Carbon Monoxide Induces Cardiac Arrhythmia via Induction of the Late Na⁺ Current

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Rationale: Clinical reports describe life-threatening cardiac arrhythmias after environmental exposure to carbon monoxide (CO) or accidental CO poisoning. Numerous case studies describe disruption of repolarization and prolongation of the QT interval, yet the mechanisms underlying CO-induced arrhythmias are unknown.

Objectives: To understand the cellular basis of CO-induced arrhythmias and to identify an effective therapeutic approach.

Methods: Patch-clamp electrophysiology and confocal Ca²⁺ and nitric oxide (NO) imaging in isolated ventricular myocytes was performed together with protein S-nitrosylation to investigate the effects of CO at the cellular and molecular levels, whereas telemetry was used to investigate effects of CO on electrocardiogram recordings *in vivo*.

Measurements and Main Results: CO increased the sustained (late) component of the inward Na⁺ current, resulting in prolongation of the action potential and the associated intracellular Ca²⁺ transient. In more than 50% of myocytes these changes progressed to early after-depolarization-like arrhythmias. CO elevated NO levels in myocytes and caused S-nitrosylation of the Na⁺ channel, Nav1.5. All proarrhythmic effects of CO were abolished by the NO synthase inhibitor L-NAME, and reversed by ranolazine, an inhibitor of the late Na⁺ current. Ranolazine also corrected QT variability and arrhythmias induced by CO *in vivo*, as monitored by telemetry.

Conclusions: Our data indicate that the proarrhythmic effects of CO arise from activation of NO synthase, leading to NO-mediated nitrosylation of Nav1.5 and to induction of the late Na⁺ current. We also show that the antianginal drug ranolazine can abolish CO-induced early after-depolarizations, highlighting a novel approach to the treatment of CO-induced arrhythmias.

Keywords: carbon monoxide; arrhythmia; late Na⁺ channel; nitric oxide; S-nitrosylation

Carbon monoxide (CO) is long established as a highly toxic gas, and its contribution to the hazardous effects of increasing air

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

It is long established that exposure to carbon monoxide, either accidental or arising from environmental pollution, can lead to cardiac arrhythmias. However, the underlying mechanisms are not understood and because of this, treatment strategies are lacking.

What This Study Adds to the Field

This study shows that the life-threatening arrhythmias that can arise because of carbon monoxide exposure are attributable primarily to the direct modulation of a specific ion channel (the cardiac sodium channel). A currently available drug is known to target this channel and may provide a clinically useful treatment for the cardiac effects of carbon monoxide exposure.

pollution is a cause of major public health concern. Exposure to CO arises from incomplete combustion of hydrocarbons. Sources include motor exhaust fumes, gas appliances, and tobacco smoke. The World Health Organization estimates that worldwide, 3 million people are killed each year by environmental air pollution associated with vehicle and industrial emissions (<http://www.who.int>; <http://www.infoforhealth.org>). A recent and extensive analysis of data collected from some 9 million people from more than 100 US urban districts over a 7-year time span identified a clear association of ambient CO exposure (distinct from other pollutant gases) with increased risk of hospitalization caused by cardiovascular complaints, including cardiac rhythm disturbances (1). This study confirmed and extended previous investigations, which have implicated environmental CO exposure in myocardial dysfunction (2, 3).

Chronic exposure to moderate (30–200 ppm) CO can induce myocardial injury and fibrosis (3–6). However, acute environmental (e.g., road traffic) (7) exposure to CO (up to 500 ppm) is associated with arrhythmias and an increased risk of sudden death (3, 8), particularly in patients with existing cardiac conditions (9, 10). Accidental exposure to higher levels of CO (>1,000 ppm) is also common in domestic and industrial settings and accounts for more than 50% of all fatal poisonings (11, 12). Patients with acute or chronic sublethal CO poisoning often present with life-threatening arrhythmias, and numerous reports of ECG alterations in CO-exposed individuals have been published. Consistent clinical features include disruption of repolarization and prolongation (accompanied by increased variability or dispersion) of the QT interval (3, 13, 14). Indeed, one report concluded that CO poisoning mimicked long-QT (LQT) syndrome (15). Such effects of CO may also contribute

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to arrhythmias during cigarette smoking, which are associated with increased QT dispersion (16, 17). Experimentally, prolongation of the QT interval by CO inhalation has been reported in conscious rats (18), suggesting they would make a good experimental model for studying the proarrhythmic effects of CO.

Because the affinity of hemoglobin for CO is higher than that for O₂ (2), sustained exposure to high levels of CO can result in tissue hypoxia. However, clinical findings have generally excluded hypoxia as the cause of arrhythmias because carboxyhemoglobin levels do not correlate with the observed ECG changes (3, 19). Given the clear clinical need to understand and treat the cardiac effects of acute CO exposure, we aimed to establish the mechanism underlying CO-induced ventricular arrhythmias. We report that CO induces early after-depolarizations (EADs) in isolated ventricular myocytes. Furthermore, in conscious, freely moving animals, we demonstrate that CO increases QT variability and can induce fatal arrhythmias during β_1 -adrenoceptor stimulation. This arrhythmic activity seems attributable to activation of the late Na⁺ current, triggered by an increase in cellular nitric oxide (NO), causing nitrosylation of the Na⁺ channel protein, Na_v1.5. Finally, we show that ranolazine, an inhibitor of the late Na⁺ current, can prevent CO-induced EADs, suggesting a novel therapeutic strategy for the treatment of CO-induced arrhythmias. Some of the results of these studies have been previously reported in the form of abstracts (20, 21).

METHODS

For a detailed description of all protocols, *see* the METHODS section in the online supplement.

Confocal Imaging of Ventricular Myocytes

Cardiac myocytes were isolated from rat ventricles by collagenase digestion as previously described (22). Myocytes were loaded with the fluorescent Ca²⁺ indicator fluo-3 (6 μ M; Sigma, Poole, UK) or the NO indicator DAF2 (5 μ M; Calbiochem, Nottingham, UK) by incubation with the

acetoxymethyl ester forms of each dye for 15 and 20 minutes, respectively. Dyes were excited with the 488-nm line of a 20-mW coherent sapphire laser (attenuated by $\sim$ 90%) and emitted fluorescence was measured at more than 515 nm. Line scan images were acquired at 6 milliseconds or 2-millisecond intervals. When monitoring intracellular Ca²⁺ levels, myocytes were stimulated at 0.2 Hz by platinum electrodes. For some experiments (*see* Figure E3 in the online supplement), cells were saponin-permeabilized (*see* the METHODS section in the online supplement).

Electrophysiology

Whole-cell patch clamp recordings were obtained from myocytes in voltage or current clamp mode using an Axopatch 200A amplifier/Digidata 1200 interface and Clampex/Clampex 9 software (Molecular Devices, Wokingham, UK). Voltage clamp signals were sampled at 50 kHz and filtered at 20 kHz. Current clamp signals were sampled at 10 kHz and filtered at 2 kHz. Action potentials (APs) were evoked by 10-millisecond current pulses (0.5 Hz) using patch electrode and perfusate solutions (*see* the METHODS section in the online supplement). Na⁺ currents were recorded from myocytes voltage-clamped at -80 mV using patch electrode and perfusate solutions (*see* the METHODS section in the online supplement). Na⁺ currents were evoked by a 100-millisecond depolarizing step to -30 mV, and measured at their peak and over the last 10 milliseconds of the depolarizing step (for late Na⁺ current).

Biotin Switch Assay

Protein S-nitrosylation was detected using a modified biotin-switch assay (23). The complete protocol is detailed in the METHODS section in the online supplement.

CO Application and Measurement

Cells were exposed to CO by three means. (1) CO was equilibrated by bubbling solutions with CO gas and then diluted appropriately. (2) CO-releasing molecule, CORM-2 ([Ru(CO)₃Cl₂]₂, tricarbonyldichlororuthenium [II] dimer; Sigma) was added to solutions (24). The inactive breakdown product (RuCl₂[DMSO]₄; synthesized in-house) of CORM-2, referred to as iCORM, was used as a control. (3) Because

Figure 1. Carbon monoxide (CO) disrupts intracellular Ca²⁺ handling in ventricular myocytes. Line-scan images of [Ca²⁺]_i in fluo-3 loaded myocyte before (control) and during superfusion with CO (87.6 ± 4.2 μ M) (A) or CO-releasing molecule (CORM-3) (20 μ M) (B) for 2 and 6 minutes. Arrowhead indicates time of electrical stimulation. Scale bars apply to all images. Bottom, normalized superimposed line-scan images illustrating the effect of CO (A) or CORM-3 (B) on the descending phase of the Ca²⁺ transient. In approximately 50% of cells the increase in duration of the descending phase developed into a plateau with clear [Ca²⁺]_i oscillations. Bar graph, mean (± SEM bars; n = 14) increase of Ca²⁺ transient duration (at 50% of maximum) caused by CO. **P < 0.01, ***P < 0.001, paired t test (A). (B) As A, except the myocyte was exposed to 20 μ M CORM-3 rather than CO. Bar graph shows mean (± SEM bars; n = 14) increase of Ca²⁺ transient duration (at 50% of maximum) caused by CORM-3. **P < 0.01, ***P < 0.001, paired t test.

Figure 2. Carbon monoxide (CO) prolongs the cardiac action potential (AP) by increasing the amplitude of the late Na⁺ current. (A) APs recorded before (black) and during (red) perfusion with CO-releasing molecule (CORM-2) (30 μM). Note reduced peak amplitude and increased duration (upper traces) or oscillations (lower traces). Bar graph, mean % change (± SEM; n = 6) in AP duration at the points indicated. ***P < 0.01 (paired *t* test). (B) Na⁺ currents evoked by depolarizations from -80 to -30 mV before and during exposure to 30 μM CORM-2, CO (87.6 ± 4.2 μM), or 30-μM inactive breakdown product (RuCl₂[DMSO]₄) of CORM-2 (iCORM), as indicated. Scale bars apply to all traces. Note enhanced late Na⁺ current caused by CO, shown more clearly in traces below. Bar graph, mean (± SEM; n = 8 for CORM-2, 7 for CO, and 6 for iCORM) % change in peak and late Na⁺ current evoked by CORM-2, CO, and iCORM, as indicated. ***P < 0.01, paired *t* test.

lipophilic CORM-2 interfered with Ca²⁺ imaging, we used the water-soluble donor, CORM-3 (gifted by R. Motterlini and synthesized in-house). CO concentrations were measured by spectrophotometric measurement of carbonmonoxymyoglobin (25).

Electrocardiogram Recordings and Analyses

Male Wistar rats were equipped with CA-F40 telemetric transmitters (DSI, St. Paul, MN) under general anesthesia and allowed to recover

Figure 3. Inhibiting nitric oxide (NO) formation prevents the proarrhythmic effects of carbon monoxide (CO). (A) Top, line scan images of [Ca²⁺]_i before and during exposure to CO (87.6 ± 4.2 μM). In this experiment, 1 mM L-NAME was present throughout and for 120 minutes before recording. Bottom, normalized fluorescence plotted as a function of time. Bar graph, mean (± SEM; n = 6) duration of Ca²⁺ transients (at 50% of maximum) caused by CO in the presence of L-NAME. (B) Na⁺ currents recorded before and during exposure to CO-releasing molecule (CORM-2) (30 μM), as indicated. Throughout the experiment, and 30 minutes before recording, the myocyte was exposed to 1 mM L-NAME. Bar graph, mean (± SEM; n = 7) % change in peak and late Na⁺ current amplitudes evoked by 30 μM CORM-2 in the absence and presence of L-NAME, as indicated. ***P < 0.01, paired *t* test. (C) Action potentials (APs) recorded before and during perfusion with 30 μM CORM-2. L-NAME (1 mM) was present throughout and 30 minutes before recording. Bar graph, mean % change (± SEM; n = 7) in AP duration at points indicated.

for 8 days. They were then submitted to filtered air either with or without 500 ppm CO for 1 hour in an airtight exposure container. Some rats were administered ranolazine (30 mg/kg intraperitoneally; Sigma) 15 minutes before CO exposure. ECGs were recorded continuously throughout the experiments using a signal transmitter-receiver system (see the METHODS section in the online supplement). In some experiments rats were further challenged with the β-adrenergic agonist isoproterenol (1 mg/kg in NaCl 0.9%, intraperitoneally; Sigma). Ventricular ectopic beats, the occurrence of ventricular tachycardia (VT), ventricular fibrillation (VF), and sudden cardiac death were also noted.

Statistical Analyses

All results are presented as means ± SEM, and statistical analysis performed using paired or unpaired Student *t* tests as indicated, where *P* less than 0.05 was considered statistically significant.

Figure 4. Carbon monoxide (CO) induces nitric oxide (NO) formation and S-nitrosylation of Nav1.5. (A) *Top*, fluorescence images of myocytes loaded with the NO indicator, DAF-2: *left*, superfusion with CO ($87.6 \pm 4.2 \mu\text{M}$) alone; *right*, superfusion with CO after preexposure to 1 mM L-NAME. *Bottom*, graph plotting mean ($\pm$ SEM; n = 6) changes in DAF-2 fluorescence during exposure to CO in the absence or presence L-NAME, as indicated. * $P < 0.05$; ** $P < 0.01$, unpaired t tests. (B) Identification of S-nitrosylated Nav1.5 in cardiac myocyte extracts made from untreated myocytes or myocytes after treatment with inactive breakdown product ($\text{RuCl}_2[\text{DMSO}]_4$) of CORM-2 (iCORM) or CORM-2 (each at $30 \mu\text{M}$), or CysNO ($50 \mu\text{M}$). (C) Effects of the reactive disulfide 2,2'-dithiobis(5-nitropyridine) (5-NO-2-PDS; $50 \mu\text{M}$) on the peak and late Na⁺ current in rat ventricular myocytes: example currents evoked by step depolarizations from -80 to -30 mV are shown before (control) and during exposure to 5-NO-2-PDS. Bar graph shows mean ($\pm$ SEM; n = 5 cells) percent change in peak and late current amplitude caused by 5-NO-2-PDS. (D) Line profiles obtained from line-scan images illustrating the effect of CO on cardiac myocytes. *Top*, evoked Ca²⁺ transients before and during exposure to CO ($87 \mu\text{M}$), applied for the period indicated by the bar above the trace. Two evoked transients are shown on an expanded time scale to highlight the early after-depolarizations (EAD)-like changes in transients typical of the effects of CO. *Bottom*, experiment conducted exactly as shown in the upper example, except that 2 mM dithiothreitol (DTT) was present throughout the experiment. Note the lack of appearance of EAD-like events.

RESULTS

Effects of CO on Cardiac Myocyte Intracellular Ca²⁺ Handling

Laser scanning confocal images (line scan mode) show that after exposure to either CO (Figure 1A) or the CO-releasing molecule CORM-3 (Figure 1B), the electrically stimulated Ca²⁺ transient duration increased progressively. In approximately 50% of cells (n = 14) a sustained plateau or [Ca²⁺]_i oscillation developed during the descending phase of the triggered Ca²⁺ transient, as illustrated in the superimposed normalized line scan profiles and associated accumulated data (Figures 1A and 1B). The [Ca²⁺] transient prolongation, with or without oscillations during the descending phase, is a characteristic feature of EADs. Because one established common underlying cause of EADs is activation of the late component of the Na⁺ current (which leads to APD

prolongation and reactivation of the L-type Ca²⁺ current), we investigated the effects of CO on APD and Na⁺ current properties in cardiac myocytes.

Proarrhythmic Effects of CO on APD and the Late Na⁺ Current

Evoked APs in ventricular myocytes were modulated in two distinct ways during exposure to the lipophilic CO donor CORM-2 ($30 \mu\text{M}$): AP amplitudes were significantly reduced ($P < 0.05$) from 97.5 ± 2.9 mV to 80.2 ± 4 mV (n = 20). But, more strikingly, their duration increased (Figure 2A), and in 11 of 20 recordings, EAD-like oscillations were observed during the plateau phase (Figure 2A, lower examples). CORM-2 was without significant effect on resting membrane potential (-70.4 ± 3.3 mV before and -75.3 ± 2.9 mV during CORM-2

exposure; $n = 8$). Peak Na^+ current amplitudes recorded under voltage-clamp were reduced by $53.4 \pm 7.7\%$ ($n = 6$) and $58 \pm 5.6\%$ ($n = 6$) in the presence of CORM-2 and dissolved CO, respectively. The mechanism underlying the reduction in peak Na^+ current amplitude has not been explored in detail. However, construction of steady-state inactivation curves showed that CO caused a hyperpolarizing shift, such that currents evoked from a holding potential of -80 mV were reduced in amplitude (see Figure E1), an observation that can account at least in part for reductions in peak Na^+ current amplitude caused by CO. More importantly, CO dramatically increased the noninactivating ("late") component of the current, as shown more clearly on the longer timescale traces (Figure 2B, lower and bar graph). This action of CO is reminiscent of the effects of Na^+ channel mutations underlying LQT-3 type EAD arrhythmias (26, 27). Similarly, the water-soluble CO donor CORM-3 also reduced AP amplitudes and prolonged AP duration, and enhanced the late Na^+ current (see Figure E2).

The Cellular Effects of CO Are Dependent on NO Formation

CO is known to activate NO synthase (NOS), thereby increasing NO production (28). Because dysfunctional NO signaling can lead to LQT-3-like symptoms and even sudden cardiac death (29, 30), we investigated whether NO might mediate the actions of CO on the late Na^+ current. To do this, we prevented NO formation by exposing cells to L-NAME for 30 minutes before and during CO exposure. Under these conditions, CO had no significant effect on Ca^{2+} transients (Figure 3A), the Na^+ current (Figure 3B), or evoked APs in isolated myocytes (Figure 3C). These findings strongly suggest that the proarrhythmic actions of CO are mediated by NO. Furthermore, we found that exposure to CO produced a time-dependent increase in the production of NO in DAF-2 loaded myocytes, which was prevented by L-NAME (Figure 4A). Because increases in the cardiac late Na^+ current have been associated previously with channel S-nitrosylation as a result of compartmentalized dysregulation of NO signaling (29), we investigated whether CO could lead to such modification of the channel protein. Using the biotin switch assay, we found that exposure of myocytes to either CORM-2 or the NO donor CysNO (but not iCORM) led to clearly detectable S-nitrosylation of the cardiac Na^+ channel, $\text{Na}_v1.5$ (Figure 4B). In addition, exposure to 2,2'-dithiobis(5-nitropyridine), a compound that mimics the S-nitrosylation by NO of TRPC5 channels (31), significantly reduced the peak Na^+ current and augmented the late Na^+ current in myocytes (Figure 4C). In addition, the reducing agent dithiothreitol (DTT), which can reverse S-nitrosylation, abolished CO-induced, EAD-like prolongations and oscillations of evoked Ca^{2+} transients (Figure 4D). In the absence of DTT, oscillatory effects were observed in seven (78%) of nine cells examined (see Figure E1), but in the presence of 2-mM DTT the incidence was dramatically reduced to 27% (3 of 11 cells; $P < 0.05$; chi-square test). This effect of DTT is not likely to be attributable to its additional action as an antioxidant, because the underlying mechanism of the action of CO (an increase in the amplitude of the late Na^+ current) was unaffected by 30-minute pretreatment of cells with another antioxidant, Mn(III)tetrakis(1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP; $100 \mu\text{M}$) (see Figure E3), which has previously been shown to prevent the reactive oxygen species (ROS)-mediated inhibition of the cardiac L-type current by CO (24). Together these data implicate increased NO formation, S-nitrosylation of $\text{Na}_v1.5$, and activation of the late Na^+ current in CO-induced EADs.

Ranolazine Reverses the Proarrhythmic Effects of CO in Myocytes

Ranolazine is an antiangina and antiarrhythmic drug in current therapeutic use. Although ranolazine can modulate a number of

Figure 5. Ranolazine (Ran) reverses the cellular effects of carbon monoxide (CO). (A) Line scan images of $[\text{Ca}^{2+}]_i$ before (control) and during superfusion with CO ($87.6 \pm 4.2 \mu\text{M}$ + CO) then during addition of $20 \mu\text{M}$ ranolazine (+CO + Ran). Right, normalized fluorescence taken from the same three images. Note CO induced oscillations, which were abolished by Ran. Below, bar graph, mean ($\pm$ SEM; bars, $n = 10$) increase in transient duration (at 50% of maximum) caused by CO, and reversal by Ran ($20 \mu\text{M}$). $^{**}P < 0.01$, paired t test (B) Na^+ currents evoked by depolarizations from -80 to -30 mV before and during exposure to $30 \mu\text{M}$ CO-releasing molecule (CORM)-2 alone, then in the additional presence of Ran. Bar graph; mean ($\pm$ SEM; $n = 7$) % change in peak and late Na^+ current evoked by CORM-2, before and during exposure to Ran. $^{**}P < 0.01$. (C) Action potentials (APs) before and during perfusion with $30 \mu\text{M}$ CORM-2 alone or in the presence of Ran ($20 \mu\text{M}$). Bar graph plots mean % change ($\pm$ SEM; bars) in normalized AP duration ($n = 7$) at the points of the AP indicated. $^{*}P < 0.05$; $^{**}P < 0.01$ (paired t test).

ion channel types, its dominant influence in the heart is inhibition of the late Na^+ current (26, 32, 33). Ranolazine (20 μM) abolished CO-induced changes in the Ca^{2+} transient and the late Na^+ current (Figures 5A and 5B), and prevented AP prolongation by CORM-2 (Figure 5C). These data suggest that such drugs as ranolazine, which target the late Na^+ current, might have therapeutic benefit in patients presenting with CO-induced cardiac rhythm disturbances.

CO Inhalation Is Proarrhythmic *In Vivo*

CO inhalation (500 ppm) (see the METHODS section in the online supplement) raised carboxyhemoglobin levels from $0.9 \pm 0.5\%$ ($n = 3$) to $32.4 \pm 1.6\%$ ($n = 5$) ($P < 0.001$) and consistently modified the ECG profile of freely moving rats within 10 minutes (Figure 6A). Heart rate increased only slightly and transiently during CO exposure (see Figure E4). The heart rate variability index SDNN was decreased (34.0 ± 1.45 milliseconds in sham vs. 25.3 ± 1.28 milliseconds, 30–60 min after CO exposure). The QTc interval was dramatically increased by CO (Figure 6B), without any modification of QRS duration, suggesting a prolonged repolarization time. QT variability was also increased strikingly by CO inhalation, as shown by representative example Poincaré plots (Figure 6C). The QT_{STV} were, respectively, 0.74 ± 0.14 milliseconds in sham versus 1.90 ± 0.25 milliseconds in CO rats ($P < 0.001$).

In accordance with these observations, the occurrence of ventricular extrasystoles was markedly increased in CO-treated animals compared with sham animals (Figure 7A). Remarkably, the CO-exposed rats exhibited spontaneous unsustained VT (two of six), whereas sham-treated rats were free of such arrhythmias (zero of eight). We further explored the CO-induced increase in susceptibility to arrhythmias by stimulating the β_1 -adrenergic pathway by injection of isoproterenol. During isoproterenol challenge, all rats exposed to CO (six of six) exhibited VT that developed into VF in all but one rat (Figure 7B). None of the sham animals developed VT or VF. A representative record of this fatal arrhythmic sequence is presented in Figure E5. Of the five animals that developed VF, three died and two were spontaneously cardioconverted (Figure 7B).

Ranolazine prevented lengthening of the QTc interval (Figure 6B) and the drastic increase of QT variability induced by CO exposure (Figure 6C). This improvement occurred independently of heart rate modification (see Figure E4) but concomitantly with an increase of the SDNN (29.05 ± 1.28 ; $P < 0.05$ vs. CO-exposed animals). The QT_{STV} during CO exposure in the ranolazine group was normalized to 1.26 ± 0.14 milliseconds ($P < 0.01$ vs. CO animals). Therefore, ranolazine decreased the number of ventricular extrasystoles occurring spontaneously, and also after isoproterenol injection (Figure 7A). None of the animals treated with ranolazine developed spontaneous VT. During the challenge with isoproterenol, ranolazine dramatically reduced the proarrhythmic effect of CO: only two rats developed VT, and only one degenerated into VF followed by death (Figure 7B).

DISCUSSION

Environmental sources of CO (e.g., road traffic) can result in exposure to CO levels up to 500 ppm (7). *In vivo* measurements have shown that after exposure to 500 ppm CO, the level of CO measured in rodent heart tissue is approximately 100 μM (34, 35). Therefore, in the present study, the levels of CO exposure (500 ppm *in vivo*; 87 μM *in vitro*) mimic the upper range of environmental exposure to CO and are below the levels typically considered representative of accidental poisoning (>1,000 ppm). We provide compelling evidence that levels of CO within this range can induce arrhythmias, which are consistent with clinical reports of abnormal ECG patterns in individuals exposed to CO (3, 13–15).

Our study is the first to suggest that CO-induced arrhythmias arise because of the stimulation of NO production by CO and a consequent increase in the late Na^+ current. The central role of NO/NOS is supported by evidence showing that (1) CO increases NO formation (Figure 4); (2) L-NAME blocks NO formation and all effects of CO on the late Na^+ current and Ca^{2+} transients (Figure 3); (3) the effects of CO on the late Na^+ current are similar to those of 5-NO-2-PDS (Figure 4C), a compound known to mimic S-nitrosylation of channels (31); and (4) the reducing agent DTT reverses the detrimental effects of CO (Figure 4D).

Figure 6. Carbon monoxide (CO) increases the QT interval and variability *in vivo*. (A) Typical examples of ECGs recorded in sham, CO-exposed, and CO-exposed ranolazine (Ran)-treated rats. (B) Bar graph showing mean ($\pm$ SEM; bars) QTc intervals under control conditions, or after CO exposure (30 and 60 min) and after isoproterenol challenge ($1 \text{ mg} \cdot \text{kg}^{-1}$ intraperitoneally), as indicated, in sham (black bars), CO-exposed (white bars), and CO-exposed Ran-treated groups (hatched bars). $**P < 0.01$ and $***P < 0.001$ versus sham; $\#P < 0.05$, $##P < 0.01$, and $###P < 0.001$ versus preceding period in the same group; $\Psi\Psi P < 0.01$ versus untreated Ran animals. (C) Representative traces of QT variability, presented as Poincaré plots.

Figure 7. Isoproterenol potentiates the proarrhythmic effects of carbon monoxide (CO). (A) Mean ($\pm$ SEM; bars) number of ventricular ectopic beats (VEB) occurring spontaneously or during isoproterenol challenge in the same experimental groups as indicated in Figure 6B. (B) Percentage of animals developing ventricular tachycardia (VT), ventricular fibrillation (VF), and sudden arrhythmic death during the test of arrhythmogenic susceptibility by isoproterenol injection (1 mg/kg intraperitoneally) in the same experimental groups as indicated in Figure 6B. *** P < 0.001 versus sham; ## P < 0.01 and ### P < 0.001 versus preceding period in the same group; Ψ P < 0.05, $\Psi\Psi$ P < 0.01, and $\Psi\Psi\Psi$ P < 0.001 versus untreated ranolazine animals.

Cardiac Na^+ channels form part of a macromolecular complex, which also includes a Ca-ATPase, syntrophin, and nNOS, and previous work has shown that NO generated within this complex can lead to nitrosylation of the channel protein and induction of the late Na^+ current (29). Indeed, exogenous NO can also induce a persistent cardiac Na^+ current (36). In light of these studies, we propose that CO exposure likely stimulates nNOS in cardiac myocytes, causing a localized rise of NO, leading to nitrosylation, augmentation of the late Na^+ current, and consequent arrhythmias. This interpretation is consistent with the fact that the only known biologic targets of CO are heme-containing proteins (37), such as NOS isoforms, and that CO has been proposed as an activator of NO production (38, 39).

It is likely that CO might have other related effects within the cell, and some of these could also contribute to activation of the late Na^+ current (e.g., previous work has shown that nNOS knockout or inhibition reduces spark frequency and lowers fractional SR Ca^{2+} release in mice) (40). CO induced activation of SR localized nNOS might therefore be expected to increase the SR Ca^{2+} leak and fractional Ca^{2+} release. Such effects might then lead to activation of CaMKII, which has been linked to an

increase in the late Na^+ current (41). However, in the present study, CO had no significant effect on resting $[\text{Ca}^{2+}]_i$, the amplitude of the triggered Ca^{2+} transient, or fractional Ca^{2+} release (see Figure E6) before an EAD had first occurred (after initiation of EADs, enhanced Ca^{2+} entry will have secondary effects). CO also failed to have any significant impact CO on spontaneous Ca^{2+} waves, SR Ca^{2+} content, and Ca^{2+} spark characteristics in saponin-permeabilized myocytes (see Figure E7). Furthermore, inhibition of CaMKII failed to prevent CO-initiation of EADs (see Figure E8). This suggests that changes in SR function and consequent activation of CaMKII are not primary events leading to activation of EADs by CO. Another possibility is that CO might activate eNOS. However, eNOS activation has been shown to blunt β_1 -adrenergic responses and inhibit EADs (42), which is not consistent with the effects of CO reported here.

In addition to its toxic actions, CO is recognized as physiologically important, exerting effects by interactions with intracellular signaling pathways and target proteins (37, 43). We previously suggested that CO may have an inhibitory effect on the L-type Ca^{2+} channel current in myocytes (24). This action resulted from elevated mitochondrial ROS and may provide a degree of myocardial protection, which likely contributes to the known beneficial effects of heme oxygenase-1, the inducible form of the enzyme, which generates endogenous CO as a result of heme degradation: HO-1 overexpression is protective against cardiac ischemia and reperfusion injury (44). The fact that CO has dual and (in some circumstances) opposing effects on the myocardium may explain why clinical reports of CO-induced arrhythmias have not always been reproduced in experimental models (i.e., the probability of developing CO-induced arrhythmias may depend on the relative balance between opposing actions of CO on the L-type Ca^{2+} and the late Na^+ current). However, in the present study, proarrhythmic effects of CO were consistently observed in conscious rats, suggesting that effects on the late Na^+ current dominate in this model (Figures 6 and 7). Furthermore, CO exposure led to a pronounced lengthening of ventricular repolarization time (QTc interval) and an increased QT variability (QT_{STV}), factors that are associated with high occurrences of spontaneous ventricular arrhythmias. QT lengthening and an increase of QT variability are also well-known prognostic markers of ventricular arrhythmic events likely to be associated with an increase of the late Na^+ current (45, 46).

Ranolazine is a well-characterized inhibitor of the late Na^+ current with significant selectivity over the transient Na^+ current (26, 33). In addition to its action on Nav1.5, ranolazine is known to modulate other ion channels, including K^+ channels (32). However, inhibition of K^+ channels is in itself proarrhythmic. Therefore, the ability of ranolazine to prevent the effects of CO is most consistent with the ability of the drug to inhibit the late Na^+ current. More importantly, however, our data suggest that ranolazine is likely to be of value in the treatment of CO-induced arrhythmias; all of the proarrhythmic effects of CO were either prevented completely by ranolazine or dramatically reduced (Figures 5–7). The beneficial effects of ranolazine were most striking *in vivo*, where the incidence of CO-induced sudden arrhythmic death decreased markedly. Furthermore, the ability of the drug to normalize repolarization time (Figure 6) is consistent with its proposed antiarrhythmic mechanism (46). A recent large-scale epidemiologic study has highlighted the fact that, in addition to common occurrences of acute CO poisoning, environmental CO exposure is a major cause of emergency hospital admissions, with cardiac rhythm disturbances identified as a prominent feature (1). We propose that ranolazine or related drugs that act by inhibiting the late Na^+ current may have therapeutic benefit in such cases.

In summary, the present study has identified the molecular basis for the proarrhythmic effects of acute CO exposure, which is distinct from chronic CO-induced remodeling of cardiac function (6) and is increasingly recognized as a common phenomenon of clinical importance. We also provide compelling evidence that CO exposure *in vivo* can, particularly during additional stress, such as increased β adrenergic stimulation, lead to fatal arrhythmias. Our data indicate that CO causes a striking increase in the late Na^+ current arising from NOS activation, NO formation, and S-nitrosylation of the $\text{Na}_v1.5$ channel protein. We propose that such compounds as ranolazine, which target the late Na^+ current, should be explored further as an antiarrhythmic approach for the treatment of acute CO exposure.

Author disclosures are available with the text of this article at www.atsjournals.org.

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