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Moderate exercise prevents impaired Ca\(^{2+}\) handling in heart of CO-exposed rat: implication for sensitivity to ischemia-reperfusion


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Sustained urban carbon monoxide (CO) exposure exacerbates heart vulnerability to ischemia-reperfusion via deleterious effects on the antioxidant status and Ca\(^{2+}\) homeostasis of cardiomyocytes. The aim of this work was to evaluate whether moderate exercise training prevents these effects. Wistar rats were randomly assigned to a control group and to CO groups, living during 4 wk in simulated urban CO pollution (30–100 parts/million, 12 h/day) with (CO-Ex) or sedentary without exercise (CO-Sed). The exercise procedure began 4 wk before CO exposure and was maintained twice a week in standard filtered air during CO exposure. On one set of rats, myocardial ischemia (30 min) and reperfusion (120 min) were performed on isolated perfused rat hearts. On another set of rats, myocardial antioxidant status and Ca\(^{2+}\) handling were evaluated following environmental exposure. As a result, exercise training prevented CO-induced myocardial phenotypical changes. Indeed, exercise induced myocardial antioxidant status recovery in CO-exposed rats, which is accomplished by a normalization of sarcomer/endoplasmic reticulum Ca\(^{2+}\)-ATPase 2a expression and then of Ca\(^{2+}\) handling. Importantly, in CO-exposed rats, the normalization of cardiomyocyte phenotype with moderate exercise was associated with a restored sensitivity of the myocardium to ischemia-reperfusion. Indeed, CO-Ex rats presented a lower infarct size and a significant decrease of reperfusion arrhythmias compared with their sedentary counterparts. To conclude, moderate exercise, by preventing CO-induced Ca\(^{2+}\) handling and myocardial antioxidant status alterations, reduces heart vulnerability to ischemia-reperfusion.

METHODS

All investigations complied with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publications No. 85-23, Revised 1996) and with the approval of the French Ministry of Agriculture. All experiments have been approved by the Local Research Ethics Committee (Comité Régional d’Éthique). Animals

Adult male Wistar rats (n = 57; 384 ± 3 g; Charles River) were randomly assigned to three experimental groups: 1) sedentary control group (Ctrl-Sed rats), 2) sedentary CO-exposed group (CO-Sed rats), and 3) exercise-trained CO-exposed group (CO-Ex rats). All experimental groups were maintained on a 12-h:12-h light-dark cycle and provided rat chow and water ad libitum.
**Exercise Training Protocol**

Moderate exercise training protocol was performed on a motor-driven treadmill for 4 wk, 5 days/wk, at a relative work rate corresponding to 50% of maximal aerobic velocity (20 m/min; 40 min/day). During the following 4 wk corresponding to the CO exposure period, to preserve the exercise training benefits, exercise training was maintained in CO-Ex rats on a frequency of 2 days/wk.

**CO Exposure**

The CO groups were exposed to simulated CO urban pollution for 4 wk, 12 h/day, during the night phase. CO rats were housed in an airtight exposure container, and exposure was performed as follows: 1) during CO exposure, a CO concentration of 30 parts/million (ppm) was maintained in the airtight container and monitored with an aspirative CO analyzer (CHEMGARD Infrared Gas Monitor NEMA 4 Version, MSA), and this initial concentration was completed with five 1-h peaks at 100 ppm CO; and 2) during ambient air exposure, the animals were placed in the laboratory animal house at a CO concentration of 0 ppm. Throughout this CO exposure period, Ctrl-Sed rats were confined in the laboratory animal house and were subjected to the same restraint as the CO-Sed rats. At the end of the 4 wk of CO exposure, the rats were housed for 24 h in standard filtered air before euthanasia to avoid any acute effects of CO on the myocardium.

**Ca^{2+} Handling in Cardiomyocytes**

In the first set of rats (n = 4 rats/group; 10 myocytes/rat), an evaluation of exercise training and CO exposure on excitation-contraction was performed on single ventricular cardiomyocytes isolated by enzymatic digestion (21). 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+}). Sarcomere length (SL) and fluorescence (405 and 480 nm) were simultaneously recorded (IonOptix system, Hilton) (1).

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### Figure 1: Effects of exercise training and carbon monoxide (CO) exposure on sarcomere length (SL) shortening and Ca^{2+} handling in cardiomyocytes.

**A**: representative contraction of intact cardiomyocytes measured by sarcomere shortening at 0.5 Hz. **B**: amplitude of SL shortening. Data are presented as percentages of baseline. **C**: representative Ca^{2+} transient during cardiomyocyte excitation-contraction. **D**: **F**: Ca^{2+} reuptake kinetics. **G**: diastolic intracellular Ca^{2+}. **E**: Ca^{2+} transient amplitude. **F**: Ca^{2+} transient amplitude (F405/F480). **G**: representative expression of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2a). The GAPDH blot (37 kDa) was used as a loading control to normalize SERCA2a protein expression. Data are presented as means ± SE (n = 5 per group, one-way ANOVA). §P < 0.05, control (Ctrl)-sedentary (Sed) vs. CO-Sed; *P < 0.05, CO-Sed vs. CO-exercise trained (Ex).
Regional Myocardial I/R Protocol on Isolated Perfused Heart

In a second set of rats (n = 10/group), a regional myocardial I/R protocol on an isolated-perfused heart was performed (18). This model was chosen to abrogate the potential effects of CO exposure on hormonal, circulating, and nervous parameters. The coronary occlusion-induced myocardial regional ischemia was maintained for 30 min. Subsequently, the heart was allowed to reperfuse during 120 min. Lactate dehydrogenase (LDH) release and incidence of ventricular fibrillation (VF) was evaluated during the reperfusion period. At the end of the perfusion, the hearts were divided into five slices perpendicular to the apex-base axis and 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. The tissue slices were photographed, and the risk and infarcted areas were then determined using a computer-based system (ImageJ, NIH).

Biochemical Assays

Heart antioxidant enzyme activity. To assess the effects of exercise training and/or CO exposure on the antioxidant capacity, the heart enzymatic antioxidant status was measured in the third set of rats as previously described (n = 5/group) (18). Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) enzymatic activities were evaluated.

LDH activity in coronary effluents. LDH activity, used as an index of cell membrane damage, was measured in coronary effluents at 5 min of reperfusion. LDH activity was measured spectrophotometrically using an LDH kit (LDH-P, BIOLABO SA, France).

Western blot analysis. Proteins were separated using 4–20% SDS-PAGE and blotted onto a nitrocellulose membrane (Protran, Schleicher and Schuell, Dassel, Germany). Membranes were incubated overnight at 4°C with the sarco(endoplasmic reticulum Ca²⁺-ATPase 2a (SERCA2a) antibody (A0110-20, Badrilla, UK), and the levels were expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) content on the same membrane. Immunodetection was carried out using the ECL Plus system (Amersham Pharmacia, Little Chalfont Buckinghamshire, UK).

Statistics

Data were analyzed using either one-way factorial or repeated-measures ANOVA. When significant interactions were found, a Student-Newman-Keuls test was applied. Binomially distributed variables (such as incidence of VFs) were analyzed using a nonparametric Yate’s χ²-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

Contraction and Ca²⁺ Handling in Single Cells

Chronic exposure to CO pollution decreased SL shortening (Fig. 1, A and B), increased diastolic cytosolic Ca²⁺ (Fig. 1, C and D), and decreased the amplitude of the Ca²⁺ transient (Fig. 1, C and E). In addition, the decay kinetics (τ) of the Ca²⁺ transient were impaired (Fig. 1F), which was explained by a decrease in SERCA2a expression in CO rats (Fig. 1G). Exercise training prevented both SERCA2a reduction (Fig. 1G) and Ca²⁺-handling alterations (Fig. 1, D–F). Exercise training therefore preserved normal SL shortening, which was similar to that of rats living in standard filtered air (Fig. 1, A and B).

Myocardial Enzymatic Antioxidant Status

After 4 wk of sustained CO exposure, the myocardial enzymatic antioxidant status was depressed. The SOD and GPx activities were reduced in CO-Sed rats compared with Ctrl-Sed
rats (Fig. 2, A and B). Exercise training prevented these deleterious effects since SOD and GPx activities did not differ between CO-Ex rats and Ctrl-Sed rats. In contrast, no effect from CO exposure or exercise training was reported on catalase activity (Fig. 2C).

Infarct Size and Myocardial Cells Death

Cardiac cell death induced by I/R was aggravated by prolonged CO exposure (Fig. 3, A and B). Indeed, infarct size was higher in CO rats than in controls. In addition, LDH release, measured in coronary effluents at the time of postsischemic myocardial reperfusion and used as an index of cell membrane damage, was higher in CO-exposed rats than in their counterparts. The promoting effect of CO exposure on myocardial necrosis was fully prevented by regular exercise since no difference in infarct size was observed between CO-Ex and Ctrl-Sed rats (Fig. 3, A and B). Consistently, the same result was obtained regarding the effects of exercise on LDH release during postsischemic reperfusion (Fig. 3C).

Myocardial Reperfusion Arrhythmias

Although no difference was observed in the incidence of VF (33%, Ctrl-Sed vs. 50% CO-Sed), chronic CO exposure was responsible for a marked increase in the severity of postsischemic reperfusion VF. Indeed, sustained VF (Fig. 3D) occurred in 25% of CO-Sed rats, whereas this phenomenon was not observed in Ctrl-Sed rats (Fig. 3E). Interestingly, the pronounced deleterious effect of CO exposure on the severity of reperfusion arrhythmias was fully prevented by exercise training since no VF was reported in CO-Ex rats (Fig. 3E).

DISCUSSION

The major results of this study are that endurance training prevents the deleterious effects of sustained CO exposure on myocardial antioxidant status, cellular Ca\(^{2+}\)/H\(^{+}\) handling, and myocardial vulnerability to I/R injury.

CO Exposure and Cardioprotective Effects of Exercise Training

In line with previous reports, including ours, we confirmed here the deleterious effects of simulated urban CO exposure on enzymatic antioxidant status and cardiomyocyte Ca\(^{2+}\) handling (1, 4). Low and sustained levels of CO exposure promoted pathological cardiac remodeling with impaired Ca\(^{2+}\) handling due to increased diastolic Ca\(^{2+}\), decreased Ca\(^{2+}\) transient, and Ca\(^{2+}\) reuptake in the sarcoplasmic reticulum (SR) due to reduced SERCA2a expression (1, 4). These changes are related to CO-induced oxidative stress (18, 37).

Fig. 3. Effects of exercise training and CO exposure on ischemia-reperfusion-induced cellular death and posts ischemic ventricular fibrillations (VFs). A: representative sections of area at risk (AAR) and infarct size of rat hearts stained, respectively, with Evans blue and triphenyltetrazolium chloride after 30 min regional ischemia and 120 min reperfusion from isolated heart experiments in each experimental group. B: infarct sizes expressed as percentages of AAR. Data are presented as means ± SE (Ctrl-Sed, n = 7; CO-Sed, n = 6; and CO-Ex, n = 6; one-way ANOVA); C: LDH activity observed in coronary effluents at 5 min of reperfusion and used as a marker of cell death. Data are presented as means ± SE (Ctrl-Sed, n = 9; CO-Sed, n = 7; and CO-Ex, n = 10; one-way ANOVA); D: representative plot of VF; E: incidence of VFs occurring during the first 5 min of reperfusion. Data are presented as percentages of rats per experimental group (Ctrl-Sed, n = 6; CO-Sed, n = 8; and CO-Ex, n = 9; nonparametric Yates' \(\chi^2\)-test). §P < 0.05, Ctrl-Sed vs. CO-Sed; *P < 0.05, Ctrl-Sed vs. CO-Ex.
Endurance Training and Sensitivity to I/R Consecutive to CO Exposure

We recently reported that a sustained CO exposure increased the vulnerability to I/R (18). Although lower infarct sizes in CO rats were observed in this study compared with the previous one (18), the present work confirms that CO exposure is associated with an increased severity of myocardial I/R injuries, characterized by an exacerbated occurrence of sustained VFs and cardiomyocyte death. These results are in line with the well-reported determinant role of oxidative stress and Ca\textsuperscript{2+} overload in the severity of postischemic reperfusion arrhythmias, cardiac dysfunction, and irreversible cardiomyocyte damages (5, 6, 22, 36). A major result of our study was that endurance training, by preventing CO-induced cellular alterations, fully prevented the worsened sensitivity of CO rats to I/R. This is in accordance with numerous studies supporting the successful cardioprotective effect of exercise training against I/R injuries (2, 7, 11, 25, 26). This beneficial effect has been largely proposed to reflect the improved myocardial enzymatic antioxidant activities (SOD and GPX). Even if the underlying mechanisms are not fully understood yet, exercise training is well recognized today as one of the most efficient cardioprotective strategies, notably through the enhancement of myocardial antioxidant status (2, 7, 12, 15, 16). Consistently, exercise preserved antioxidant status and thereby, prevented cytosolic Ca\textsuperscript{2+} overload and depressed Ca\textsuperscript{2+} transient in CO-trained rats, in line with various reports of the beneficial effects of exercise training on Ca\textsuperscript{2+} handling in a pathological population (21, 29, 32). The maintenance of normal SERCA2a expression mainly explained this benefit on Ca\textsuperscript{2+} homeostasis. Although the direct effects of exercise training on SERCA2a expression could not be ruled out (21, 33), our results are consistent with the indirect effects mediated by the normalization of enzymatic antioxidant status on this redox-sensitive protein (8, 20, 31, 34).

being associated with altered redox (1) and enzymatic antioxidant statuses (1, 18).

One main result of the present study was that moderate exercise training conducted before CO exposure successfully prevented the deleterious effects of CO exposure on myocardial enzymatic antioxidant activities (SOD and GPX). Even if the underlying mechanisms are not fully understood yet, exercise training is well recognized today as one of the most efficient cardioprotective strategies, notably through the enhancement of myocardial antioxidant status (2, 7, 12, 15, 16). Consistently, exercise preserved antioxidant status and thereby, prevented cytosolic Ca\textsuperscript{2+} overload and depressed Ca\textsuperscript{2+} transient in CO-trained rats, in line with various reports of the beneficial effects of exercise training on Ca\textsuperscript{2+} handling in a pathological population (21, 29, 32). The maintenance of normal SERCA2a expression mainly explained this benefit on Ca\textsuperscript{2+} homeostasis. Although the direct effects of exercise training on SERCA2a expression could not be ruled out (21, 33), our results are consistent with the indirect effects mediated by the normalization of enzymatic antioxidant status on this redox-sensitive protein (8, 20, 31, 34).

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REFERENCES

DISCLOSURES
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