated in our laboratory (3). CO exposure levels were controlled daily both inside and outside of the chamber with a permanent CO level controller (MX32, Oldham).

For the H+CO group, 7-wk-old rats were housed for 3 wk in the hypobaria hypoxic chamber as described for the chronic hypoxic model. During the third and last week of hypoxia for the H+CO group, animals were exposed to ~50 ppm of exogenous CO as described above for the CO group. For the H+CO 3 wk group, rats were exposed to CO from the beginning of hypoxia exposure and for 3 wk. Animals were removed from the chamber twice a week (for ~10 min) to change cages, food, and water.

Echocardiographic measurements. After exposure to the different environmental stresses (hypoxia, exogenous CO, hypoxia + CO), animals were removed from the chamber, weighed, and deeply anesthetized with a ketamine-xylazine solution (100 mg/kg and 7.5 mg/kg, respectively, ip). The animal chest was shaved, and contact gel was applied to improve ultrasound transmission. Echocardiography was performed with a Sequoia ultrasound system equipped with a 15-MHz transducer (Acuson, Mountain View, CA).

Both systolic and diastolic functions of the RV and the left ventricle (LV) were studied with echocardiography in bidimensional (BD), mode pulsed Doppler mode, and time-motion (TM) mode. RV systolic function was assessed in TM mode on a modified parasternal long-axis view to make the RV cavity as well as the RV free wall visible. RV diastolic function was measured as an index of hypertrophy. RV end-systolic- and end-diastolic surfaces were also measured in BD mode recorded on a four-chamber apical view to reveal the presence of cardiac dilation. RV systolic function was estimated by calculating shortening fraction (SF; %) from RV surfaces as ([end-diastolic surfaces] − [end-systolic surfaces])/(end-diastolic surfaces). Moreover, RV systolic function was assessed by measuring the pulmonary outflow in pulsed Doppler mode recorded on a parasternal short-axis view. The Doppler beam was aligned with the pulmonary artery trunk axis. In the Doppler window, the pulmonary outflow spectrum was adjusted to permit assessment of maximal velocities. Peak velocity and acceleration times were recorded in aortic outflow spectra.

LV diastolic function was assessed by measuring the LV filling mitral inflow in pulsed Doppler mode on an apical four-chamber view. On the Doppler window, early diastolic E wave and atrial contraction A wave were recorded. Peak velocities and E/A were measured.

Simultaneous electrocardiograms were recorded during echocardiographic exam, and time measurements were normalized to the R-R interval. All measurements represent the mean of three cardiac cycles. Doppler tracings were recorded at a speed of 200 mm/s.

Hemodynamic measurements. RV and LV transpatal pressures were recorded with intraventricular catheterization as previously described (11), with modifications. Briefly, after tracheotomy, rats were intubated and ventilated with normoxic air at 10 ml/kg volume-inflow and 48 cycles/min with a respirator (C. F. Palmer, London, UK). A surgical incision of ~20 mm was made along the abdomen, exposing the abdominal chamber. The diaphragm was incised to access the thoracic cavity. Resting intraventricular pressures were then recorded through a 20-cm polyethylene catheter (PE-50) filled with heparinized (5% heparin) physiological salt solution (in mM: 138.6 NaCl, 5.4 KCl, 1.8 CaCl2, 1.2 MgCl2, 0.33 NaH2PO4, 10 HEPES, and 11 D-glucose; pH adjusted to 7.4 with NaOH) and attached to a hypodermic needle (Butterfly-23, Venisystems, Abbott Ireland, Sligo, Ireland). The PE-50 was linked to a calibrated pressure sensor (Baxter Uniflow). Pressures were calibrated to zero at the midstest and were acquired with a digitizing card (PCI-MOI-16XE-10, National Instruments). Intraventricular pressures were visualized simultaneously on a recorder (Hewlett Packard 7834A, Palo Alto, CA) and a paper plotter (Gould, Ballainvilliers, France).

Myocardial perfusion assessment by fluorescent microsphere injection. Fluorescent microspheres (15 ± 0.2-μm diameter, Dye-Track Fluorescent Yellow part 130–0485, Triton Technology) were used for myocardial perfusion assessment as previously reported (8). The number of microspheres injected was calculated to secure delivery of ~400 microspheres to the whole heart (~1 g) according to $N_{min} = (400/mL)/(Q_{min}/Q_{r})$, where $N_{min}$ is the minimum number of microspheres, $m$ is the organ/tissue mass (in g), $Q_{r}$ is the organ/tissue flow (in ml·min⁻¹·g⁻¹), and $Q_{min}$ is the total flow (in ml/min). Coronary flow represents ~4% of total flow, as quantified with magnetic resonance imaging in rats (7). These calculations yielded a minimum requirement of 10⁷ microspheres. This number has previously been shown to provide 95% confidence that the flow measured is within

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Values are means ± SE. CO, carbon monoxide exposure; RV, right ventricle; LV, left ventricle; S, septum. *P < 0.05 vs. Control; †P < 0.05 vs. non-CO.
10% of the actual flow (2). We injected 0.2 ml of the suspension (~2 \times 10^5 microspheres) in the LV though the PE-50 over a period of 1 min. The LV pressure waveform was monitored continuously to verify proper intracavitary delivery of microspheres. Remnant microspheres were flushed into the ventricular cavity with heparinized physiological salt solution. The heart was immediately removed and placed in cold physiological salt solution for ventricular dissection. The RV and LV (including septum) were placed for 48 h in 5 ml of 4 N KOH containing 2% Tween 80 for tissue digestion. Microspheres were physically separated from digested tissues by negative pressure filtration. Filters containing microspheres (part 31079, DYE-TRAK, Triton Technology) were placed in 2 ml of solvent (2-ethoxyethyl acetate, Sigma-Aldrich). The peak fluorescent spectrum was read at \(~485\) nm for an excitation wavelength of 450 nm with a spectrofluorometer (F-4500, Hitachi). The relative perfusion for each ventricle was calculated as the fluorescence in the ventricle divided by the fluorescence in the whole heart (RV + LV).

**Blood sample analysis, necropsy, and histopathology.** A 1-ml sample of heparinized blood was removed by direct intracardiac puncture. Blood samples were analyzed with a spectrophotometer (Rapidlab 855, Bayer) to measure hematocrit (Hct) and carboxyhemoglobin (COHb) levels. After death of the animal, the heart was excised, dried, and then carefully removed. The RV and LV (including septum) were placed for 48 h in 5 ml of 5 ml of 5 ml of 5 ml of 5 ml of 5 ml of physiological salt solution. The heart was immediately removed and placed in cold physiological salt solution for ventricular dissection. The RV and LV (including septum) were placed for 48 h in 5 ml of 4 N KOH containing 2% Tween 80 for tissue digestion. Microspheres were physically separated from digested tissues by negative pressure filtration. Filters containing microspheres (part 31079, DYE-TRAK, Triton Technology) were placed in 2 ml of solvent (2-ethoxyethyl acetate, Sigma-Aldrich). The peak fluorescent spectrum was read at \(~485\) nm for an excitation wavelength of 450 nm with a spectrofluorometer (F-4500, Hitachi). The relative perfusion for each ventricle was calculated as the fluorescence in the ventricle divided by the fluorescence in the whole heart (RV + LV).

**Results**

**Effects of CO inhalation on hematologic parameters.** Blood samples were collected in order to assess the hematologic changes induced by chronic hypoxia and CO inhalation. In particular, Hct and COHb levels were measured (Table 1). Hct was increased in the H group compared with control (60 \pm 1% vs. 41 \pm 1%; \textit{P} < 0.05). Additional exposure to CO induced a significant increase of Hct in the CO-exposed group (46 \pm 1%; \textit{P} < 0.05) as well as the H+CO group (63 \pm 1%; \textit{P} < 0.05) compared with control rats. Indeed, the effects of chronic hypoxia and CO on Hct level were cumulative. The COHb level was 0.4 \pm 0.4% in the Control group and 0.5 \pm 0.4% in the H group. These values were consistent with physiological COHb levels due to endogenous production of CO.

CO exposure induced an increase of COHb in the CO group (4.1 \pm 0.8%; \textit{P} < 0.05) and the H+CO group (2.4 \pm 0.3%; \textit{P} < 0.05) compared with controls. Nevertheless, COHb level was lower in the H+CO group compared with the CO group.

**Effects of CO inhalation on PHT and RV function.** Pulmonary flow was recorded by Doppler echocardiography (Fig. 1). A mid-systolic notch was detected on the pulmonary artery spectrum in the hypoxic (H and H+CO) groups (not shown). Moreover, the pulmonary artery acceleration time was significantly reduced in both H and H+CO groups compared with the Control and CO groups (Fig. 1A). In addition, pulmonary peak velocity was increased in both H and H+CO groups.

**Fig. 1.** Effect of hypoxia and carbon monoxide (CO) on pulmonary flow recorded with Doppler echocardiography. A: pulmonary flow acceleration time is markedly decreased in both hypobaric hypoxia (H) and hypoxia + CO exposure (H+CO) groups compared with the Control and CO exposure (CO) groups. B: moreover, pulmonary peak velocity is increased in the H and H+CO groups compared with the Control and CO groups. \(*\text{P} < 0.05\).

**Fig. 2.** Right ventricular (RV) systolic function was assessed by echocardiography and transparietal catheterization. A: RV shortening fraction (RVSF) was increased in the H group compared with the Control and CO groups. In the H+CO group, RVSF was decreased compared with the H group. B: RV end-systolic blood pressure (RVESP) was increased in the H group compared with the Control and CO groups. However, CO exposure in rats with pulmonary hypertension (PHT) induced a decrease of RVESP compared with the H group. In the H+CO group, RVESP was still higher than in the Control and CO groups. \(*\text{P} < 0.05\).
compared with Control and CO groups (Fig. 1B). Nevertheless, exposure to 50 ppm CO had no additional effect on pulmonary artery recorded flow in the CO group compared with the Control group or in the H group compared with the H+CO group.

RV end-systolic pressure (RVESP) (Fig. 2B) was increased in the H group (54 ± 6 mmHg) compared with the Control group (19 ± 2 mmHg; \( P < 0.05 \)). As for RV shortening fraction (RVSF), RVESP was significantly decreased in the H+CO group (38 ± 3 mmHg; \( P < 0.05 \)) compared with the H group. Despite this, RVESP was still high compared with the Control group and the CO group.

RV function was attested by echocardiography. RVSF (Fig. 2A) was increased in the H group (71 ± 12%) compared with the Control group (41 ± 2%; \( P < 0.05 \)). However, RVSF remained unchanged in the CO group (39 ± 4%; \( P > 0.05 \)) compared with the Control group. In the H+CO group, RVSF was significantly decreased (45 ± 3%; \( P < 0.05 \)) compared with the H group. Moreover, RVSF measured in the H+CO group was similar to that in the Control and CO groups.

Interestingly, in 2 of 10 rats of the H+CO group, we observed localized necrosis of the RV wall, whereas none of the rats from the H group exhibited this necrosis (Fig. 3).

**Effect of CO inhalation on LV function.** Neither LV shortening fraction (LVSF) nor LV end-systolic pressure (LVESP) was altered after chronic hypoxia (Table 2). In contrast, LVSF was decreased in the CO group (41 ± 3%) compared with the Control group (48 ± 3%; \( P < 0.05 \)). Nonetheless, LVESPs were not different in all groups. Mitral E-to-A wave ratio was significantly decreased in the CO group (1.5 ± 0.5) compared with the Control group (2.2 ± 0.4; \( P < 0.05 \)). This was due to a decrease of the LV relaxing E wave peak velocity in the CO group (0.66 ± 0.03 m/s) compared with the Control group (0.79 ± 0.03 m/s; \( P < 0.05 \)). Together, these results showed that CO inhalation had induced a LV filling impairment in the CO group. In the H+CO group both mitral E-to-A wave ratio (3.0 ± 0.4) and E wave peak velocity (0.81 ± 0.02 m/s) were increased compared with the CO group (\( P < 0.05 \)) and were similar to control values.

**Effect of CO inhalation on myocardial perfusion.** The RV relative perfusion is shown in Fig. 4A, and the LV relative perfusion is shown in Fig. 4B. In the Control group, the RV relative perfusion was 22 ± 2% and the LV relative perfusion was 78 ± 2%. After chronic hypoxia, the RV relative perfusion was increased (36 ± 3%; \( P < 0.05 \)) and the LV relative perfusion was decreased (64 ± 3%; \( P < 0.05 \)). The myocardial perfusion profile was not altered in the CO group compared with the Control group. Nevertheless, in the H+CO group, the myocardial perfusion profile was altered compared with the H group. Indeed, the relative RV perfusion was significantly decreased (21 ± 5%; \( P < 0.05 \)) and the relative LV perfusion was significantly increased (79 ± 5%; \( P < 0.05 \)) compared with the H group.

**Morphological and histological study.** No modifications were observed in LV in the four groups. RV hypertrophy was...
observed in both H and H+CO groups as attested by RV/(LV+S) increase (Table 1). Interestingly, in five of eight cases, we found lesions of localized fibrosis predominantly localized at the level of tricuspid papillary muscle insertion in the H+CO group (Fig. 5). These abnormalities were considered severe in two cases, with an aspect of extended cell loss mimicking RV infarction. These abnormalities were observed macroscopically and correspond to echocardiography observations. In contrast, in the H group only RV hypertrophy was observed without fibrosis lesions. Moreover, no modification of the histological appearance of the RV was observed in the CO group compared with controls. We then exposed a last group of rats to CO over 3 wk concurrently with hypoxia in order to examine a toxic effect of CO due to hypoxia association. Within the H+CO 3 wk group, RV pressures were slightly decreased compared with the 1-wk CO group (RVESP = 26.6 ± 3 vs. 38 ± 3 mmHg; P < 0.05) and COHb levels were slightly increased (COHb = 4.2 ± 2% vs. 2.4 ± 0.3%; P < 0.05). We observed in one of six cases an extended necrosis area and in two of six cases a mild fibrosis infiltration localized essentially around the coronary arteries (Fig. 5).

Fig. 4. Myocardial perfusion was estimated by the fluorescent microsphere method. Relative perfusion was calculated as the fluorescence in the ventricle divided by the fluorescence in the whole heart (RV + left ventricle (LV)). A: RV relative perfusion was increased in the H group compared with the Control group. There was no difference between the CO group and the Control group. However, RV relative perfusion was decreased in the H+CO group compared with the H group and remained similar to the Control and CO groups. B: relative LV perfusion was decreased in the H group compared with the Control group. There was no difference between the CO group and the Control group. However, LV relative perfusion was increased in the H+CO group compared with the H group and remained similar to the Control and CO groups. *P < 0.05.

Fig. 5. Histology confirmation of RV necrosis occurring in a hypoxic rat after CO exposure. A: normal aspect of RV wall in optical microscopy. B: macroscopic examination of RV showing necrosis area (arrow). C: RV fibrosis infiltration was observed when hypoxic rats were concurrently exposed to low doses of CO (trichrome coloration: turquoise blue = fibrosis). D: RV necrosis (arrow) in optical microscopy in H+CO group (trichrome coloration).
In the present study, we showed that a 1-wk exposure to 50 ppm exogenous CO had deleterious effect on RV myocardial perfusion adaptation to chronic hypoxia and pressure overload.

**Relevance of CO exposure.** CO is an endogenously generated gas as well as a ubiquitous environmental pollutant. In this study, CO exposure was 50 ppm for 1 wk, which is physiologically relevant for external CO exposure (25). For example, a cigarette smoker can be exposed to 500 ppm CO (17). The dose effects seem essential for the occurrence of pathological or potential beneficial effects, and it also seems crucial to study the effects of identical concentrations of CO in inhaled air in both hypoxic and control groups. This induced different COHb levels in the two groups because hypoxia was obtained with hypobaric conditions. However, even if the group with hypoxic CO had a lower level (2.4 ± 0.3%) of COHb than the normoxic group (4.1 ± 0.8%), it exhibited more severe pathological lesions.

**Model of experimental PHT.** In the present study, PHT was induced by 3 wk of hypobaric hypoxia exposure, a model that has been validated previously (1). As described, chronic hypoxia induced an elevation of pulmonary vascular resistance leading to PHT and RV hypertrophy (18). Enhancement of RV perfusion during hypoxic PHT has already been reported and is attributed to an increased number of capillaries per myocyte due to sustained VEGF expression in the RV (15). This beneficial adaptation of perfusion appeared despite a critical elevation in Hct (21).

**Effect of CO exposure on normoxic rats.** Exogenous CO exposure has been reported to induce cardiac remodeling and hypertrophy in both ventricles after a 1-wk exposure to 100 ppm, which corresponded to 12% COHb (10). In the present study, average COHb was 4.1% in normoxic rats and Hct was slightly increased. Four percent COHb has been shown to correspond to daily inhalation of ~6–14 cigarettes (5) and is higher than the guideline value for CO exposure of 2.5% from the World Health Organization (28). Increases of Hct following exposure to exogenous CO have been reported previously (11). This modification did not modify RV and LV perfusion, and no histological lesions were observed in the RV. Modifications in mitral flow profile could evoke diastolic function alteration and could induce a fall in the LV filling pressure due to CO-induced vasodilation.

**Effect of CO exposure on hypoxic rats.** In H+CO rats, COHb was 2.4%, which appears lower but was not statistically different compared with rats in the CO group. In hypoxic rats, 1-wk exposure to 50 ppm CO induced an increase of Hct compared with other groups, indicating that the effects of chronic hypoxia and chronic exposure to CO are additive. In the present study, low doses of CO did not modify cardiac masses in hypoxic rats. However, an increase of LVSF was observed in H+CO rats by TM-mode echocardiography as previously shown by Melin et al. (11). This effect was in accordance with the absence of histological lesions in the LV. Surprisingly, RVESSPs were significantly decreased despite the short period of exposure to CO. CO is a biologically active molecule with potent vasorelaxant properties. As such, CO inhalation has been proposed as a therapeutic agent against hypertensive pathologies such as PHT (3) and systemic hypertension (14). CO was shown to reduce mean pulmonary arterial pressure increase as well as RV hypertrophy when it was applied during exposure to hypoxia (3). In the present study, CO was applied during the third week of hypoxia, when PHT was established. We demonstrated that a short exposure to 50 ppm CO was also able to reduce RV pressure overload. This beneficial effect on PHT was counterbalanced by impairment of RV perfusion with a redistribution of perfusion toward the LV.

**Possible mechanisms of CO-induced cardiotoxicity.** Adaptation to chronic hypoxia resulted in RV hypertrophy associated with enhanced RV perfusion. Chronic hypoxia triggered the induction of genes encoding for VEGF and nitric oxide synthase (NOS), leading to angiogenesis and vasodilation to maintain an adequate myocardial perfusion. This occurs under the activation of hypoxia-inducible factor 1. Both of these mechanisms contributed to maintain cardiac function despite the increased workload. The main finding of this study was that 50 ppm CO was able to alter the adaptive response of the RV to hypoxia and induced ischemic lesions. The same lesions were also observed when CO was applied during 3-week hypoxia. As mentioned above, CO has been extensively studied as a gasotransmitter leading to vasodilation. CO is also known to have antiapoptotic properties and to promote angiogenesis (26). However, this controversial molecule is a potent environmental pollutant and poison. Importantly, environmentally relevant concentrations of CO induce apoptotic cell death in endothelial cells (23). This apoptotic effect of CO was due to increase of nitric oxide (NO), oxidant stress, and possibly peroxynitrite in the cell (23, 24). The interaction between CO and NO could be a key mechanism of the CO-induced cardiotoxicity in the remodeled RV. For example, previous study has shown that exogenous CO suppresses endothelial NOS (24), which is involved in hypoxia-induced angiogenesis (12). One mechanism could be that the CO altered the NO metabolism in coronary arteries. This could inhibit angiogenesis and induce a “maladaptive” response to hypoxia including decrease of the RV perfusion responsible for ischemic injuries.

**Conclusions.** In the present study, we determined that a low dose of CO did not have a deleterious effect on the heart under normoxic conditions despite elevation of Hct. In contrast, a short exposure to a low dose of CO mimicking effects due to atmospheric pollution under hypoxic conditions can induce a deleterious effect on RV perfusion despite a beneficial effect on PHT.

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