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To cite this version:

G. Meyer, L. André, T. Sandré, J. Boissiere, C. Farah, et al.. Simulated urban carbon monoxide air pollution exacerbates rat heart ischemia-reperfusion injury. AJP - Heart and Circulatory Physiology, 2010, 298 (5), pp.H1445 - H1453. 10.1152/ajpheart.01194.2009 . hal-01824345

HAL Id: hal-01824345

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Submitted on 13 Apr 2020

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Simulated urban carbon monoxide air pollution exacerbates rat heart ischemia-reperfusion injury

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Meyer G, André L, Tanguy S, Boissiere J, Farah C, Lopez-Lauri F, Gayrard S, Richard S, Boucher F, Cazorla O, Obert P, Reboul C. Simulated urban carbon monoxide air pollution exacerbates rat heart ischemia-reperfusion injury.

—Myocardial damages due to ischemia-reperfusion (I/R) are recognized to be the result of a complex interplay between genetic and environmental factors. Epidemiological studies suggested that, among environmental factors, carbon monoxide (CO) urban pollution can be linked to cardiac diseases and mortality. The aim of this work was to evaluate the impact of exposure to CO pollution on cardiac sensitivity to I/R. Regional myocardial I/R was performed on isolated perfused hearts from rats exposed for 4 wk to air enriched with CO (30–100 ppm). Functional variables, reperfusion ventricular arrhythmias (VA) and cellular damages (infarct size, lactate dehydrogenase release) were assessed. Sarcomere length shortening and Ca^{2+} handling were evaluated in intact isolated cardiomyocytes during a cellular anoxia-reoxygenation protocol. The major results show that prolonged CO exposure worsens myocardial I/R injuries, resulting in increased severity of postischemic VA, impaired recovery of myocardial function, and increased infarct size (60 ± 5 vs. 33 ± 2% of ischemic zone). The aggravating effects of CO exposure on I/R could be explained by a reduced myocardial enzymatic antioxidant status (superoxide dismutase −45%; glutathione peroxidase −49%) associated with impaired intracellular Ca^{2+} handling. In conclusion, our results are consistent with the idea that chronic CO pollution dramatically increases the severity of myocardial I/R injuries.

environmental pollution; myocardial infarction; antioxidant status

MYOCARDIAL DAMAGES RESULTING from ischemia-reperfusion (I/R) are a major cause of morbidity and mortality in western nations. Those myocardial I/R injuries result in cardiac dysfunction, arrhythmias, as well as irreversible myocyte damages (25, 28). The sensitivity of the myocardium to I/R-induced cellular injuries is recognized today to be the result of a complex interplay between genetic, pathological, and environmental factors. Moreover, it appears to be aggravated in several diseases, including hypertension, metabolic disorders, and complications from cigarette smoking and environmental pollution.

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Among the environmental factors that could influence the development of cardiovascular diseases, several epidemiological studies have recently suggested that urban atmospheric pollution may exert adverse effects on cardiovascular health (7, 11, 12, 15). Among the numerous pollutants, carbon monoxide (CO) has been described as one of the main pollutants responsible for the development of cardiovascular diseases (9, 35). In urban environments, CO concentration usually varies from 2 to 40 ppm, but during heavy traffic it may be as high as 170 ppm (10, 34, 40). At this level, CO exposure has been correlated with hospital admissions, mortality, and morbidity related to cardiovascular diseases (9, 13, 29). Today, although the pathophysiological mechanisms regarding acute CO poisoning are well known, mechanisms associated with chronic exposure to lower concentrations of CO, consistent with urban environmental pollution, remain unclear. We (2) and Bye et al. (14) have recently reported that prolonged CO exposure induces a pathological myocardial cellular phenotype characterized by a major remodeling of the excitation-contraction coupling. Such deleterious consequences may worsen the effect of cardiovascular diseases.

The aim of this experimental work was to challenge the impact of a chronic exposure to simulated CO urban pollution on the sensitivity of the myocardium to I/R in a rat model. The major result shows that prolonged exposure to CO at a level found in the urban environment has a dramatic deleterious impact on the sensitivity of the myocardium to I/R.

METHODS

Experiments complied with the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health (National Institutes of Health Publications no. 85-23, revised 1996) and was approved by the French Ministry of Agriculture.

Animals and CO Exposure

Adult, male Wistar rats (n = 64; 345 ± 7 g; Charles River Laboratories) were randomly assigned to the following two groups: CO rats (exposed for 4 wk to simulated CO urban pollution, n = 32) and control animals (Ctrl rats, exposed to standard filtered air, n = 32). CO rats were housed in an airtight exposure container for 4 wk. Exposure was performed according to a 12:12-h CO in the air-ambient air cycle. To simulate CO urban pollution, exposure was performed as follows: during CO exposure, a CO concentration of 30 ppm was maintained in the airtight container and monitored with an aspirative CO analyzer (CHEMGARD Infrared Gas Monitor NEMA 4 Version;

MSA). This initial concentration was supplemented with five 1-h peaks at 100 ppm CO. During ambient air exposure, animals were placed in the laboratory animal house with a CO concentration of 0 ppm. Ctrl rats were confined in the laboratory animal house for 4 wk and were manipulated daily. At the end of the 4-wk CO exposure, rats were housed 24 h in standard filtered air to avoid the acute effects of CO on the myocardium.

Regional Myocardial Ischemia and Reperfusion on Isolated Perfused Rat Heart

Rats were anaesthetized with pentobarbital sodium (50 mg/kg ip). A thoracotomy was performed, and the heart was rapidly removed, by cutting the great vessels, and immersed in ice-cold Krebs solution. The heart was transferred to the perfusion apparatus, and the aorta was cannulated for perfusion with oxygenated (95% O_2-5% CO_2) Krebs solution (37°C) composed of (in mM) 118.3 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, and 2.5 CaCl (pH = 7.4). The hearts were perfused at a constant pressure (80 mmHg).

Evaluation of postischemic reperfusion injury. On a first set of rats (n = 8/group), the atrioventricular node was crushed using fine forceps, the right atrium was excised, and the hearts were paced at a rate of 300 beats/min with an electrical stimulator (low voltage stimulator, BSL MP35 SS58L, 3V). An ultrathin, water-filled balloon was inserted in the left ventricle via the mitral valve, and the balloon volume was adjusted to achieve a left ventricular (LV) end diastolic pressure of 5 mmHg. Coronary blood flow was measured by collection of the infiltration effluents. A suture on a round-bodied needle was placed around the left anterior descending coronary artery (LAD), and the suture ends were placed around a small length of tubing to form a snare. The heart was allowed to stabilize for 30 min. Following the stabilization period, the LAD was occluded for 30 min. Subsequently, the snare occluder was released to allow reperfusion of the previously ischemic vascular bed for 120 min. The LAD was then reoccluded, and Evans blue dye solution (5 ml, 2%) was injected in the left ventricle to allow perfused (stained blue) and nonperfused (unstained) areas of the heart to be distinguished. After removal of the hearts, it was divided into five slices perpendicular to the apex-base axis. Triphenyltetrazolium chloride staining (0.5 mg/ml for 20 min at 37°C) was used to assess myocardial tissue viability and to determine myocardial infarct size. Tissue slices were photographed, and area at risk and infarcted area were then determined using a computer-based system (ImageJ; NIH).

Evaluation of postischemic reperfusion-induced ventricular arrhythmias. On a second set of rats (n = 14/group), arrhythmic events were evaluated on isolated nonpaced hearts. The heart was mounted, and the LAD occlusion was performed as previously described. A computerized electrocardiogram was obtained continuously during the protocol. The various types of arrhythmias were defined as described in the Lambeth conventions (39). The analysis of the electrocardiogram enabled assessment of the incidence (percent no. of hearts exhibiting a given type of arrhythmia) of different types of ventricular arrhythmias (Fig. 1*A*) as follows: sinus rhythm, ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). To allow the comparison of the severity of rhythm disturbances occurring upon reperfusion, each individual heart was characterized according to a five-point arrhythmic score previously described by Tanguy et al. (37) and designed so that the more severe the arrhythmia, the larger the number (Fig. 1*B*). Such a simplified scoring system allows the assignment of a single number to each heart, and the comparison of the distribution of the hearts by various scores.

Cardiomyocyte Excitation-Contraction Analysis After Cellular Anoxia-Reoxygenation

On a third set of rats (n = 4/group), single ventricular cardiomyocytes were isolated by enzymatic digestion as previously described (24). Cardiomyocytes were transferred to a glass petri dish and placed in an anoxic chamber (O_2 level ~2%) for 60 min, followed by a 60-min reoxygenation in ambient air (O_2 ~19.4%). Unloaded cell shortening and Ca^{2+} concentration (indo 1 dye) were measured using field stimulation (0.5 Hz, 22°C, 1.8 mM external Ca^{2+}) before and after anoxia-reoxygenation (A/R). Sarcomere length (SL) and fluorescence (405 and 480 nm) were recorded simultaneously (IonOptix system; Hilton). The A/R experiment was then carried out in the presence or absence of a nonspecific antioxidant [*N*-acetylcystein (NAC), 20 μM].

Biochemical Assays

Heart antioxidant enzyme activity. A fourth set of rats (n = 6/group) was used to evaluate the enzymatic antioxidant status of the myocardium consecutive to chronic CO exposure and before I/R. After the end of CO exposure (24 h), the hearts were freeze-clamped, and the frozen ventricular tissue was homogenized in Tris·HCl buffer (60 mM Tris·HCl and 1 mM diethyltriaminopentaacetic acid, pH 7.4, 10 ml/g wet wt) using a Teflon potter homogenizer. Tissue homogenates were then centrifuged for 10 min at 200 *g* at 4°C to remove all nuclear debris. Cardiac superoxide dismutase (SOD) activity was assessed in the supernatant according to the method described by Marklund (22). Cardiac glutathione peroxidase (GPx) activity was assessed spectrophotometrically on the cytosolic fraction according to the method described by Flohe and Günzler (18). Catalase activity was determined according to the method described by Beers and Sizer (8). All enzyme activities were expressed in units per milligram protein (U/mg protein). The modified method of Lowry et al. (21) was used to determine protein content of tissue homogenates, using BSA as standard.

Lactate dehydrogenase in coronary effluents. Lactate dehydrogenase (LDH) activity was measured in coronary effluents by spectrophotometry using an LDH kit (LDH-P; BIOLABO). Measurements were made at the end of stabilization and at 10, 30, and 60 min of reperfusion. LDH activity was normalized to coronary blood flow.

Thiobarbituric acid-reactive substances in LV tissues. Thiobarbituric acid-reactive substances (TBARS) were assessed in LV tissues after 30 min of reperfusion using the thiobarbituric acid (TBA) test. Frozen heart tissue (120 mg) was homogenized in 1 ml 0.1% TCA solution. The homogenate was centrifuged at 12,000 *g* for 15 min, and 0.5 ml of the supernatant was added to 1 ml of 0.5% TBA in 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction was stopped by placing the reaction tubes in an ice bath. Tubes were briefly vortexed, triplicate 200-μl aliquots were taken from each tube and placed in 96-well plates, and the supernatant absorbance was read at 532 nm in a microplate reader. The value for nonspecific absorption at 600 nm was subtracted. The amount of TBARS (red pigment) was calculated using an extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$.

Statistics

Data were analyzed using one-way ANOVA between groups and repeated-measures ANOVA when necessary. When significant interactions were found, a Tukey-Kramer test was applied. The distribution of the hearts based on the various arrhythmic scores was analyzed by a nonparametric Mann-Whitney *U*-test. Binomially distributed variables (such as incidence of VF) were analyzed using nonparametric Yates' chi square test (Statview; Adept Scientific, Letchworth, UK). A level of $P < 0.05$ was considered statistically significant. Data are expressed as group means or group mean fractions of baseline ± SE.

RESULTS

Effects of CO Exposure on Myocardial Reperfusion Ventricular Arrhythmias

The time course of reperfusion-induced VPB, VT, and VF in individual hearts is shown in Fig. 1*C*. Figure 1*D* shows a

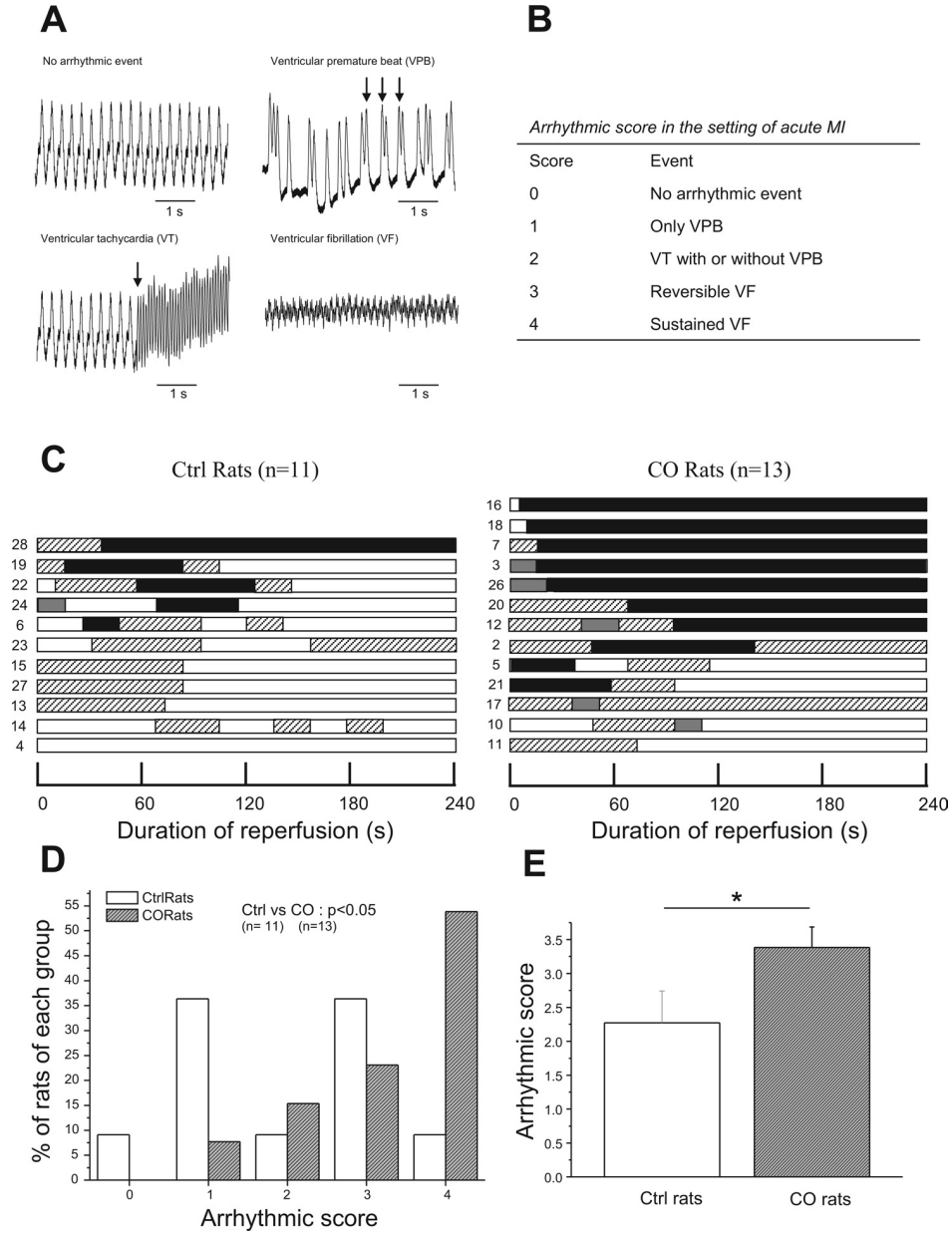

Fig. 1. Effects of carbon monoxide (CO) exposure on the severity of postischemic reperfusion-induced arrhythmic events. Electrocardiographic recordings (*A*) of normal sinus rhythm and of arrhythmias identified during postischemic reperfusion as described in the Lambeth Convention [sinus rhythm, ventricular premature beat (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF)] and related arrhythmic scores (*B*). *C*: time course of reperfusion-induced VPB (crossed out bars), VT (gray bars), and VF (black bars) of each heart of the control (Ctrl) group (*left*) and CO (*right*) group (nos. on *left* refer to the order of the experiments). *D*: categorization of arrhythmia classes (score) according to *B*. Values are expressed as a percentage of the total no. of hearts for each group (Ctrl rats $n = 11$, CO rats $n = 13$, distribution was tested using nonparametric Mann-Whitney's test, Ctrl vs. CO rats: $P < 0.05$). MI, myocardial infarction. *E*: effects of CO exposure on mean arrhythmic score (data are presented as means ± SE, Ctrl rats $n = 11$, CO rats $n = 13$, one-way ANOVA, $*P < 0.05$, Ctrl vs. CO rats).

significant difference in the distribution of the arrhythmic scores observed in both experimental groups. According to the increased mean arrhythmic score observed in CO rats during reperfusion, the arrhythmia severity was higher in CO rats compared with Ctrl rats (Fig. 1*E*). Moreover, sustained VF were triggered more frequently in the CO rats compared with Ctrl rats (54 vs. 9%, $P < 0.05$ nonparametric Yates' chi square test).

Effects of CO Exposure on Postischemic Recovery of Myocardial Function

Any difference regarding cardiac function was reported between CO and Ctrl rats before ischemia (Table 1). Postischemic recovery of LV developed pressure and contractility, assessed during reperfusion, was significantly lower in CO rats compared with Ctrl (Fig. 2). Indeed, the postischemic recovery of LV developed pressure, $+dP/dt_{max}$, and $-dP/dt_{max}$ were significantly altered in the CO rats compared with Ctrl rats (Fig. 2, *A–C*).

These functional results were paired with some deleterious effects of CO exposure on postischemic myocardial coronary blood flow recovery (Fig. 2*D*). Indeed, although no difference in coronary blood flow was reported between CO and Ctrl rats before ischemia (Ctrl rats: 11.5 ± 0.9 ml/min vs. CO rats: 11.8 ± 1.4 ml/min), coronary blood flow was significantly lower in CO rats compared with Ctrl rats during reperfusion.

Effects of CO Exposure on Myocardial Postischemic Reperfusion-Induced Cellular Death

The deleterious effects of CO exposure on I/R-induced injury were characterized by an increase in myocardial infarct

Table 1. *Myocardial function during regional ischemia-reperfusion protocol in Ctrl and CO rats*

	LVDP, mmHg	$+dP/dt_{max}$, mmHg/s	$-dP/dt_{max}$, mmHg/s
Baseline			
Ctrl rats	91.79 ± 7.47	3,365 ± 304	−1,944 ± 123
CO rats	93.85 ± 12.11	2,932 ± 369	−1,994 ± 219
30 min of Ischemia			
Ctrl rats	65.50 ± 4.82	2,304 ± 487	−1,447 ± 199
CO rats	53.29 ± 5.66	1,757 ± 240	−1,111 ± 162
5 min of Reperfusion			
Ctrl rats	61.28 ± 3.05	2,169 ± 318	−1,255 ± 103
CO rats	41.86 ± 3.61*	1,372 ± 276*	−902 ± 131*
30 min of Reperfusion			
Ctrl rats	63.31 ± 5.15	2,376 ± 331	−1,344 ± 114
CO rats	43.71 ± 5.66*	1,541 ± 364*	−887 ± 216*
60 min of Reperfusion			
Ctrl rats	50.99 ± 1.85	1,996 ± 320	−1,063 ± 81
CO rats	36.10 ± 6.62*	1,332 ± 365	−761 ± 115*
90 min of Reperfusion			
Ctrl rats	43.54 ± 1.33	1,747 ± 376	−920 ± 120
CO rats	31.78 ± 9.76	1,203 ± 331	−703 ± 121
120 min of Reperfusion			
Ctrl rats	39.85 ± 2.52	1,609 ± 258	−832 ± 110
CO rats	24.52 ± 5.91*	1,028 ± 237*	−570 ± 169*

Values are means ± SE; LVDP, left ventricular developed pressure; $+dP/dt_{max}$, maximal derivative of left ventricular pressure; $-dP/dt_{min}$, minimal derivative of left ventricular pressure; Ctrl rats, rats exposed to filtered standard air; CO rats, rats exposed to carbon monoxide. $*P < 0.05$ vs. Ctrl rats.

size in CO rats compared with Ctrl rats (Fig. 3, *A* and *B*). Indeed, no difference regarding the risk zone (ischemic zone) was observed between CO and Ctrl rats after 120 min of reperfusion. However, the infarct size was markedly increased in CO rats (60 ± 5 vs. 33 ± 2% of the risk zone; $P < 0.05$). LDH released in coronary effluents, used as an index of cell death, was significantly augmented at the onset of reperfusion in both Ctrl and CO rats (1.91 ± 0.30 to 5.84 ± 1.45 U/min for Ctrl rats; 2.69 ± 0.54 to 14.26 ± 3.20 U/min for CO rats; $P < 0.05$). Moreover, the peak of LDH release at the onset of reperfusion was significantly higher in CO rats than in Ctrl rats. Finally, LDH release remained significantly higher in CO rats after 30 and 60 min of reperfusion. No difference regarding LDH release was observed during the stabilization period (Fig. 3*C*). LV TBARS concentration was significantly increased in CO compared with Ctrl rats after 30 min of reperfusion (6.43 ± 0.76 vs. 4.40 ± 0.35 nmol/g; Fig. 3*D*).

Effects of CO Exposure on Cardiomyocyte Excitation-Contraction Coupling Before and After A/R

At the cellular level, prolonged CO exposure induced impairments of ventricular myocyte function (Fig. 4). Indeed, before A/R, SL shortening (Fig. 4, *A* and *B*) as well as Ca^{2+} transient (Fig. 4, *C* and *D*) were significantly impaired in CO rats compared with Ctrl rats. In addition, diastolic cytosolic Ca^{2+} was markedly higher in CO compared with Ctrl rats (Fig. 4*E*). Consecutively to A/R, SL shortening was decreased in both groups (Fig. 4, *A* and *B*) and remained significantly lower in CO compared with Ctrl rats. This decreased SL shortening was associated with a decreased Ca^{2+} transient in both groups consecutive to A/R. It is of note that Ca^{2+} transient from CO rats remained lower than Ca^{2+} transient from Ctrl rats (Fig. 4, *C* and *D*). Following A/R, diastolic Ca^{2+} increased in the two experimental groups; however, for this parameter, statistical significance was reported only in Ctrl rats. Consequently,

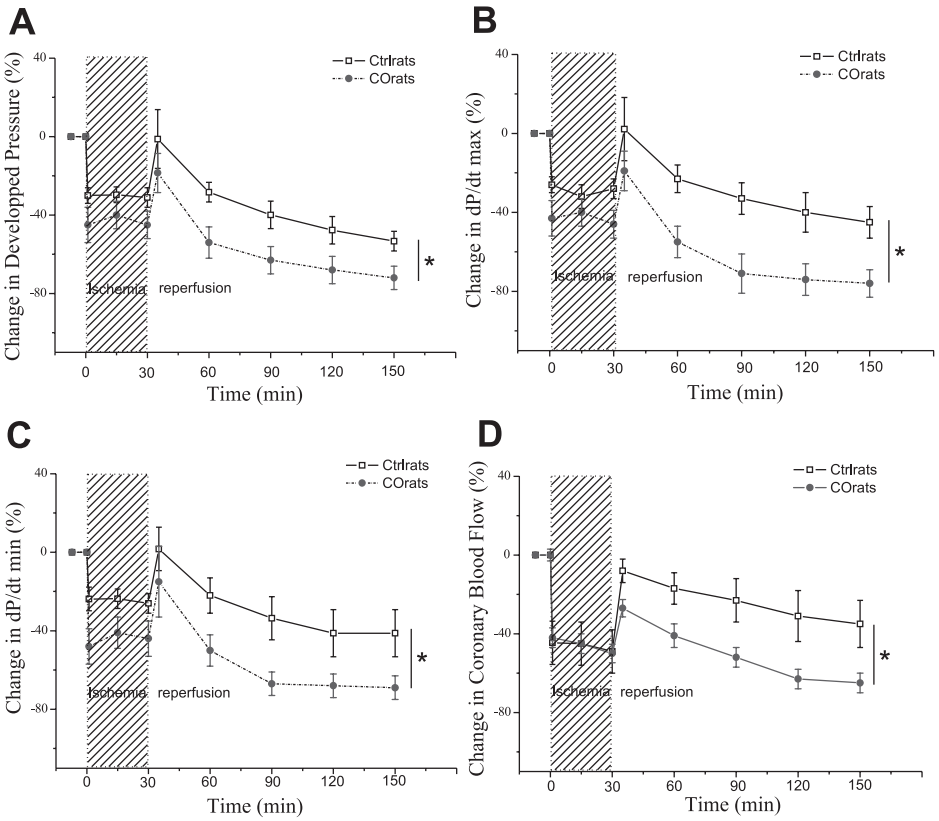

Fig. 2. Effects of CO exposure on the time course of changes in left ventricular function parameters during a 30-min ischemia and 120 min of reperfusion. Change in left ventricle developed pressure (*A*), maximal ($+dP/dt_{max}$, *B*), and minimal ($-dP/dt_{max}$, *C*) derivative of left ventricular pressure over time, and coronary blood flow (*D*). Data are presented as mean fraction of baseline ± SE ($n = 6$ rats/group, repeated-measures ANOVA, $*P < 0.05$, Ctrl vs. CO rats).

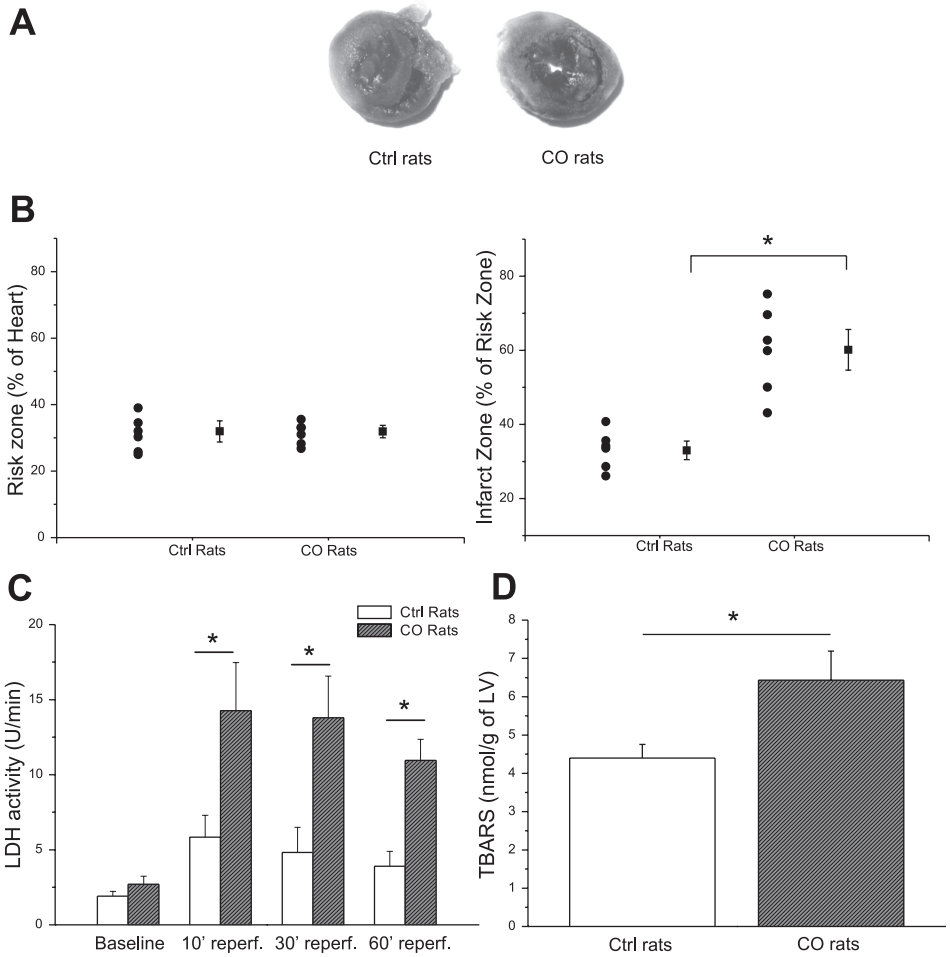

Fig. 3. Effects of CO exposure on I/R-induced cellular death. *A*: representative sections of rat hearts stained with triphenyltetrazolium chloride (TTC) after a regional ischemia of 30 and 120 min reperfusion from the isolated heart experiments in Ctrl and CO rats. *B*: risk zone expressed as a percentage of heart area (*left*) and infarct zone expressed as a percentage of risk zone (*right*). Data are presented as means ± SE (n = 6/group, one-way ANOVA, $*P < 0.05$, Ctrl vs. CO rats). *C*: lactate dehydrogenase (LDH) release in coronary effluents during stabilization and reperfusion, and used as a marker of cell death. *D*: thiobarbituric acid-reactive substances (TBARS) concentration in left ventricular (LV) tissues after 30 min of postischemic reperfusion, and used as a marker of lipid peroxidation. Data are presented as means ± SE (n = 6 rats/group, repeated-measures ANOVA, $*P < 0.05$, Ctrl vs. CO rats).

following A/R, no difference was observed concerning intracellular diastolic Ca^{2+} between Ctrl and CO rats (Fig. 4*E*).

Following A/R, the incubation of NAC (a nonspecific antioxidant) reduced the impairment of cardiac cell contraction in both Ctrl and CO rats. In addition, NAC infusion partly blunted the higher sensitivity of CO rat cardiomyocytes to A/R, since in this condition no difference was reported between Ctrl and CO groups regarding SL shortening (Fig. 5*A*). As far as the alterations of Ca^{2+} handling are concerned, the incubation of NAC improved Ca^{2+} transient in both Ctrl and CO rats. However, the difference between the two experimental populations was still reported (Fig. 5*B*). Finally, diastolic cytosolic Ca^{2+} was significantly reduced in CO rat cardiomyocytes in the presence of NAC compared with the corresponding controls (Fig. 5*C*). In the presence of NAC, no difference was reported regarding this variable between Ctrl and CO rats.

Effects of CO Exposure on Myocardial Antioxidant Enzyme Activity

In CO rats, following 4 wk of exposure to simulated urban CO pollution, cardiac SOD and GPx activities were significantly lower compared with Ctrl rats (Fig. 6, *A* and *B*). No significant change in catalase activity was observed in this model of CO exposure (Fig. 6*C*).

DISCUSSION

To the best of our knowledge, our study is the first to investigate the effects of prolonged exposure to simulated urban environmental CO pollution on myocardial sensitivity to I/R. The major result is that prolonged exposure to simulated urban CO pollution worsens myocardial I/R injuries, promoting a major increase in the severity of arrhythmic events, an impairment of myocardial function observed at both global and cellular levels, and an increase in the infarct size. These phenomena could be mainly related to hidden effects of CO exposure on myocardial phenotype, including *1*) an impairment of cellular Ca^{2+} handling and *2*) an altered antioxidant status of the myocardium.

CO Exposure and Myocardial I/R

Eventhough CO urban pollution has been associated with increased cardiovascular disease and cardiac mortality (9, 13, 29), we demonstrate here that chronic CO exposure renders the heart more vulnerable to I/R. Previous experimental studies have only documented the effect of acute CO exposure or to CO-releasing molecules used as preconditioning strategies to protect the myocardium against I/R injury (3, 6, 19). It was also reported that endogenous CO production could protect the heart from I/R injuries. These beneficial effects of endogenous

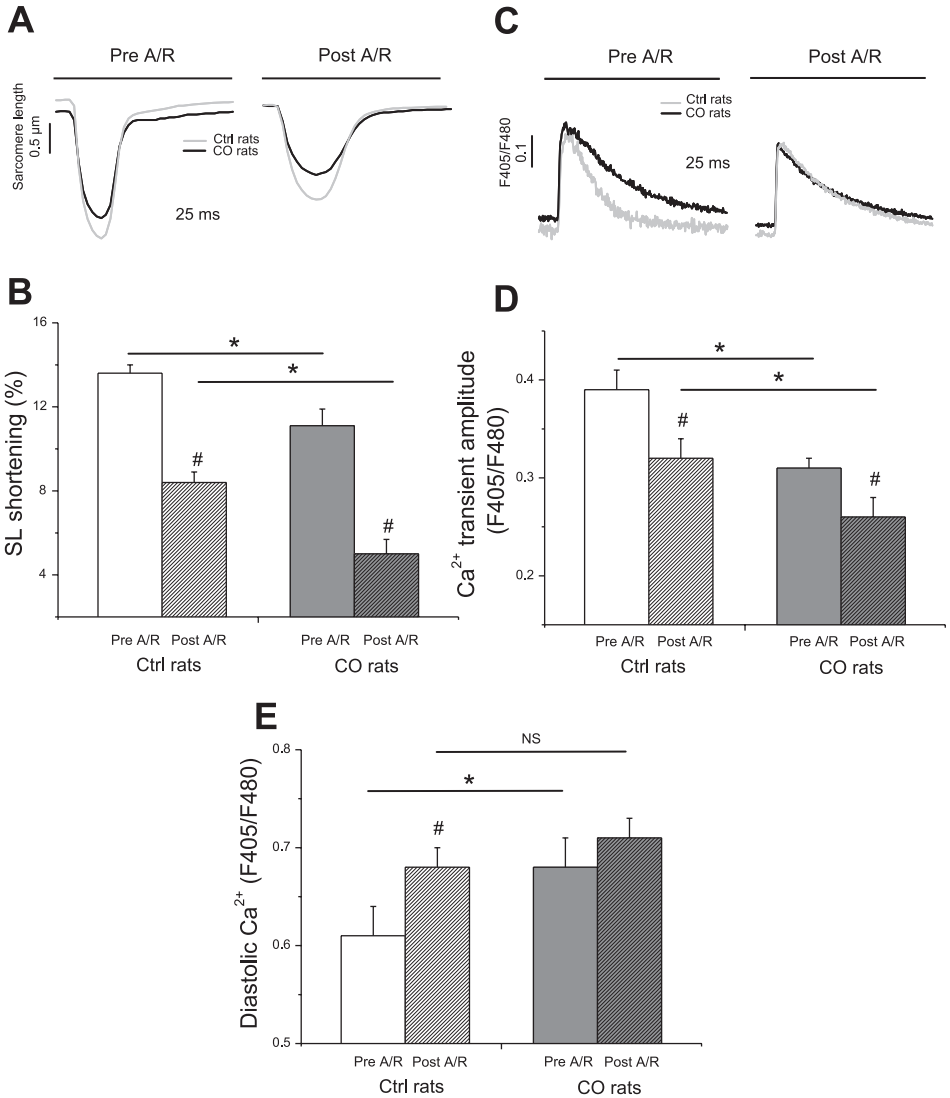

Fig. 4. Effects of CO exposure on cardiomyocyte excitation-contraction before and after cellular anoxia-reoxygenation (A/R). *A*: representative contraction of intact myocytes. *B*: contraction of intact myocytes, measured by sarcomere length (SL) shortening. SL shortening is presented as a mean of the percentage of shortening ± SE [n = 4 rats/group, 2-way ANOVA; $P < 0.05$, pre-A/R vs. post-A/R (#) and Ctrl vs. CO rats (*)]. *C*: representative superimposed Ca^{2+} transients in intact myocytes. Ca^{2+} transients (*D*) and diastolic Ca^{2+} (*E*) in intact myocytes. Ca^{2+} transients and diastolic Ca^{2+} are presented as means ± SE [n = 4 rats/group, 2-way ANOVA; $P < 0.05$, pre-A/R vs. post-A/R (#) and Ctrl vs. CO rats (*)]. NS, not significant.

CO production during I/R could be because of a decrease in oxidative stress (26), an enhanced Ca^{2+} handling (36), and CO anti-inflammatory and anti-apoptotic properties (20), which may ultimately lead to a decrease in I/R-induced VF (4, 5). In our model, no difference regarding cardiac function and coronary blood flow was evidenced between Ctrl and CO rats before I/R. Remarkably, only postischemic reperfusion allowed us to distinguish the hearts of CO-exposed rats from those of Ctrl rats, thereby pointing out the deleterious effect of CO. These results are in line with our previous study (2), which exhibited no difference of cardiac function in the basal condition but highlighted functional impairments in CO rats in stressful conditions (i.e., β-adrenergic stimulation). Therefore, discrepancies between our results and studies reporting the protective role of CO exposure could be explained by differences in the duration and severity of CO exposure (3, 6, 19). In our model, CO rats experimented a chronic (4 wk with 12 h daily exposure) CO exposure mimicking urban concentrations (30–100 ppm). In addition, it has to be noted that, in our model, to avoid the acute effects of CO on the myocardium, rats were housed for 24 h in standard filtered air before I/R study. Therefore, under our experimental conditions, no difference of carboxyhemoglobin was made obvious between Ctrl and CO rats at the time of the I/R procedure (carboxyhemoglobin 24 h after exposure, CO rats: 1.2 ± 0.4% vs. Ctrl rats 1.0 ± 0.5%; not significant).

Effects of CO Exposure on Postischemic Myocardial Injuries

The higher susceptibility of the myocardium to I/R in CO-exposed rats was notably characterized by higher propensity to arrhythmic events at the onset of reperfusion. Higher arrhythmic score and higher occurrence of sustained VF (54 vs. 9%) were evidenced. Arrhythmias, and more particularly sustained VF, are the most dangerous consequences of myocardial I/R, since they induce an impairment of blood circulation and ultimately lead to sudden cardiac death. Sheps et al. (31, 32) have already highlighted the proarrhythmic effect of CO exposure (1 day at 100 or 200 ppm) in patients with documented coronary artery diseases. However, as mentioned above, in our model, no difference of carboxyhemoglobin was reported between Ctrl and CO rats

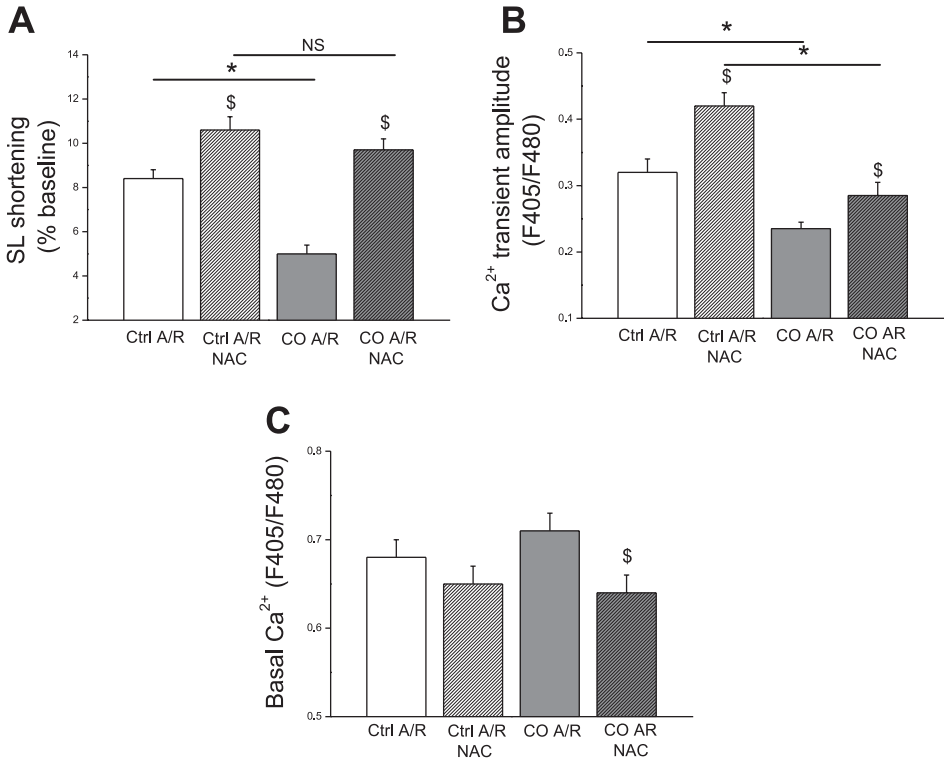

Fig. 5. Effects of *N*-acetylcystein (NAC) incubation on the sensitivity to A/R of cardiomyocytes from Ctrl and CO rats. *A*: contraction of intact myocytes, measured by SL shortening. SL shortening is presented as a mean of the percentage of shortening ± SE. *B* and *C*: Ca^{2+} transients (*B*) and (C) diastolic Ca^{2+} (*C*) in intact myocytes. Ca^{2+} transients and diastolic Ca^{2+} are presented as means ± SE [$n = 4$ rats/group, 2-way ANOVA; $P < 0.05$, with NAC vs. without NAC ($) and Ctrl vs. CO rats (*)].

at the time of the I/R procedure, avoiding then the acute effects of CO on the myocardium. In CO rats, the higher sensitivity of the myocardium was also characterized by an impairment of postischemic recovery of myocardial function. This could be related to the significantly impaired cellular contractile function observed, consecutive to A/R, on isolated cardiomyocytes of CO rats compared with Ctrl rats. This result suggests that cardiomyocyte damages lead to a reduced postischemic recovery of cardiac function. It seems, however, that the main factor involved in the explanation of this result was that CO pollution is associated, in our model, with a marked increase of I/R-induced cardiac cell death. I/R injuries, including cardiomyocyte impairments as well as arrhythmic events, were mainly explained, in past literature, by increased oxidative stress and Ca^{2+}-handling alteration (25, 41).

Implication of Ca^{2+} and Oxidative Stress

The increased sensitivity to I/R of the myocardium after CO exposure was characterized by the increase of arrhythmic events, increased functional disturbances, and cell death. The cellular mechanisms underlying theses aspects of the reperfusion syndrome may involve impairments of Ca^{2+} handling or overproduction of oxygen-derived free radicals. In a recent study, we have shown that CO pollution by itself initiates a pathological phenotype of the cardiomyocytes involving a profound remodeling of the excitation-contraction coupling through Ca^{2+}-handling alteration (2), which was confirmed in the present work. This remodeling was characterized by an impairment of cardiomyocyte shortening that was not observed at the whole heart level. This phenomenon, which has already been reported by our team, may involve compensatory mechanisms (2) that remain to be investigated. The cardiomyocytes also exhibit an increase in diastolic Ca^{2+} resulting from an altered Ca^{2+} reuptake in the sarcoplasmic reticulum (SR) due to a decreased sarco-(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) 2a expression and also potentially from a Ca^{2+} leak from the SR through the ryanodine receptors (2). Alterations of Ca^{2+} handling may be involved in cardiomyocyte dysfunction and ultimately in cell death during I/R (1, 33, 38). It is therefore very likely that these alterations, associated with CO exposure, increase the severity of cardiomyocytes and whole heart injuries related to I/R. Indeed, in our study, at the whole heart level, CO exposure increases cardiomyocyte death after I/R. Considering only viable myocytes, we report here that SL shortening and Ca^{2+} handling are altered by A/R to the same extent in both Ctrl and CO rats, resulting therefore in more pronounced dysfunctions in CO rats compared with Ctrl rats. Therefore, it seems that Ca^{2+} handling in viable cardiomyocytes was not more impaired by A/R in CO rats, but that the lower amplitude of Ca^{2+} transient reported in this population following A/R was the result of prolonged CO exposure-induced phenotypical changes. Consequently, a major finding of the present study was that the impaired Ca^{2+} handling observed before to A/R is a key factor in the development of functional impairments and arrhythmic events observed at the onset of reperfusion in CO rats and is therefore a candidate to explain higher sensitivity of CO rat myocardium to I/R.

The essential role of oxidative stress in the pathogenesis of myocardial I/R injury has been largely reported (16, 30, 41). Indeed, exacerbated oxidative stress during I/R is a key factor in the worsening of postischemic arrhythmias, cardiac dysfunction, and irreversible cardiomyocyte damages (16, 17, 25, 41). Oxidative stress is defined as an imbalance between the pro-

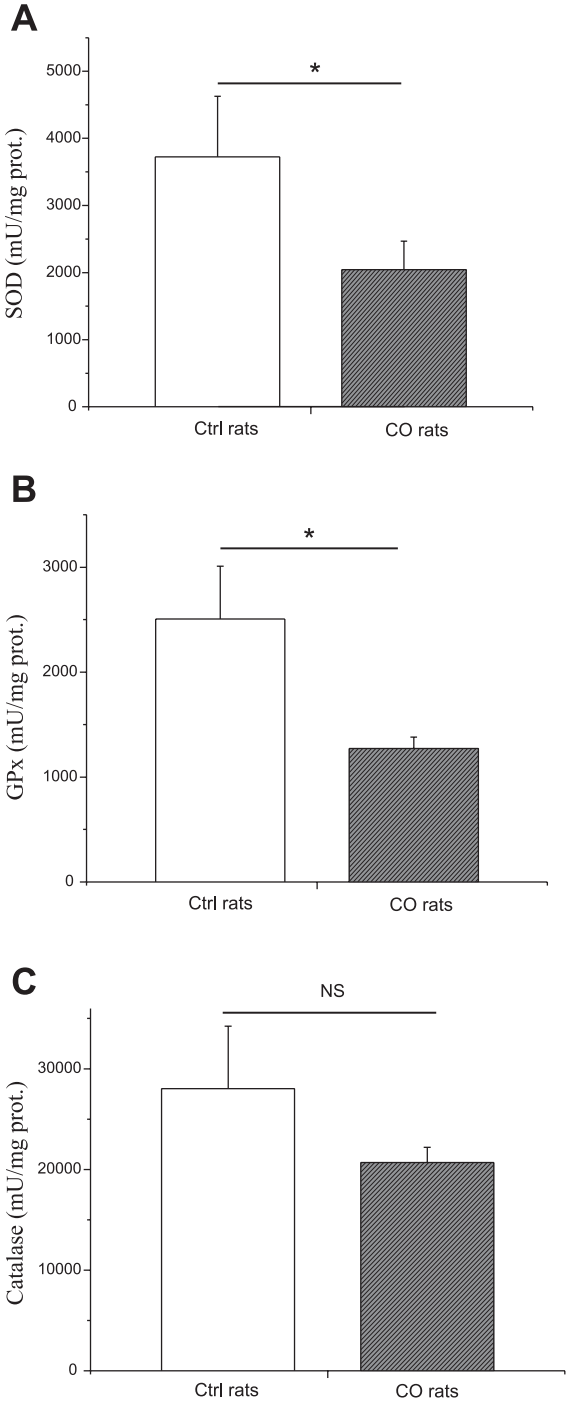

Fig. 6. Effects of CO exposure on superoxide dismutase (SOD, *A*), glutathione peroxidase (GPx, *B*), and catalase (*C*) activities expressed in mU/mg protein. Data are presented as means ± SE (n = 6 rats/group, 1-way ANOVA, $*P < 0.05$, Ctrl vs. CO rats).

duction of reactive oxygen species and biological antioxidant systems that are involved in the detoxification of reactive intermediates. An important result of our study is the impaired enzymatic antioxidant status of the myocardium, including a major decreased activity of SOD (−45%), GPx (−49%), and catalase (−26%, not statistically significant). We previously discussed the potential role of altered redox status of the myocardium to explain the effects of prolonged exposure to CO on excitation-contraction coupling (2). Indeed, since proteins involved in Ca^{2+} handling are potential targets for redox alterations, decrease of antioxidant defenses associated with the increased activity of thioredoxin reductase observed in our previous study (2) confirmed a CO-induced oxidative stress. Therefore, those alterations of enzymatic antioxidant defense could play a major role in phenotypical changes of CO-exposed rat myocardium, mainly affecting Ca^{2+} homeostasy. Besides, SOD prevents changes in myocardial function, and Ca^{2+} homeostasis in isolated hearts subjected to I/R (27), catalase, and GPx plays a major role in protecting the myocardium from I/R injury (16). We have observed an increase in TBARS production, used as a marker of lipid peroxidation, and therefore oxidative stress, in CO-exposed rats following I/R. Therefore, we can postulate that the lower enzymatic antioxidant defense that was observed consecutively to CO exposure could contribute to the increased sensitivity of the myocardium to I/R damages. To prevent oxidative stress-induced alterations, an acute antioxidant strategy, using a nonspecific antioxidant (NAC), was performed during cellular A/R. This acute antioxidant strategy was found able to prevent the deleterious effects of CO exposure on SL shortening during A/R. This result highlights the implication of an increased cardiac oxidative stress in our model of CO-exposed rats. However, the deleterious effects of CO exposure on Ca^{2+} transient were not prevented by this acute antioxidant strategy. This observation is not surprising, since prolonged exposure to CO pollution results in phenotypical remodeling of Ca^{2+}-handling proteins, such as decreased SERCA2a expression (2). This profound remodeling of the Ca^{2+}-handling phenotype, which was reported in CO rat myocardium, could not be reversed by such an acute antioxidant strategy. Therefore, chronic antioxidant therapy, notably by preventing myocardial phenotypical changes, could be a promising strategy for protecting the heart against the deleterious effects of chronic CO exposure on myocardial sensitivity to I/R.

In conclusion, this study shows that chronic exposure to CO consistent with air pollution from the urban type significantly increases the effects of a myocardial infarction in rats and could be considered as a major health risk. Indeed, the World Health Organization estimates that air pollution is responsible for 800,000 premature deaths worldwide each year, and, particularly, exposure to air pollution increases the risk of mortality from cardiovascular disease by 76% (23). Among the numerous pollutants, CO has been described as one of the main pollutants responsible for the development of cardiovascular diseases (9, 35). Prolonged CO exposure worsens I/R-linked cardiac injuries and therefore provides an experimental rationale to explain the increased risk of cardiac mortality observed in exposed populations. In summary, prolonged CO exposure-induced cardiac phenotypical changes, such as an imbalance in the cardiomyocyte oxidant status and an impairment of Ca^{2+} handling, are likely to predispose the heart to I/R injuries.

GRANTS

This work was funded by a French National Research Agency grant (COMYOCARD).

DISCLOSURES

No competing financial interests exist.

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