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Carbon Monoxide and Lethal Arrhythmias

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Includes the Commentary of the Institute's Health Review Committee

Research Report Number 36

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HEALTH REVIEW COMMITTEE'S COMMENTARY Health Effects Institute

Carbon Monoxide and Lethal Arrhythmias

Jay P. Farber[1], Peter J. Schwartz, Emilio Vanoli, Marco Stramba-Badiale, Gaetano M. De Ferrari

ABSTRACT

The effect of acute exposure to carbon monoxide on ventricular arrhythmias was studied in a previously described chronically maintained animal model of sudden cardiac death. In 60 percent of dogs with a healed anterior myocardial infarction, the combination of mild exercise and acute myocardial ischemia induces ventricular fibrillation. The events in this model are highly reproducible, thus allowing study by internal control analysis. Dogs that develop ventricular fibrillation during the test of exercise and acute myocardial ischemia are considered at high risk for sudden death and are defined as "susceptible"; dogs that survive the test without a fatal arrhythmia are considered at low risk for sudden death and are defined as "resistant."

In the current study, the effects of carboxyhemoglobin levels ranging from 5 to 15 percent were tested in resistant and susceptible dogs. A trend toward higher heart rates was observed at all levels of carboxyhemoglobin, although significant differences were observed only with 15 percent carboxyhemoglobin. This trend was observed at rest and during exercise in both resistant and susceptible dogs. In resistant animals, in which acute myocardial ischemia is typically associated with bradycardia even under the control condition, this reflex response occurred earlier and was augmented after exposure to carbon monoxide. This effect may depend on the increased hypoxic challenge caused by carbon monoxide, and thus on an augmentation of the neural reflex activation or a sensitization of the sinus node to acetylcholine induced by hypoxia. In both resistant and susceptible dogs, carbon monoxide exposure induced a worsening of ventricular arrhythmias in a minority of cases. This worsening was not reproducible in subsequent trials.

These data indicate that acute exposure to carbon monoxide is seldom arrhythmogenic in dogs that have survived myocardial infarction. Nevertheless, the observation that carbon monoxide exposure increases heart rate at rest and during moderate exercise may have clinical implications relevant to patients with coronary artery disease.

INTRODUCTION

Carbon monoxide (CO)[2] is a widely spread pollutant; it is a constituent of car and heating system exhaust and of cigarette smoke. A causal link between high concentrations of CO in the atmosphere and an increase in cardiovascular mortality has been proposed (Cohen et al. 1969; Eversole et al. 1974; Scharf et al. 1974; Kaufman et al. 1983; Atkins and Baker 1985). Indeed, it has been suggested that even acute air pollution episodes are hazardous to patients with cardiovascular problems. A significant association between community carbon monoxide concentration and mortality has been reported in Los Angeles (Lester and Seskin 1970; Hexter and Goldsmith 1971). A pilot study in Denver reported an increased risk for cardiorespiratory complaints on those winter days with a higher atmospheric concentration of CO (Eversole et al. 1974). These studies could not specifically determine whether or not environmental CO exposure contributes to the more than 400,000 sudden cardiac deaths occurring each year in the United States, mainly in patients with coronary artery disease.

A sudden reduction of the oxygen supply to the heart is often the critical event underlying the occurrence of ventricular fibrillation and sudden cardiac death. Many patients die within minutes after the appearance of symptoms of myocardial ischemia (Baum et al. 1974), and evidence for the occurrence of an ischemic episode just prior to the development of lethal arrhythmias (Roelandt et al. 1984) is growing.

Elevated sympathetic activity may facilitate the occurrence of an ischemic episode and is arrhythmogenic (Janse et al. 1985). Acute myocardial ischemia reflexively increases sympathetic activity thus enhancing arrhythmogenicity (Malliani et al. 1969; Schwartz et al. 1976). The role of the sympathetic nervous system in the genesis of malignant arrhythmias has been demonstrated and reviewed in detail (Schwartz and Priori 1990). Under normal conditions, the heart derives over 90 percent of its energy requirements from aerobic metabolism (Kobayashi and Neely 1979). Within a minute after occlusion of a coronary artery, all aspects of cardiac function are impaired and a marked electrical dishomogeneity of the myocardium occurs; in this condition, sympathetic hyperactivity further increases cardiac electrical instability, and ventricular fibrillation is more likely to develop.

Oxygen supply to the acutely ischemic myocardium, through collaterals, may reduce the effective size of the ischemic area and thereby reduce the risk of life-threatening arrhythmias. Maximal dilatation of collaterals has been shown to occur within seconds after an acute coronary oc-

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[2] A list of abbreviations appears at the end of this report for your reference.

clusion in dogs (Bishop et al. 1976; Marcus et al. 1976). Even moderate levels of carboxyhemoglobin (COHb) may impair this compensatory mechanism by reducing the oxygen-carrying capacity of the blood and thus its ability to deliver oxygen to the heart tissue.

Little is known of the potential relationship between CO and cardiac arrhythmias. A few experimental studies have suggested that CO lowers the threshold for ventricular fibrillation during acute myocardial ischemia or during the first few days after myocardial infarction (Bellet et al. 1972; DeBias 1976; DeBias et al. 1976; Aronow et al. 1978). The clinical relevance of the studies is limited by the use of anesthesia (Vatner 1978), and by the use of a controversial technique to estimate the ventricular fibrillation threshold (Elharrar and Zipes 1977; Verrier et al. 1978). A clinically relevant experimental model for sudden death has been developed over the last few years in our laboratories (Schwartz et al. 1984). This model incorporates three main factors known to be involved in the genesis of sudden cardiac death: a healed myocardial infarction, acute myocardial ischemia, and physiologically elevated sympathetic activity.

In this experimental preparation, dogs with a prior myocardial infarction engage in a submaximal exercise test, at the end of which a two-minute coronary occlusion is initiated while the dogs are still running. The outcome of the exercise-plus-ischemia test (survival or ventricular fibrillation) allows the identification of two subgroups of animals, one with low risk and one with high risk of sudden death. The events in this model are highly reproducible over time, and allow an internal control analysis, thus overcoming the limitations of group comparison studies.

In conscious post-myocardial-infarction dogs at low risk and at high risk of sudden death, we have investigated the effects of acute exposure to CO on the response to myocardial ischemia during exercise. Results have been presented (Vanoli et al. 1986; De Ferrari et al. 1987) and published (Vanoli et al. 1989).

SPECIFIC AIMS

The overall objective of this study was to determine whether or not acute exposure to CO would increase susceptibility to life-threatening arrhythmias in dogs with a healed anterior myocardial infarction.

This hypothesis was tested in two separate groups of dogs: one at low risk of sudden death, in which the combination of acute myocardial ischemia and exercise had not induced ventricular tachyarrhythmias under control conditions; the other at high risk of sudden death, in which ventricular fibrillation had occurred during the exercise-plus-ischemia test performed under control conditions.

Both groups of animals were challenged with CO levels consistent with and slightly higher than those observed in heavy smokers.

METHODS

Mongrel dogs (102) of either gender weighing 15 to 25 kg were used for the study. Very young (less than one year) and old dogs were excluded by dentition.

SURGICAL PREPARATION

Anesthesia was induced with 25 mg of intravenously administered thiopental sodium (Pentothal; Abbott Laboratories, North Chicago, IL) per kilogram of body weight and was maintained by the inhalation of a mixture of halothane, nitrous oxide, and oxygen. With aseptic procedures, a left thoracotomy was performed in the fourth intercostal space. The pericardium was opened and the heart was suspended in a cradle. The left circumflex coronary artery was carefully dissected from the surrounding epicardial fat, and both a 20-MHz continuous-wave Doppler flow transducer and a hydraulic occluder were placed around the vessel. Isolated silver-coated copper wires were sutured to the epicardial surface of both the left and right ventricles to record the electrocardiogram. A Tygon catheter was positioned in the aortic arch to record blood pressure and to collect blood samples.

A modified two-stage occlusion was performed on the left anterior descending coronary artery below the first diagonal branch to produce a myocardial infarction. The vessel was partially occluded for 20 minutes and then completely tied off.

All lead wires were tunneled under the skin to exit from the dorsal surface of the neck. Pentazocine lactate (Talwin, 30 mg; Winthrop Laboratories, New York, NY) was given intramuscularly approximately every 8 hours for the first 24 hours to control postoperative pain. We adhered to the guidelines of the American Physiological Society on the care and treatment of laboratory animals. A Konigsberg pressure cell (Konigsberg Instruments, Pasadena, CA) was successfully implanted in the left ventricle of three animals to measure contractile function (dP/dt).

EXPERIMENTAL PROTOCOL

One month after myocardial infarction was induced, all animals performed a submaximal exercise stress test. The

animals ran on a motor-driven treadmill for 12 to 18 minutes while the workload increased every 3 minutes (4.8 kmph, 0 percent grade during first 3 minutes; 6.4 kmph, 16 percent grade during the last 3-minute period). At the beginning of the last minute of exercise, the left circumflex coronary artery was occluded; the treadmill was then suddenly stopped and the occlusion was maintained for another minute. Coronary occlusion was begun either when the animal completed 17 minutes of exercise or when the heart rate had reached a level close to 220 beats/minute. Large steel plates (Model 62w17, American Optical Corp.) were placed across the animal's chest so that electrical defibrillation could be performed with minimal delay.

Dogs that experienced ventricular fibrillation during this test were identified as at high risk for sudden cardiac death and defined as "susceptible." Dogs that did not have arrhythmias were identified as at low risk and defined as "resistant."

CARBON MONOXIDE PROTOCOL

Low-Risk (Resistant) Animals

The dogs that survived the exercise-plus-ischemia test (low-risk subgroup) performed two or three additional exercise-plus-ischemia tests with different levels of COHb saturation, in a random sequence. Forty-eight hours were allowed for recovery between tests. Before the test, the dogs were exposed to a mixture of 1,500 parts per million (ppm) CO in air (from a premixed cylinder) through a custom-made facial mask. The facial mask and the cylinder were connected by a demand valve and a low-resistance respiratory valve. This procedure raised blood COHb levels by approximately 0.5 percent saturation per minute. Initially, we studied COHb saturations of 10 and 15 percent; on the basis of those responses, the 5 percent COHb saturation level was added subsequently. Blood samples for COHb measurements were taken from the aortic catheter at the beginning and end of the exposure and at the end of the exercise test.

High-Risk (Susceptible) Animals

In each animal that developed ventricular fibrillation during the control exercise-plus-ischemia test, the threshold level of work for malignant ventricular arrhythmias (that is, the heaviest workload that was not associated with ventricular tachycardia or fibrillation) was determined.

To accomplish this goal, we repeated the exercise-plus-ischemia test, progressively shortening the duration of exercise and therefore reducing the workload at which the coronary artery occlusion was performed. If ventricular fibrillation still occurred at the lowest workload (five minutes of exercise at 4.8 kmph and 0 percent grade), the duration of the coronary artery occlusion was reduced stepwise by 30 seconds to a minimum of 30 seconds (15 seconds while the dog was running and 15 seconds after the treadmill stopped). If ventricular fibrillation still occurred in this setting, a two-minute coronary artery occlusion (or a shorter occlusion if a two-minute occlusion was associated with malignant arrhythmias) was performed at rest. Dogs were allowed to recover for at least 48 hours between tests.

Once the threshold level for arrhythmias was determined, this same level was used after exposure to CO (as described in the Low-Risk [Resistant] Animals section) with 15 percent COHb. Another test without CO exposure was then performed to ensure that ventricular arrhythmias did not occur at the threshold. Finally, to confirm the reproducibility of the model, the dogs were tested again to confirm that malignant arrhythmias were still present during occlusions above the threshold (see Table 4).

DATA RECORDING

During each test, flow velocity in the left circumflex coronary artery, arterial blood pressure, electrocardiogram, and heart rate were recorded on an eight-channel R 612 Beckman (Palo Alto, CA) direct-writing oscillograph. The signals were also recorded on a magnetic tape (FR-1300; Ampex, Redwood City, CA) for later analysis.

CARBON MONOXIDE ANALYSIS

The arterial blood samples were drawn in 3-mL plastic syringes in which the dead space was filled with heparinized saline. The samples were capped using three-way stopcocks. Most of the samples were analyzed for COHb levels immediately after being drawn. In those few instances where analysis could not be done immediately, the samples were temporarily placed on ice. Analyses from initial studies were performed using an IL 182 CO-Oximeter (Instrumentation Laboratories, Lexington, MA); this instrument was replaced with an IL 282 model. The CO-Oximeters had analytical circuitry designed to evaluate dog hemoglobin. The instruments were periodically recalibrated by factory technicians. To check the instruments and perform the user-adjustable calibrations, the applicable standard and test solutions were utilized according to the basic recommendations of Instrumentation Laboratories. Calibration and standard checks were performed more frequently, depending upon use, stability, and maintenance of the instrument (for example, cuvette cleaning). As an added check on the reliability of the IL 182, frequent cross analysis of blood samples was performed against an IL 282.

STATISTICAL ANALYSIS

Data are reported in the text as means ± SD. Statistical analysis was performed by the analysis of variance (ANOVA) for repeated measures followed by the Tukey test for multiple comparisons. Student's t test was used for paired observations. In the resistant group, the fact that a few animals did not perform exercise-plus-ischemia tests at all levels of COHb posed a problem. We analyzed together (using ANOVA) the 11 dogs that were studied at control, 10 percent, and 15 percent COHb levels. Three dogs that did not undergo the 10 percent study were not included in the analysis for statistical reasons; however, their results under control conditions and at 15 percent COHb were indistinguishable from those of the other animals. We also analyzed together (using ANOVA) the six dogs that were studied at the control and 5 percent COHb levels; one of these animals served as control in both groups because it was studied at all levels of COHb. The significance level was $p < 0.05$.

RESULTS

Data are presented separately for resistant and susceptible dogs. In the first part of the study only resistant dogs were used, and in the second part only susceptible dogs were used.

LOW-RISK (RESISTANT) ANIMALS

To obtain a low-risk population of animals, 50 dogs underwent surgery to place the permanent coronary occlusion and chronic instrumentation. Fourteen of them (28 percent) died within the first 72 hours after myocardial infarction. Six animals (12 percent) were excluded for technical reasons (mainly due to malfunction of the occluder positioned around the circumflex coronary artery). One month after surgery, the remaining 30 dogs underwent the exercise-plus-ischemia test. Of these, 14 dogs (47 percent) had ventricular fibrillation; they were defibrillated and, having been identified as animals at high risk, were assigned to another protocol. The CO exposure protocol was performed in the 16 dogs that did not have life-threatening arrhythmias during the control test and were, therefore, identified as animals at low risk for sudden death. The outline of the events after surgery and of the study design is represented in Figure 1.

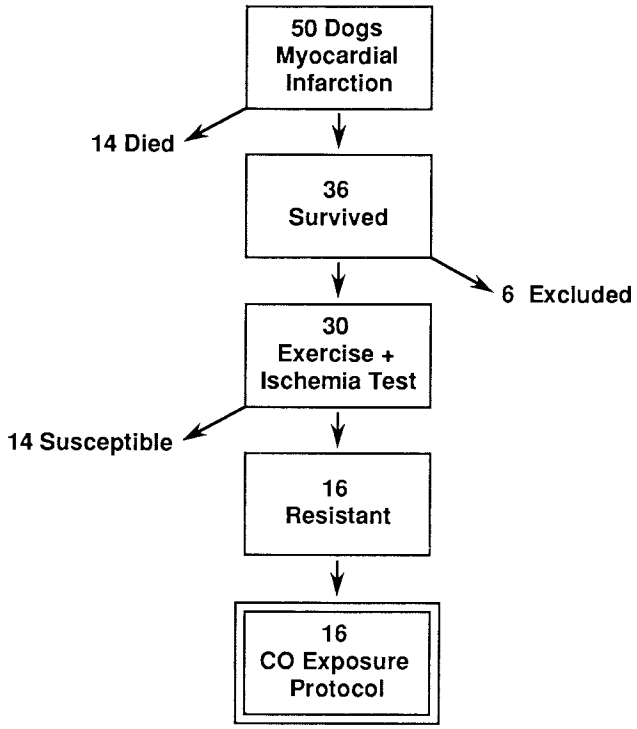

Figure 1. Outline of the study of resistant animals. Fourteen dogs died during the first 72 hours after myocardial infarction and six were subsequently excluded for technical reasons. The 16 dogs that proved to be resistant to ventricular fibrillation—and were, therefore, identified as low-risk animals—constitute the population of the first part of the study.

Carboxyhemoglobin Levels

The dogs were exposed to the CO-air mixture for sufficient time to allow COHb saturation to reach approximately 5, 10, or 15 percent. At the beginning of the exercise-plus-ischemia test, the COHb saturation levels were actually 6.0 ± 0.6 percent, 13.1 ± 1.6 percent, and 20.8 ± 1.8 percent, respectively. At the end of the coronary occlusion (one minute after cessation of exercise), the COHb saturation levels were 5.0 ± 0.6 percent, 10.2 ± 1.7 percent, and 15.0 ± 1.2 percent, respectively. For simplicity, the results will be presented as being obtained with 5, 10, and 15 percent COHb levels.

Ten dogs were studied at 10 and 15 percent COHb levels, three at 5 and 15 percent levels, two at the 5 percent level, and one at 5, 10, and 15 percent levels. This nonuniform pattern resulted from the fact that in a few dogs, after some trials performed according to the randomized sequence, the hydraulic occluder ruptured, so the remaining trials could not be performed.

Heart Rate

With 15 percent COHb, heart rate was higher ($p < 0.05$) when compared to the control values both at rest (139 ± 22 versus 117 ± 28 beats/minute) and during exercise (245 ±

18 versus 219 ± 11 beats/minute at the highest workload). Also, with 5 and 10 percent COHb, heart rate was higher than in the control condition, but the difference did not reach statistical significance (Figures 2 and 3; Tables 1 and 2).

The heart rate response to acute myocardial ischemia during exercise was altered importantly by all three levels of COHb (Table 3). For all resistant animals (n = 16) in the control coronary occlusion trials, heart rate during the first 30 seconds of ischemia was practically unmodified (+2 ± 30 beats/minute versus the heart rate level just prior to coronary artery occlusion); heart rate declined by the end of the first minute of ischemia (−13 ± 30 beats/minute). After exposure to CO, the reduction in heart rate occurred much earlier and was of greater magnitude (Figures 4 and 5). The difference in the heart rate response after 30 seconds of ischemia was significant ($p < 0.05$) for all three levels of COHb: 5 percent, −9 ± 21 beats/minute; 10 percent, −19 ± 36 beats/minute; and 15 percent, −30 ± 26 beats/minute. An example is shown in Figure 6.

After one minute of ischemia, a difference in heart rate, which was not statistically significant, was present only with the 15 percent level of COHb.

Blood Pressure

Systolic arterial pressure was not affected by CO exposure at any level of saturation, either at rest (control, 138 ± 11 mm Hg; 5 percent COHb, 129 ± 15 mm Hg; 10 percent COHb, 134 ± 2 mm Hg; 15 percent COHb, 139 ± 8 mm Hg) or at the maximum workload during exercise (control, 159 ± 11 mm Hg; 5 percent COHb, 158 ± 24 mm Hg; 10 percent COHb, 156 ± 14 mm Hg; 15 percent COHb, 153 ± 20 mm Hg).

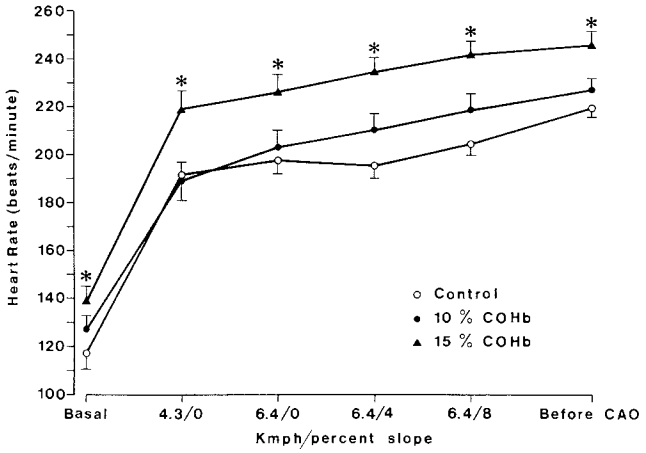

Figure 2. Analysis of heart rate during exercise for the 11 resistant animals that performed the control test with 10 and 15 percent COHb. Heart rate was significantly higher (* = $p < 0.05$) with 15 percent COHb both at rest and throughout exercise.

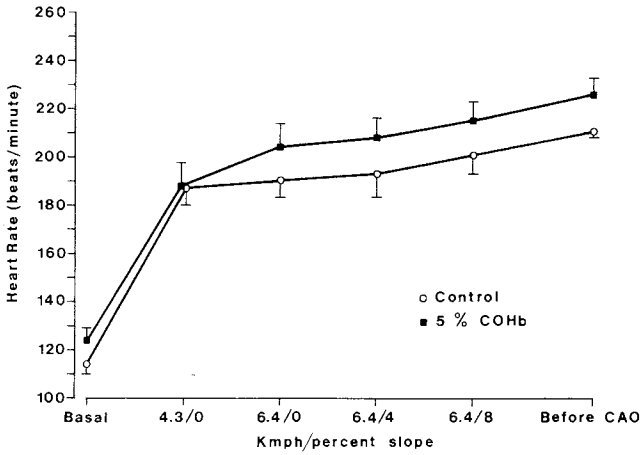

Figure 3. Analysis of heart rate during exercise for the six resistant animals that performed the control and 5 percent COHb tests. Heart rate was always higher after CO exposure, although the difference was not significant.

During myocardial ischemia the reduction in blood pressure was not significantly different among the various groups.

Cardiac Arrhythmias

During the control trials, no arrhythmias (fewer than five isolated premature ventricular contractions) were observed in 14 out of 16 animals (87.5 percent). The other two animals (12.5 percent) had repetitive nonfatal ventricular arrhythmias (three to four ventricular couplets) during the exercise-plus-ischemia test. After exposure to CO, one of the two animals that had repetitive ventricular arrhythmias under control conditions developed ventricular tachycardia and the other developed ventricular fibrillation (Figures 7 and 8). These life-threatening arrhythmias occurred at the 15 percent level of COHb. During the CO test at the 15 percent COHb level, 1 of the 14 animals that had no arrhythmias in the control test developed electromechanical dissociation, a situation in which electrical activation of the heart does not produce the consequent mechanical activation. This animal required cardiac massage to prevent irreversible brain damage and death. However, in these three animals, subsequent tests with room air or 15 percent COHb failed to induce arrhythmias.

The remaining 13 animals (81 percent) that had no arrhythmias during the control trial showed the same pattern after CO exposure. At any level, they remained resistant.

HIGH-RISK (SUSCEPTIBLE) ANIMALS

In the second part of the study (second year), fifty-two randomly selected dogs underwent surgery (Figure 9).

Table 1. Heart Rates (in Beats per Minute) of Resistant Dogs at Rest and During Exercise Under Control Conditions and with 10 and 15 Percent Carboxyhemoglobin Levels

Dog No.	Control	10 Percent COHb	15 Percent COHb
Rest			
CO1	100	120	150
CO4	110	110	130
CO9	115	120	125
CO10	105	105	120
CO13	180	150	185
CO15	100	120	110
CO21	120	135	135
CO23	120	150	120
SD299	120	120	150
SD298	100	110	140
SD303	120	160	160
Average	117	127	139
SD	23	19	22
4.8 kmph/0 Percent Slope			
CO1	210	200	245
CO4	162	170	210
CO9	165	160	180
CO10	195	160	205
CO13	210	210	230
CO15	190	200	235
CO21	190	215	240
CO23	205	165	185
SD299	200	175	210
SD298	190	180	210
SD303	190	250	260
Average	192	190	219
SD	16	28	25
6.4 kmph/0 Percent Slope			
CO1	215	200	255
CO4	195	200	210
CO9	175	170	195
CO10	190	175	210
CO13	215	220	240
CO15	190	210	230
CO21	190	220	248
CO23	205	190	190
SD299	200	200	230
SD298	200	200	220
SD303	200	250	260
Average	198	203	226
SD	12	22	24

(*Table continues next column.*)

Table 1. (*continued*)

Dog No.	Control	10 Percent COHb	15 Percent COHb
6.4 kmph/4 Percent Slope			
CO1	215	215	245
CO4	185	220	225
CO9	190	180	195
CO10	175	185	245
CO13	210	215	235
CO15	180	210	235
CO21	210	225	250
CO23	170	190	210
SD299	200	200	260
SD298	210	210	220
SD303	200	260	260
Average	195	210	235
SD	16	22	21
6.4 kmph/8 Percent Slope			
CO1	210	220	250
CO4	190	230	230
CO9	195	190	215
CO10	180	180	240
CO13	210	225	235
CO15	190	230	250
CO21	235	230	250
CO23	205	190	210
SD299	210	230	270
SD298	210	220	230
SD303	215	260	270
Average	205	219	241
SD	15	23	20
Before Coronary Artery Occlusion			
CO1	230	234	255
CO4	215	230	240
CO9	220	210	230
CO10	210	210	255
CO13	220	230	235
CO15	230	240	250
CO21	245	235	250
CO23	210	190	210
SD299	210	230	270
SD298	210	220	230
SD303	215	260	245
Average	220	226	245
SD	11	18	18

Seventeen died within the first 72 hours after myocardial infarction. Five dogs were excluded for technical reasons (mainly due to malfunction of the occluder positioned around the left circumflex coronary artery). One month after myocardial infarction, the remaining 30 dogs underwent the exercise-plus-ischemia test already described.

Table 2. Heart Rates (in Beats per Minute) of Resistant Dogs at Rest and During Exercise Under Control Conditions and with 5 Percent Carboxyhemoglobin Level

Dog No.	Control	5 Percent COHb
Rest		
SD295	110	105
SD297	120	130
SD298	100	120
SD299	120	120
SD303	115	120
SD304	120	140
Average	114	123
SD	8	12
4.8 kmph/0 Percent Slope		
SD295	160	170
SD297	200	200
SD298	190	190
SD299	200	170
SD303	180	180
SD304	190	220
Average	187	188
SD	15	19
6.4 kmph/0 Percent Slope		
SD295	160	175
SD297	200	210
SD298	200	215
SD299	200	215
SD303	180	180
SD304	200	230
Average	190	204
SD	16	22
6.4 kmph/4 Percent Slope		
SD295	160	195
SD297	200	200
SD298	200	210
SD299	210	215
SD303	180	190
SD304	200	235
Average	192	208
SD	18	16
6.4 kmph/8 Percent Slope		
SD295	180	200
SD297	210	200
SD298	210	220
SD299	210	240
SD303	180	195
SD304	215	245
Average	201	217
SD	16	22
Before Coronary Artery Occlusion		
SD295	210	210
SD297	210	210
SD298	210	220
SD299	210	240
SD303	210	230
SD304	215	245
Average	211	226
SD	2	15

(Table continues next column.)

Of these 30 dogs, 13 did not have arrhythmias; they were defined as resistant (at low risk for sudden death) and assigned to another protocol. Seventeen dogs had ventricular fibrillation during the test; they were considered susceptible (at high risk for sudden death) and assigned to the CO exposure protocol.

These susceptible dogs underwent further exercise-plus-ischemia tests to assess the threshold level. Of the 17 dogs, 7 died of ventricular fibrillation while we attempted to identify a threshold level, and 1 dog was excluded because the occluder failed during the study. In the remaining 9 dogs, we were able to establish threshold values. In 1 dog, the threshold level was found at 6.4 kmph and 12 percent grade. In 4 dogs, the threshold level was determined at the lowest workload (4.8 kmph and 0 percent grade) with shortened occlusions (see Methods section): in 2 of the dogs, coronary artery occlusion lasted one minute, starting 30 seconds before the end of exercise; in the other 2 dogs, a coronary artery occlusion of 30 seconds, starting 15 seconds before the end of exercise, was performed. In the other 4 dogs in which ventricular fibrillation had occurred during the exercise protocol, the threshold level could only be determined at rest, even with shortened occlusion times: in 3 dogs the coronary artery occlusion lasted two minutes; in 1 dog, it was maintained for 30 seconds. Therefore, these 9 dogs entered the CO exposure protocol. An example of the CO exposure protocol is shown for 1 dog in Table 4. Of the 5 dogs that had threshold levels during exercise, 3 were also studied under resting conditions; therefore, the data for the resting coronary artery occlusion are based on 7 animals instead of only the 4 with threshold levels at rest. The purpose of studying some dogs both during exercise and at rest was to acquire information on the hemodynamic end reflex responses of high-risk dogs under different experimental conditions.

Table 3. Effects of 5, 10, and 15 Percent Carboxyhemoglobin on Heart Rate Changes During Acute Myocardial Ischemia in Resistant Dogs

Dog No.	Control	5 Percent COHb
Difference in Heart Rate, 30-Second Coronary Artery Occlusion		
SD295	- 35	- 40
SD297	10	0
SD298	20	0
SD299	0	- 30
SD303	5	0
SD304	25	15
Average	4	- 9
SD	21	21
Difference in Heart Rate, 60-Second Coronary Artery Occlusion		
SD295	- 50	- 40
SD297	- 10	10
SD298	0	0
SD299	- 45	- 60
SD303	- 50	- 50
SD304	5	5
Average	- 25	- 23
SD	26	31

Dog No.	Control	10 Percent COHb	15 Percent COHb
Difference in Heart Rate, 30-Second Coronary Artery Occlusion			
CO1	25	0	- 10
CO4	20	0	- 45
CO9	40	15	- 40
CO10	45	15	23
CO13	5	- 65	- 10
CO15	- 15	- 55	- 60
CO21	- 75	- 85	- 55
CO23	- 25	- 5	- 30
SD299	0	- 35	- 60
SD298	20	- 20	- 10
SD303	25	20	- 30
Average	6	- 20	- 30
SD	34	36	26
Difference in Heart Rate, 60-Second Coronary Artery Occlusion			
CO1	5	6	- 35
CO4	- 15	- 20	- 50
CO9	20	30	- 80
CO10	30	15	- 30
CO13	20	0	0
CO15	10	- 30	- 30
CO21	- 25	- 85	- 25
CO23	- 65	- 10	- 45
SD299	- 45	- 40	- 70
SD298	0	- 40	- 30
SD303	- 50	- 20	- 20
Average	- 11	- 18	- 37
SD	32	32	23

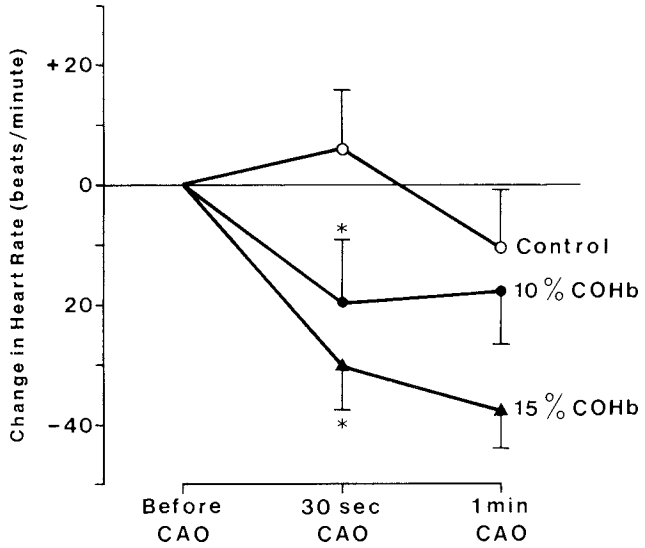

Figure 4. Heart rate response to coronary artery occlusion in the 11 resistant dogs with 10 and 15 percent COHb. Heart rate before coronary artery occlusion was 219 ± 11 beats/minute for control conditions, 226 ± 18 beats/minute for 10 percent COHb, and 245 ± 18 beats/minute for 15 percent COHb. After exposure to CO, coronary artery occlusion caused a pronounced decrease (* = $p < 0.05$) in heart rate of rapid onset despite continuation of exercise.

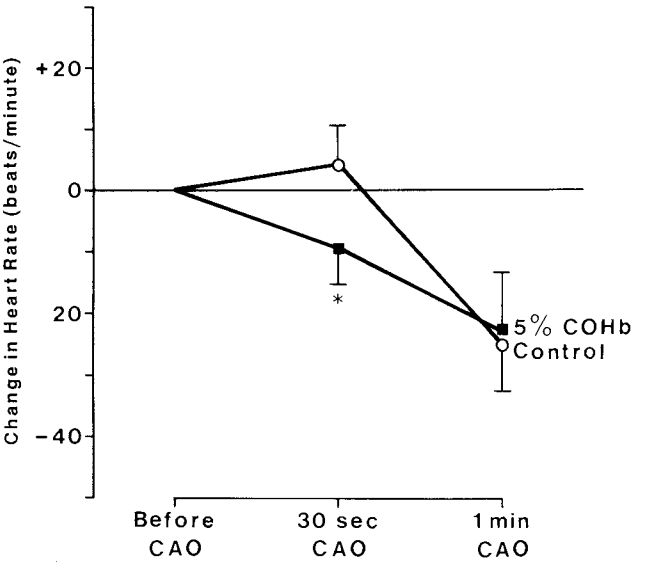

Figure 5. Heart rate response to coronary artery occlusion in the six resistant dogs with 5 percent COHb. Heart rate before coronary artery occlusion was 211 ± 2 beats/minute for control conditions, and 226 ± 15 beats/minute for 5 percent COHb. With this saturation level, the heart rate response to ischemia was significantly different (* = $p < 0.05$) 30 seconds after the coronary occlusion.

Carboxyhemoglobin Levels

In the five animals that underwent exercise-plus-ischemia tests after exposure to CO, the COHb levels were 19.7 ± 1.1 at the beginning of the exercise and 15.3 ± 1.1 after the end of the occlusion. Carboxyhemoglobin levels just prior to the

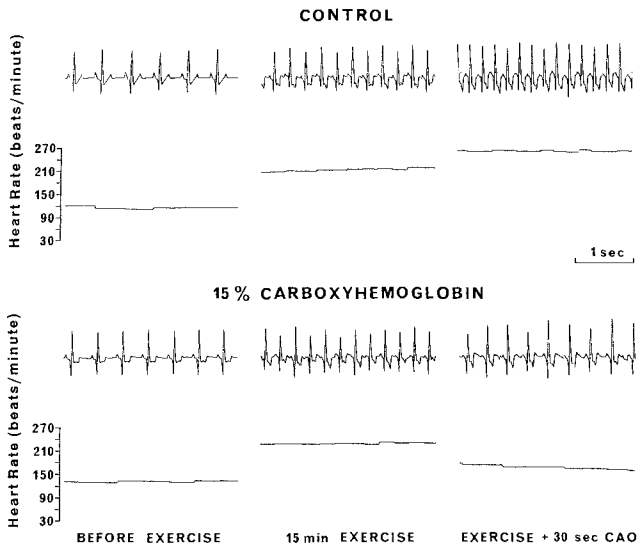

Figure 6. Electrocardiogram and tachogram from one resistant dog under control conditions and with 15 percent COHb. After CO exposure, heart rate before ischemia is slightly higher, but markedly decreases within 30 seconds after coronary artery occlusion.

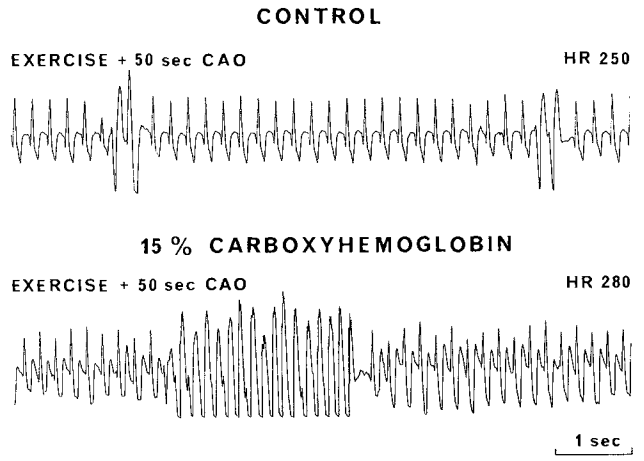

Figure 7. Electrocardiogram 50 seconds after coronary artery occlusion in a resistant dog. In the upper tracing (control conditions), a few couplets of premature ventricular contractions are evident. In the lower tracing (15 percent COHb), a nonsustained ventricular tachycardia occurs.

resting coronary artery occlusion were 15.3 ± 1.1 in the seven animals from which data were derived.

Heart Rate

The heart rate of each dog is shown in Tables 5 and 6. Heart rates were significantly higher in the CO tests compared to control conditions at rest both for the five dogs standing on the treadmill (163 ± 44 beats/minute versus 120 ± 11 beats/minute) (Figure 10) and for the seven dogs lying on the table (135 ± 28 beats/minute versus 114 ± 32 beats/minute) (Figure 11).

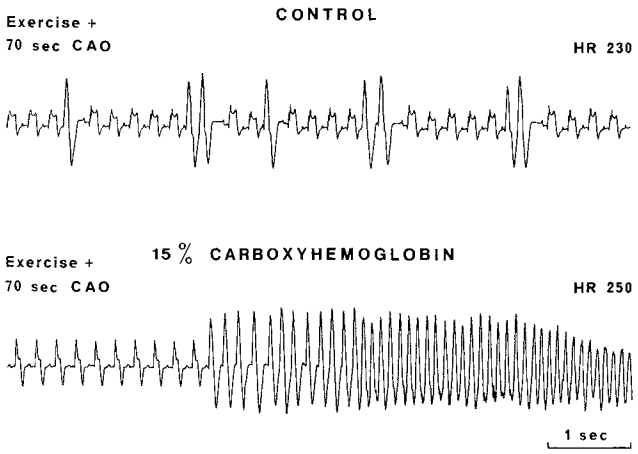

Figure 8. Electrocardiogram 70 seconds after coronary artery occlusion in a resistant dog. Under control conditions (upper tracing), a few couplets of premature ventricular beats are evident. With 15 percent COHb, ventricular tachycardia occurs and degenerates into ventricular flutter and fibrillation.

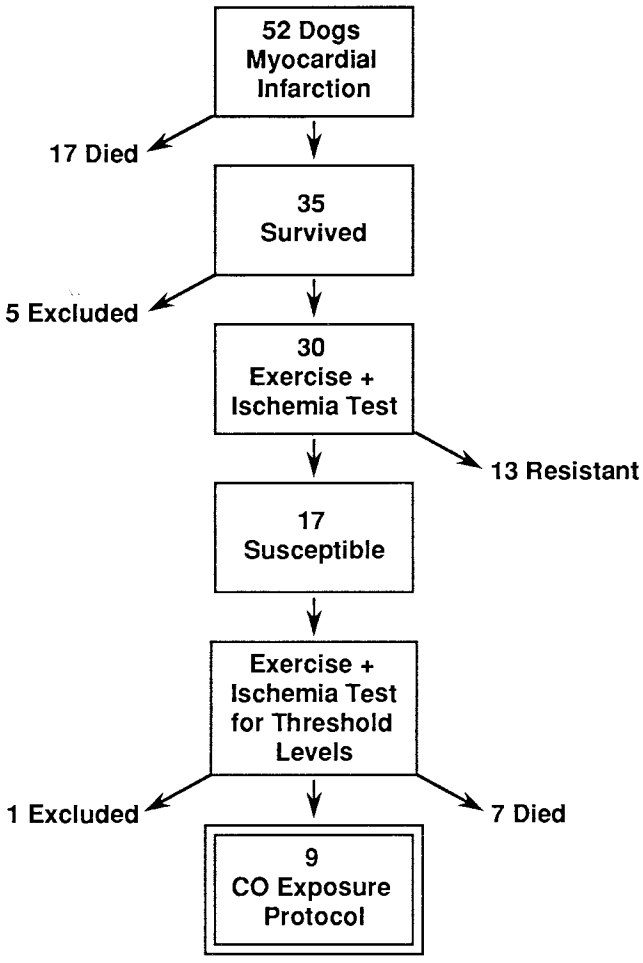

Figure 9. Outline of the study of susceptible animals. Seventeen dogs died during the first 72 hours after myocardial infarction and five were subsequently excluded for technical reasons. The 17 susceptible dogs that proved to be susceptible to ventricular fibrillation—and were, therefore, identified as high-risk animals—constitute the population of the second part of the study.

Table 4. Typical Threshold-Level Testing and Carbon Monoxide Exposure Protocol for One Susceptible Dog

Exercise-Plus-Ischemia Test	Workload of Exercise (kmph/percent slope)	Duration of Coronary Occlusion (seconds)	Outcome
Control	6.4/4	120	Ventricular fibrillation
Control	4.8/0	120	Ventricular fibrillation
Control	4.8/0	90	Ventricular fibrillation
Control	4.8/0	60	Ventricular fibrillation
Control	4.8/0	30	No arrhythmias
CO exposure	4.8/0	30	No arrhythmias
Control	4.8/0	30	No arrhythmias
Control	4.8/0	60	Ventricular fibrillation

Table 5. Heart Rate Response (in Beats per Minute) to Coronary Artery Occlusion in the Five Susceptible Dogs Studied with Exercise-Plus-Ischemia Tests Under Control Conditions and After Carbon Monoxide Exposure Resulting in 15 Percent Carboxyhemoglobin

	Rest		Before Coronary Artery Occlusion		15-Second Coronary Artery Occlusion	
Dog No.	Control	Carbon Monoxide	Control	Carbon Monoxide	Control	Carbon Monoxide
SD400	130	230	250	290	310	305
SD401	110	165	200	190	310	300
SD406	110	110	170	225	280	290
SD431	120	140	220	240	290	290
SD446	130	170	230	240	240	220
Average	120	163	214	237	286	281
SD	11	44	31	36	29	35

Table 6. Heart Rate Response (in Beats per Minute) to Coronary Artery Occlusion in the Seven Dogs Studied at Rest Under Control Conditions and with 15 Percent Carboxyhemoglobin

	Before Coronary Artery Occlusion		15-Second Coronary Artery Occlusion		30-Second Coronary Artery Occlusion	
Dog No.	Control	Carbon Monoxide	Control	Carbon Monoxide	Control	Carbon Monoxide
SD370	80	100	105	100	140	140
SD382	140	150	170	200	210	240
SD416	170	185	230	250	250	285
SD406	85	120	120	150	125	230
SD431	95	140	110	180	175	210
SD446	115	135	115	140	115	160
SD486	115	113	118	113	118	116
Average	114	135	138	162	162	197
SD	32	28	46	52	52	61

In the heart rate response to ischemia during exercise, we noted that the dogs with 15 percent COHb showed a smaller increase in heart rate 15 seconds after the beginning of the occlusion ($+44 \pm 52$ beats/minute versus $+72 \pm 43$ beats/minute) than was observed under control conditions, as illustrated in Figure 12. This phenomenon was not present when the occlusion was performed at rest. Here the heart rate was higher after CO exposure (15 percent COHb) through-

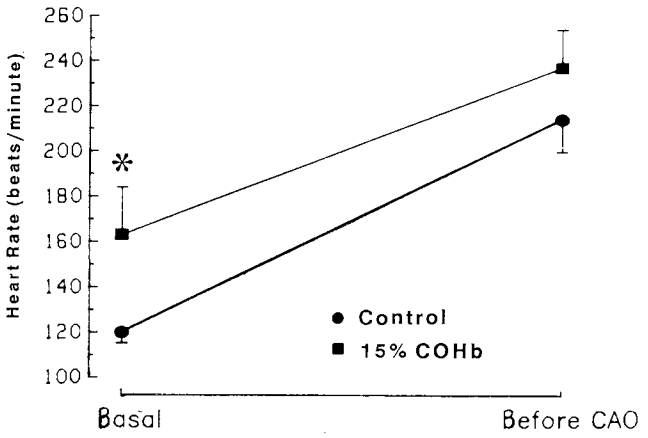

Figure 10. Analysis of heart rate during exercise for the five susceptible animals that performed the control exercise-plus-ischemia test and the test with 15 percent COHb. Heart rate was significantly higher (* = $p < 0.05$) with 15 percent COHb before the beginning of exercise.

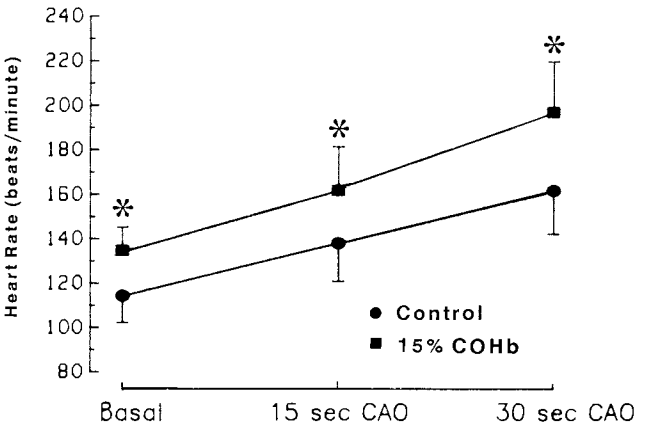

Figure 11. Analysis of heart rate during the coronary artery occlusion of the seven susceptible animals that performed the control and the 15 percent COHb tests at rest. Heart rate was always significantly higher (* = $p < 0.05$) before and during the coronary occlusion after CO exposure.

out the occlusion (Figure 11), so that the changes in heart rate 15 seconds after the beginning of the occlusion were identical in the two groups (Figure 13).

Blood Pressure

Systolic blood pressure was not affected by CO exposure either at rest (146 ± 12 mm Hg in control versus 151 ± 16 mm Hg after CO) or during exercise (157 ± 12 mm Hg in control versus 176 ± 20 mm Hg after CO). Diastolic blood pressure was similarly unchanged both at rest (96 ± 4 mm Hg in control versus 97 ± 9 mm Hg after CO) and during exercise (102 ± 12 mm Hg in control versus 96 ± 2 mm Hg after CO). Both systolic and diastolic blood pressure changes after 15 seconds of coronary artery occlusion during exercise were unaffected by CO exposure (systolic, −40 ± 9 mm

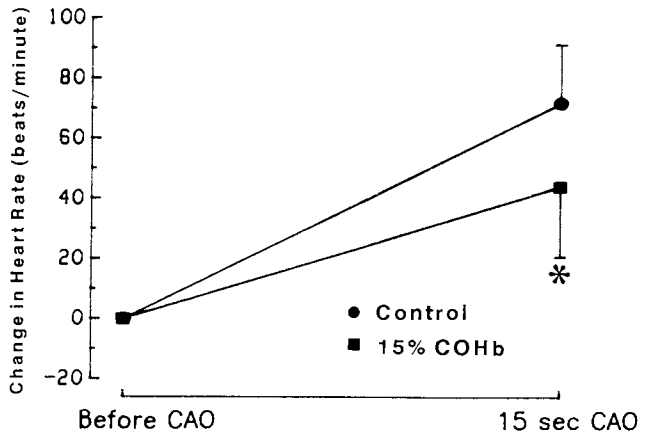

Figure 12. Heart rate response to coronary artery occlusion in the five susceptible dogs that performed the control and the 15 percent COHb tests during exercise. Heart rate before coronary artery occlusion was 214 ± 30 beats/minute under control conditions, and 237 ± 36 beats/minute with 15 percent COHb. After exposure to CO, the coronary artery occlusion causes a smaller increase (* = $p < 0.05$) in heart rate.

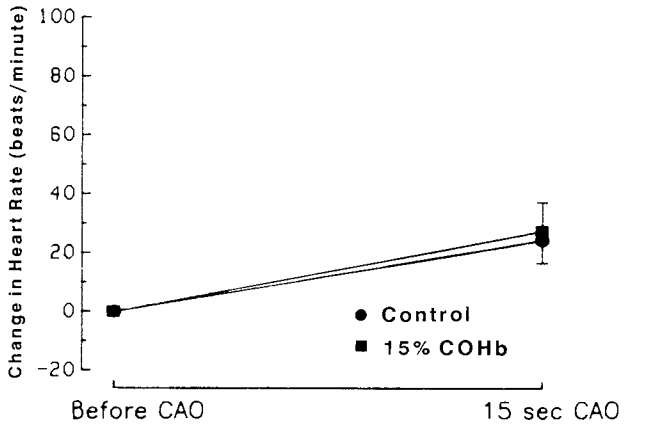

Figure 13. Heart rate response to coronary artery occlusion in the seven susceptible dogs that performed the control and 15 percent COHb tests at rest. Heart rate before coronary artery occlusion was 114 ± 32 beats/minute under control conditions, and 135 ± 28 beats/minute with 15 percent COHb. After exposure to CO, the increase in heart rate did not change.

Hg in control conditions versus −25 ± 17 mm Hg after CO exposure; diastolic, −25 ± 4 mm Hg in control conditions versus −11 ± 13 mm Hg after CO exposure). Also, the systolic and diastolic blood pressure responses 30 seconds into coronary artery occlusion at rest were similar under control conditions and after CO exposure (systolic, −7 ± 4 mm Hg in control conditions versus −13 ± 6 mm Hg after CO exposure; diastolic, −2 ± 2 mm Hg in control conditions versus −3 ± 3 mm Hg after CO exposure).

Cardiac Arrhythmias

Ventricular fibrillation occurred in all 17 susceptible

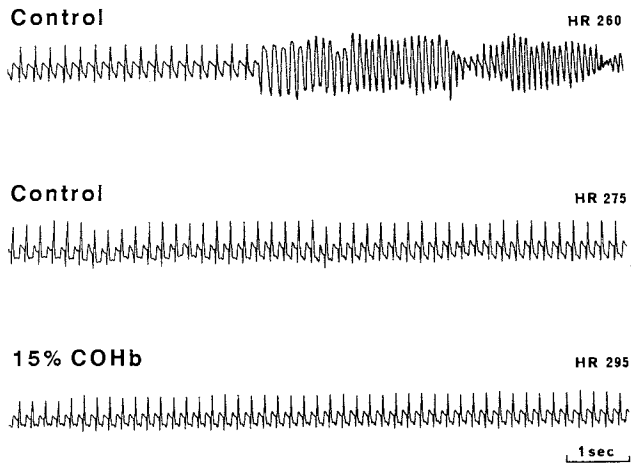

Figure 14. Electrocardiograms after coronary artery occlusion during three consecutive exercise tests (workload of 3 kmph and 0 percent grade) in a single susceptible dog. Under control conditions, a 60-second coronary artery occlusion (upper trace, recorded at 52 seconds) provoked ventricular tachycardia, which degenerated into ventricular flutter and fibrillation. A 30-second coronary artery occlusion under control conditions (second trace, recorded at 15 seconds of occlusion) did not induce arrhythmias. With 15 percent COHb, a 30-second coronary artery occlusion (third trace, recorded at 15 seconds of occlusion) produced a higher heart rate (295 beats/minute) but did not induce arrhythmias.

dogs in the first control test during acute occlusion of the circumflex coronary artery (mean time 39 ± 12 seconds). All dogs underwent additional tests to find a level of workload and ischemia not associated with ventricular fibrillation.

In five dogs, a protocol not associated with ventricular fibrillation was found by reducing the workload and the length of the coronary artery occlusion. In four dogs, the coronary artery occlusion had to be performed at rest. Whenever a protocol not associated with ventricular fibrillation was found, no other type of arrhythmias occurred. With 15 percent COHb, only one dog had a few premature ventricular contractions, and no arrhythmia occurred in the others. A typical test sequence is presented in Figure 14. After these procedures, the animals were again challenged at the lowest level of exercise and occlusion time that previously had induced a lethal arrhythmia. Lethal arrhythmias were again observed (Table 4). The animals that could be tested only at rest were challenged again with a coronary artery occlusion time that previously had induced a lethal arrhythmia. Lethal arrhythmias again occurred.

Left Ventricular Pressure and Contractile Function

Left ventricular pressure and dP/dt, an index of cardiac contractile function, were measured during exercise in three dogs. No differences were observed between the tests performed under control conditions and after exposure to CO. Carboxyhemoglobin levels of 15 percent were achieved in each case. The treadmill exercise was the same as or higher than that used for induction of cardiac arrhythmias.

DISCUSSION

These results provide novel information concerning the effects of CO on the cardiovascular system: (1) heart rate is higher at rest and during exercise at all levels of workload after exposure to CO; (2) heart rate response to acute myocardial ischemia is strikingly affected by CO; and (3) acute exposures to CO do not increase the susceptibility to ventricular tachyarrhythmias during a short episode of acute myocardial ischemia in dogs with a healed myocardial infarction.

CARBON MONOXIDE AND HEART RATE

At Rest

After exposure to CO, resting heart rate was higher in every group of animals in a manner directly proportional to the COHb saturation, even if statistical significance was reached only for the longest exposure time. These results are at variance with reports in men and dogs (Vogel et al. 1972; Wagner et al. 1978) in which brief inhalation of gases with high CO content did not induce an increase in heart rate, but they correlate closely with a study (Adams et al. 1973) in which healthy dogs were exposed to a mixture of 1,500 ppm of CO and air.

Two dogs were tested while breathing air for possible effects of the face mask on heart rate. No change in heart rate could be attributed to the face mask.

During Exercise

In our study, heart rate was higher at all workloads during exercise than under control conditions. Carboxyhemoglobin levels of 10 to 20 percent have been shown to cause a heart rate increase during moderate exercise in men but not dogs (Pirnay et al. 1971; Ekblom and Huot 1972; Vogel et al. 1972; Wagner et al. 1978). The study on dogs (Wagner et al. 1978), however, was performed with CO exposure by bolus (22 percent) in trained animals. Our study also provides the first data on animals with a previous myocardial infarction.

During Myocardial Ischemia

The heart rate response to acute myocardial ischemia in the resistant dogs was unexpectedly modified by CO at all

levels of COHb. The decrease in heart rate during the first minute of coronary artery occlusion, typical of the dogs at low risk (Schwartz et al. 1984), occurred earlier after CO exposure than in control dogs. On the other hand, in the susceptible dogs, heart rate increased more under control conditions than after CO exposure. The interpretation of these findings is uncertain. Acute myocardial ischemia simultaneously activates sympathetic (Malliani et al. 1973) and vagal (Recordati et al. 1971) sensory endings. This may result in the dominance either of excitatory sympathetic reflexes (Malliani et al. 1969) or of depressor vagal reflexes (Thoren 1978). It may be that the effects of ischemia are enhanced by CO through the attendant hypoxia. This could accelerate the alteration in cardiac geometry which, by the distortion of the sensory endings of the cardiac mechanoreceptors, leads to the excitation of both vagal and sympathetic afferent fibers. Such a sequence of events might explain why the reduction in heart rate occurs earlier with CO, but it would not help to explain why the magnitude of this decrease also tends to be greater. However, the magnitude of the heart rate decrease could depend on the simultaneous presence of higher sympathetic tone (Young and Stone 1976), which would create the conditions for the "accentuated antagonism" (Levy 1971). This potentiation of vagal effects in the presence of elevated sympathetic activity occurs in conscious animals (Stramba-Badiale et al. 1990).

Another possibility along the same lines is raised by the report that a larger ischemic area, as caused by proximal coronary artery occlusion, elicits a predominant vagal response, compared to the prevalent sympathetic response provoked by a more distal occlusion of the same vessel (Lombardi et al. 1984).

An alternative explanation, perhaps more intriguing, is that, by a hypoxia-related mechanism, CO may increase the sensitivity of the sinus node to acetylcholine (Zamfirescu et al. 1969).

The smaller increase in heart rate that was observed in the susceptible group may be due to the higher heart rate at the moment of coronary artery occlusion after CO exposure. Indeed, the heart rate reached by the susceptible dogs, with or without CO, 30 seconds into coronary artery occlusion (280 beats/minute) is almost maximal for dogs; the modification in the reflex response observed after exposure to CO simply may represent the difference between the actual value of heart rate at the moment of coronary artery occlusion and the maximal heart rate obtainable in dogs.

CARBON MONOXIDE AND CARDIAC ARRHYTHMIAS

Although the relationship between exposure to CO and cardiac arrhythmias clearly has important health-related implications, the data available in the literature are scarce and limited in significance.

Using the questionable methodology of the train of pulses, as described by Verrier and colleagues (1978), the effects of CO or of cigarette smoke inhalation on the threshold for ventricular fibrillation were examined (Bellet et al. 1972; DeBias 1976; DeBias et al. 1976; Aronow et al. 1978). It was reported that CO exposure somewhat reduced the ventricular fibrillation threshold, a result interpreted as an index of increased electrical instability. This effect was present both in normal and in ischemic animals.

By contrast, Foster (1981) could not demonstrate arrhythmogenic changes induced by CO by looking for slowed conduction and ventricular tachycardia in acutely ischemic dogs. Similarly, and of importance, given the methodologic accuracy of the study, an abstract by Verrier's group (Mills et al. 1987) indicates a complete lack of effect of CO on cardiac electrical stability. These data are consistent with the present results.

The present study differs from previous ones because we evaluated cardiac arrhythmias in a clinically relevant setting. The various aspects of assessing the effect of CO exposure at the time of an acute ischemic episode during exercise in conscious animals with a healed myocardial infarction add greatly to the physiologic meaning of the study.

Worsening of arrhythmia or the appearance of a new life-threatening event occurred in 3 out of 16 of the resistant dogs and premature ventricular contractions occurred in 1 out of 9 of the susceptible dogs. These events could never be reproduced and must be viewed as anecdotal. It is probably significant that the only two dogs that had some arrhythmias during the control test both developed malignant arrhythmias after CO exposure in the present model. Still, the nonreproducibility of this finding calls for extreme caution in concluding that there is an important arrhythmogenic effect of CO. On the other hand, the observation that after CO exposure the heart rates were higher at rest and for low levels of exercise may have clinical implications relevant to patients with coronary artery disease, in particular when exposure to CO lasts for days, as during winter in polluted areas (Lester and Seskin 1970).

We interpret these results as indicating that in dogs with healed myocardial infarction, acute exposure to CO is not arrhythmogenic, with occasional exceptions.

The limited data concerning left ventricular contractile function further suggest that moderate COHb levels may not impair cardiac performance accompanying exercise.

CRITIQUE OF THE ANIMAL MODEL

This experimental preparation incorporates the most im-

portant determinants of sudden cardiac death in humans (Myerburg et al. 1990): a healed myocardial infarction with superimposition of transient myocardial ischemia at a time of elevated sympathetic activity. The early mortality rate (30 percent) is similar to that obtained by other laboratories producing myocardial infarction in dogs (Trolese-Mongheal et al. 1985; Patterson et al. 1986), and has remained constant in our laboratory during our 10 years of experience with this model. If we add to this surgical mortality rate the number of dogs in which initial instrumentation failure occurred, we lost 41 percent of our 102 dogs in this study. In a recent study with a comparable number of dogs (91) (Hull et al. 1990), 45 percent of the animals were lost from the study for the same reasons. Additional animals (34 percent) were eliminated in the present study because of the study design, late equipment failure, or mortality as noted in the Methods section.

The two outcomes of the exercise-plus-ischemia test, survival (resistant) or sudden death (susceptible) are characteristically quite reproducible. Billman and associates (1984) showed that 7 of 8 sedentary susceptible animals remained susceptible for 6 weeks. We reviewed two relatively large studies (currently submitted for publication), involving a total of 54 susceptible dogs, in which animals were retested one to three weeks after the initial challenge; more than 85 percent of the animals remained susceptible in each of these studies. Conversion of a resistant animal to a susceptible animal upon retest, with no other intervention, is a rare event. In the present study, all 16 resistant animals and all 9 susceptible animals retained their initial designation. The occurrence of other arrhythmias (for example, premature ventricular contractions or couplets of premature beats) during coronary artery occlusion in the present model has not been reproducible. For this reason, such arrhythmias were not used to evaluate susceptible and resistant animals.

CLINICAL IMPLICATIONS

Given the inability of acute elevations of COHb (up to 15 percent) to induce fatal arrythmias in dogs with healed myocardial infarctions, it is important to note that results of studies that use the present canine model in other situations appear to be relevant to the problem of sudden cardiac death in humans. Effects in the dog model that have also been observed in cardiac patient populations include an increased risk of sudden cardiac death when baroreceptor reflex sensitivity (Schwartz et al. 1988) or heart rate variability (Hull et al. 1990) are low, and a decreased incidence of sudden death after left stellate ganglion ablation (Janse et al. 1985).

The preceding results, we believe, allow cautious extrapolation to the clinical setting. The present data suggest that many patients with infarcts may not be endangered by brief exposure to the COHb levels observed in our study. Two other possibilities should also be considered. The first is that CO does not represent the same risk in humans and dogs because of mechanistic considerations that have not yet been evaluated. A second issue is that some subpopulation of humans with myocardial infarctions may be susceptible to the effects of brief exposure to CO, even if such exposure has little effect in the majority of patients.

It is very important to note that the present data do not consider the potentially detrimental effect of chronic exposure to CO. Two particularly important issues are the role of CO in the development of atherosclerosis (Astrup and Kjeldsen 1979; Balraj 1984) and the role of CO in the development of cardiomegaly (Penney et al. 1984; Clubb et al. 1986).

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ABBREVIATIONS

ANOVA	analysis of variance
CO	carbon monoxide
COHb	carboxyhemoglobin
dP/dt	an index of contractile function
ppm	parts per million
SD	standard deviation

INTRODUCTION

In the summer of 1984, the Health Effects Institute (HEI) issued a Request for Applications (RFA 84–2) soliciting proposals for "Acute Effects of Carbon Monoxide on Cardiac Rhythm." In response to this RFA, Dr. H. Lowell Stone, of the Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, submitted a proposal entitled "Carbon Monoxide and Lethal Arrhythmias." The HEI approved the two-year project, which began in July 1985. Total expenditures were $354,674. Upon the death of Dr. Stone, Dr. Jay P. Farber, of the same department, became the principal investigator. Dr. Peter J. Schwartz, a cardiologist and a long-term collaborator with Dr. Stone, who was a coprincipal investigator on the original proposal, continued his participation in the project. The Investigators' Report was received at the HEI in October 1987 and was accepted by the Health Review Committee in January 1989. During the review of the Investigators' Report the Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary. The Commentary is intended to place the Investigators' Report in perspective as an aid to the sponsors of the HEI and to the public.

REGULATORY BACKGROUND

The Clean Air Act mandates that the U.S. Environmental Protection Agency (EPA) establish primary standards for air pollutants based on health effects and at levels "requisite to protect the public health . . . allowing an adequate margin of safety." The legislative history of the act makes it clear that in setting the National Ambient Air Quality Standards (NAAQS)[1], the EPA is required to consider the health of particularly sensitive subgroups of the population. The Senate report on the legislation states: "An ambient air quality standard . . . should be the maximum permissible air level of an air pollution agent or class of such agents (related to a period of time) which will protect the health of any group of the population" (U.S. Senate 1970).

The identification of such groups is not clearly defined, but the Senate report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens (such as bronchial asthmatics and emphysematics) who in the normal course of daily activity are exposed to the ambient environment." The report further states that "in establishing an ambient standard necessary to protect the health of these persons, reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group."

The current primary NAAQS for carbon monoxide is 9 parts per million (ppm), averaged over eight hours, and 35 ppm, averaged over one hour, both not to be exceeded more than once a year. Established in 1971, this standard was based on a study by Beard and Wertheim (1967), who reported an effect on the central nervous system (impairment in discrimination of time intervals) when the carboxyhemoglobin (COHb) levels of human subjects reached two to three percent. Several subsequent studies, however, failed to replicate these findings (Stewart et al. 1970; O'Donnell et al. 1971; Otto et al. 1979).

During the 1970s, three studies reported that carbon monoxide exposure (50 and 100 ppm for one to four hours) aggravated the symptoms associated with cardiovascular disease. The studies by Aronow and Isbell (1973) and by Anderson and coworkers (1973) reported more rapid onset of pain in angina patients, and another study by Aronow and coworkers (1974) reported a similar result in patients with intermittent claudication (ischemia of the muscles due to sclerosis with narrowing of the arteries). After these reports, the EPA proposed to lower the one-hour standard from 35 ppm to 25 ppm. This lower standard was never implemented, in part because of the inadequacy of the available data. In 1985, the EPA (U.S. Environmental Protection Agency 1985) decided not to revise the existing standard but is, at present, in the process of reviewing several human health effects studies that have been completed recently as part of their mandated periodic reevaluation of the criteria pollutant standard for carbon monoxide (Sheps et al. 1987; Adams et al. 1988; Allred et al. 1989a,b; Kleinman et al. 1989).

The known ability of carbon monoxide to interfere with tissue oxygen delivery could account for the exacerbation of cardiac symptoms. Whether or not carbon monoxide–induced hypoxia also causes heart disease, or induces potentially life-threatening effects, such as arrhythmias, has not been well studied. Investigations of the arrhythmia-producing effects of carbon monoxide exposure in experimental models of cardiovascular disease have the potential to contribute knowledge useful in evaluating probable health effects in humans. This information is essential for informed regulatory decision making required by the Clean Air Act.

[1] A list of abbreviations appears at the end of the Investigators' Report for your reference.

SCIENTIFIC BACKGROUND

CARDIAC ARRHYTHMIAS

Heart disease, the most prevalent form of which is ischemic heart disease (commonly referred to as coronary artery disease), is the leading cause of disability and death in industrialized nations (Levy and Feinleib 1984). Ischemic heart disease refers to a spectrum of clinical disorders of the heart resulting from an imbalance between the myocardial need for oxygen and the adequacy of the blood supply (Robbins and Cotran 1979). Reduction in coronary blood flow is almost always the cause of this imbalance and is most often due to atherosclerosis, the formation of lipid deposits in the large- and medium-sized arteries. Occlusion of the coronary artery by lipid deposits results in inadequate delivery of blood, and hence oxygen, to the myocardial tissue. The resulting myocardial ischemia can be further exacerbated by increased oxygen demand, as occurs with exercise, or by impairment in the oxygen-carrying capacity of the blood, as occurs with anemia or carbon monoxide–induced hypoxia. Severe myocardial ischemia can lead to myocardial infarction (heart attack).

In the normal human heart, the heartbeat originates in the sinoatrial node, with each myocardial cell then being activated in an organized programmed sequence via the heart's specialized conduction system. Abnormal cardiac rhythms may result whenever interruptions in the blood supply and oxygen delivery occur, due to disordered impulse formation or altered conduction within the myocardial tissue, or both. In abnormal conditions, an ectopic focus (a focus other than the sinoatrial node) may discharge, resulting in a beat that occurs before the expected next normal beat, thereby transiently interrupting the cardiac cycle (an extrasystole or premature beat). Conduction delay and block in ischemic myocardial tissue can also cause arrhythmias. Such a block usually results from partial obstruction of the blood flow due to either atheroma (lipid deposits characteristic of atherosclerosis) or thrombosis (a clot formed from constituents of blood).

A rapid and irregular discharge of impulses arising from ectopic foci or impaired conduction, or both, can produce atrial or ventricular fibrillation. When extrasystoles initiate fibrillation within the ventricles, the heart is unable to pump blood effectively throughout the systemic circulation and death results (Ganong 1985). Ventricular fibrillation is the most serious arrhythmia that can occur as a consequence of ischemic heart disease (Goldman 1979) and it causes more than 80 percent of the deaths due to heart attacks.

Exercise tests are often used in conjunction with other stimuli to evaluate cardiac function because exercise may induce an otherwise intermittent arrhythmia. At the onset of exercise, an increased sympathetic discharge to the heart occurs. As a result, cardiac output and heart rate increase, vascular beds in the muscles dilate, and vasculature in other parts of the body constricts. This altered peripheral resistance diverts blood flow to the exercising muscles and away from other organs (Berne and Levy 1981; West 1985).

During exercise, increased oxygen transport to the heart occurs primarily through increased heart rate. Although coronary blood flow can increase sufficiently in healthy individuals at all levels of exercise, individuals with ischemic heart disease may not be able to sustain adequate coronary blood flow during exercise. Disturbances in the coordination of heart rate and blood flow, as the heart attempts to increase cardiac output, can ultimately cause cardiac arrhythmias. Any decreased availability of oxygen within the blood due to the presence of carbon monoxide can further exacerbate the lack of oxygen delivery to the heart.

Much of what is known about derangements of cardiac rhythm has come from animal models of myocardial ischemia. As early as 1894, Porter noted that "fibrillar contractions" often followed occlusion of the coronary artery and that irregular cardiac rhythm preceded fatal ventricular fibrillation (Porter 1894). Waldo and Kaiser (1973) found that in dogs with coronary artery ligation, areas of myocardial ischemia had periods of localized fibrillation. Citing animal studies in both the dog and pig, investigators have noted that myocardial ischemia in laboratory animals affects the electrophysical properties of ventricular myocardial cells, and leads to electrical instability of the myocardium (Lown and Verrier 1976; Lazzara et al. 1978; Janse and Kléber 1981; Motté et al. 1984; Lazzara and Scherlag 1988). Extracardiac factors have also been shown to influence cardiac rhythm; stimulation of the cardiac sympathetic nerves, such as occurs in acute myocardial ischemia, can induce irregular heartbeats, and in the ischemic myocardium, can be sufficient stimulus to induce fibrillation (Han et al. 1964; Lown and Verrier 1976). Schwartz (Schwartz and Stone 1982; Schwartz 1986) and others (Lown and Verrier 1976) have shown that manipulation of the parasympathetic nervous system also affects the onset of arrhythmias in dogs with healed myocardial infarction. Thus, in animal models, the evidence supports the hypothesis that both myocardial ischemia and neural activity can alter cardiac vulnerability and predispose the heart to ventricular fibrillation.

EXPERIMENTAL MODELS

The dog has been the model of choice for studies of myocardial ischemia because the normal canine coronary vas-

cular system resembles that of humans who have developed coronary artery disease. Specifically, humans with severe ischemia can avert myocardial infarction by use of collateral vessels that are able to take over blood flow lost by occluded coronary arteries (Braunwald and Alpert 1984). In the healthy human heart, there are virtually no functional intercoronary channels; however, severe or advanced coronary stenosis (narrowing of the coronary artery) induces progressive enlargement of small arterial collaterals and development of new collateral channels from preexisting capillaries. These new blood vessels are critical in preventing ventricular fibrillation (Berne and Levy 1981; Braunwald and Alpert 1984; Epstein et al. 1989). In contrast, the normal dog heart has coronary collateral vessels that link the branches of the major coronary arteries, thereby providing immediate epicardial collateral flow after acute coronary artery occlusion. Because coronary stenosis induces the development of collateral vessels in humans, many researchers believe the dog is the best available animal model to study human coronary artery disease (Gross 1985); others, however, prefer the pig as a model because, compared to dogs, atherosclerotic plaques in swine more closely resemble those in humans (Eckstein 1954; Brooks et al. 1975; Gross 1985).

The experimental model for arrythmias that has been studied the most extensively is that of dogs in which the anterior descending coronary artery has been occluded to produce a myocardial infarction. The creation of infarction by coronary ligation allows the investigator to produce spontaneous and triggered arrhythmias. Animals with healed myocardial infarctions, such as those used by Farber and his coworkers, are considered to be good models to study stabilizing and unstabilizing influences on ventricular fibrillation. One advantage of this model is that the effects of carbon monoxide exposure could be evaluated in conscious animals, eliminating the possible confounding effects of anesthesia on the physiologic parameters being measured.

CARDIOVASCULAR EFFECTS OF CARBON MONOXIDE

Individuals with coronary artery disease are affected by alterations of coronary blood flow and oxygen transport to the heart; they are also susceptible to other factors that may influence cardiac oxygen consumption. One such factor is believed to be exposure to carbon monoxide. The health effects of carbon monoxide exposure are thought to arise largely from interference with the oxygen-carrying function of the blood.

Under normal conditions, inhaled oxygen diffuses rapidly across the alveolar walls of the lung and binds to hemoglobin inside the red blood cells. The oxyhemoglobin complex is transported in the blood, and the oxygen is eventually released into the tissues. If carbon monoxide is present in the inhaled air, the carbon monoxide and oxygen compete for the oxygen-binding sites on the hemoglobin molecules. Because the affinity of hemoglobin for carbon monoxide is 240 times greater than for oxygen, COHb forms and the oxygen-carrying capacity of the red blood cells is reduced. In addition, delivery of oxygen to the tissues is further impaired because, in the presence of carbon monoxide, hemoglobin releases oxygen to the tissues more slowly. The resulting tissue hypoxia may cause transient or permanent damage, especially in those organs that demand high oxygen delivery, such as the brain and heart.

Tissue hypoxia in the myocardium initiates a variety of compensatory responses, such as increased cardiac output and vasodilation, that are directed toward increasing the rate of oxygen delivery to the heart. Because of their limited ability to increase blood flow, individuals with coronary artery disease comprise a subpopulation that is potentially susceptible to adverse health effects when exposed to relatively low levels of carbon monoxide (U.S. Environmental Protection Agency 1985).

Clinical and animal studies have produced limited information with regard to the potential arrhythmogenic effects of carbon monoxide exposure. In men with coronary artery disease, exposure to low levels of carbon monoxide (resulting in 2 to 4 percent COHb) while exercising decreased the time to the onset of myocardial ischemia (Allred et al. 1989a,b). A study of normal subjects and subjects with coronary artery disease and no baseline ectopy (Hinderliter et al. 1989) failed to demonstrate an arrhythmogenic effect of carbon monoxide exposure. There has, however, been a recent report of an effect of carbon monoxide exposure on ventricular arrhythmias in subjects with coronary artery disease and some degree of preexisting rhythm irregularity (Sheps et al. 1990). In the latter study, subjects experienced an increase in arrhythmias when COHb levels were elevated to 6 percent during exercise. No effect was seen at rest or in exercising subjects with 4 percent COHb.

A limited number of investigations of cardiac vulnerability to ventricular fibrillation, under conditions of acute and chronic carbon monoxide exposure, have been conducted in laboratory animals. The results of these studies are equivocal. In agreement with the studies in human subjects, abnormal electrocardiograms have been reported in dogs (Preziosi et al. 1970; Sekiya et al. 1983) and in Cynomolgus monkeys (DeBias et al. 1973) exposed to carbon monoxide. DeBias and coworkers (1976) also reported that the threshold for transthoracic induction of ventricular fibrillation was reduced in normal monkeys, and in animals with experimental myocardial infarctions, after a six-hour exposure to 100 ppm of carbon monoxide (mean COHb was 9.3 percent). In the latter study, the number of animals was

small (four to six monkeys per group), and the normal animals did not receive a sham air exposure but breathed ambient laboratory air.

A reduced ventricular fibrillation threshold was also reported after a two-hour exposure to 100 ppm carbon monoxide (mean COHb was approximately 6 percent) in normal dogs (Aronow et al. 1979) and in dogs with acute myocardial infarctions (Aronow et al. 1978). Interpretation of the results of Aronow and coworkers (1978, 1979) is complicated by the fact that while the mean threshold for ventricular fibrillation after exposure to carbon monoxide was lower than the preexposure threshold, the threshold increased by the same amount in the control animals after exposure to compressed air, a factor that may have biased interpretation of the data. In contrast to these reports, Foster (1981) found no effects of carbon monoxide exposure (mean COHb was 10.4 percent) on ventricular arrhythmias or on ischemic conduction delay in dogs with acute experimental myocardial ischemia.

JUSTIFICATION FOR THE STUDY

The effects of carbon monoxide exposure on cardiac function have been examined in a number of experimental systems. It is not, however, known whether or not exposure to carbon monoxide affects the incidence of ventricular arrhythmias in normal subjects or in subjects with cardiovascular disease. The HEI solicited proposals under RFA 84-2, "Acute Effects of Carbon Monoxide on Cardiac Rhythm," for research on the effects of carbon monoxide exposure, at or near ambient levels, on myocardial excitability. Investigations using animal models of susceptible human populations, and clinical or epidemiological studies of susceptible individuals, were all considered relevant.

After reviewing the proposals submitted under RFA 84-2, the HEI Research Committee decided to support four research projects, two studies to determine the effects of carbon monoxide exposure on cardiac electrical stability in animal models, and two human clinical studies to evaluate the effects of carbon monoxide exposure on subjects with ischemic heart disease and ventricular arrhythmias. By supporting diverse biological models and end points, the Institute's goal was to develop a comprehensive research program to determine the effects of carbon monoxide exposure on the occurrence of arrhythmias and on susceptibility to ventricular fibrillation.

OBJECTIVES AND STUDY DESIGN

The primary objective of the study was to determine if exposure to carbon monoxide has an effect on cardiac electrical stability in dogs that had survived an acute myocardial infarction. The investigators used a previously described animal model that incorporated two factors that are known to increase the risk of ventricular fibrillation, healed myocardial infarction and acute myocardial ischemia (Schwartz et al. 1984; Schwartz 1986). In this model, unanesthetized dogs with a healed anterior wall myocardial infarction are challenged with mild exercise and acute coronary artery occlusion. Those dogs that experience ventricular fibrillation during the brief coronary occlusion are considered by the investigators as "susceptible" to arrhythmias and subsequent sudden death; those dogs that do not experience ventricular fibrillation are defined as "resistant."

The investigators had previously demonstrated differences in the reflex heart rate of susceptible and resistant dogs during the ischemia-plus-exercise protocol. The reflex heart rate usually decreases in animals that survive and increases in dogs that develop ventricular fibrillation (Schwartz 1984). They hypothesized that two related factors, the activity of the autonomic nervous system and the ischemia-induced reduction in heart rate, were responsible for the difference in electrical stability between the susceptible and resistant animals (Schwartz et al. 1984; Schwartz 1986). They postulated, further, as the basis for this study, that elevated levels of COHb would increase the extension and severity of myocardial ischemia after coronary artery ligation, making the animals more susceptible to cardiac arrhythmias.

Two separate groups of animals were used to study resistant and susceptible dogs. First, myocardial infarctions were produced in mongrel dogs (50 to 52 per group) by occluding the left anterior descending coronary artery. During surgery, a pneumatic occluder was also placed around the left circumflex coronary artery. One month later, the surviving animals with healed myocardial infarctions (30 per group) performed a submaximal exercise test (12 to 18 minutes of exercise with increasing workload). Acute myocardial ischemia was induced by occluding the left circumflex coronary artery for two minutes, beginning at the last minute of exercise and extending through the first minute after exercise ceased. Animals that experienced ventricular fibrillation during the exercise-plus-ischemia test were identified as being at high risk for ventricular fibrillation and were defined as "susceptible"; animals that did not have arrhythmias were defined as "resistant." Seventeen dogs entered the study as susceptible animals, sixteen as resistant.

The investigators then evaluated the possible arrhythmogenic effect of exposure to carbon monoxide in the two groups of animals. Resistant animals were exposed to relatively high levels of carbon monoxide (1,500 ppm) for the period of time needed to achieve 5, 10, or 15 percent COHb

saturation. The exercise-plus-ischemia test was performed immediately after the carbon monoxide exposure. Carboxyhemoglobin levels, heart rate, blood pressure, and the electrocardiographic response were measured at different time intervals during the procedure.

Because the susceptible animals, by definition, developed arrhythmias during the standard exercise-plus-ischemia test, they were treated in a somewhat different manner. The investigators first determined the threshold for fatal arrhythmias for the exercise-plus-ischemia protocol for each animal. Susceptible animals were then exposed to carbon monoxide (resulting in 15 percent COHb), either at rest or during exercise, using the predetermined threshold conditions. The cardiac parameters described above were measured during and after the coronary artery occlusion.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

The authors conducted experiments studying the effects of three carbon monoxide–exposure conditions on dogs with surgically induced myocardial ischemia. A limited number of animals, defined as either "susceptible" or "resistant" to fatal arrhythmias, were tested under each protocol. The technical and experimental limitations of the study, including unstable COHb levels and uncertain infarct size, as well as the small sample sizes, limit the interpretation of the investigators' findings.

ASSESSMENT OF METHODS AND STUDY DESIGN

Farber and his coworkers used a postinfarction sudden-death model, in which brief occlusion of the coronary artery is coupled with submaximal exercise in conscious dogs with a healed arterior wall myocardial infarction. The study design hinged on the postsurgical division of mongrel dogs into two groups, "susceptible" and "resistant", on the basis of whether or not the animals developed ventricular fibrillation after preliminary challenge procedures; these procedures included exercise and an acute episode of myocardial ischemia (Schwartz et al. 1984; Schwartz 1984).

The investigators had extensive experience with the postinfarction sudden-death model (Schwartz et al. 1984; Schwartz 1986). There are, however, concerns regarding the experimental protocol, sample size, and the generalizability of the reported responses.

A total of 102 dogs entered the study. The surgical procedures required to induce the initial myocardial infarction and to apply the instrumentation resulted in a high mortality rate (30 percent) and a technical failure rate of 11 percent. In addition, 41 percent of the animals identified as susceptible died during subsequent testing for the threshold for ventricular fibrillation. The authors state that these results are consistent with previous experience in their laboratory. Due to the high operative and experimental mortality, only 25 animals (25 percent of the original sample) were exposed to carbon monoxide, 16 resistant dogs and 9 susceptible animals. The number of animals in individual subgroups was often below acceptable levels for statistical analysis. For example, the total number of dogs exposed to all three concentrations of COHb is one for the resistant group and none for the susceptible group. The model previously had been characterized with regard to the reproducibility of the rates of survival after the surgical procedures, and the reproducibility of ventricular fibrillation during the exercise-plus-ischemia test in susceptible and resistant animals (Billman et al. 1984).

The high mortality rate in this study, although consistent with the investigators' previous experience, raises concerns about possible bias in the sample used for testing. Measurements of cardiological parameters could only be performed in animals that survived the experimental procedure (25 percent). The data, therefore, may not reflect the true effects of myocardial ischemia and exposure to carbon monoxide, thus restricting the generalizability of the results.

While the authors provided sufficient details about the surgical procedures, other technical aspects were not adequately documented. One weakness of this report is the lack of detail regarding the carbon monoxide exposures. No information was provided on the design and monitoring of the exposure system, the duration of individual exposures, respiration rates, and individual COHb data. As will be discussed below, there was a high degree of variability in the reported mean COHb levels, but because of the lack of experimental detail, it cannot be determined if the variability was the result of inaccurate COHb measurements, the design of the exposure apparatus, or the exposure protocol. A sham exposure was not part of the study design, although two dogs so tested while breathing air showed no effects of the face mask on heart rate. A sham exposure for all animals would have been useful to determine if the experimental procedures affected heart rate.

The animals were exposed to high concentrations of carbon monoxide (1,500 ppm) for short periods of time to achieve before-exercise COHb values of 5, 10, or 15 percent, as determined by CO-Oximeter measurements of arterial blood samples. The authors state the COHb concentrations actually measured in resistant dogs were 6.1 $\pm$ 1.3 percent for the 5 percent COHb target level, 13 $\pm$ 1.6 percent for the 10 percent COHb target level, and 20 $\pm$ 1.8 percent for the 15 percent COHb target level at the beginning of the

exercise-plus-ischemia test. In addition, they report that after exercise and a 30-second coronary artery occlusion, COHb fell from 20 to 15 percent. More rigorous control of the carbon monoxide exposure and monitoring conditions might have reduced the observed variability in target COHb values. Although achievement of steady-state conditions was not a goal of this study, the rapid rate of carbon monoxide uptake (0.5 percent COHb saturation per minute) may have led to inhomogeneities in coronary perfusion, a factor that can affect cardiac susceptibility and fibrillation.

Although the authors cite an earlier study in which the degree of heart muscle damage was characterized by infarct size (Schwartz 1986), the degree of heart muscle damage in the experimental dogs in the present study was evaluated only by the placement of the initial ligature. Interpretation of the current study would have been improved if the investigators had included a measure of the extent of the infarction in this report.

STATISTICAL METHODS

As originally designed, this study had two components: a repeated measures analysis of variance comparing control, 10 percent, and 15 percent COHb target exposures in resistant animals, and a paired comparison of control and 15 percent COHb target exposures in susceptible animals. During the study, a protocol was added at the request of the HEI to study resistant animals at the 5 percent COHb target exposure. The Statistical Methods section of the report states that statistical analysis was performed by analysis of variance for repeated measurements followed by Tukey's test for multiple comparisons. In fact, this methodology was used only for the resistant group, and for those animals, only in the analysis of the 10 percent and 15 percent target COHb doses. As noted by the authors, the six animals receiving both control and 5 percent target COHb doses were analyzed separately by paired *t* test or repeated measures analysis of variance.

The large number of missing observations at each dose resulted in a fragmented analysis in which the sample size varied from analysis to analysis. Thus, the authors relied on paired comparisons for many analyses. Even though the authors used multiple comparisons procedures to compare control, 10 percent, and 15 percent target COHb exposures for each endpoint, the separate analysis of resistant and susceptible animals, and the multiplicity of outcome variables, imply that the study was subject to a multiple comparisons problem. The large number of analyses performed implies that some statistically significant findings could be anticipated to occur by chance. Thus, interpretation of the study should rely on patterns of positive findings and dose-response relations, rather than isolated statistically significant findings. The findings regarding heart rate changes meet these more stringent criteria.

INTERPRETATION OF RESULTS

The results of the study by Farber and his coworkers suggest, but do not prove, that acute exposures to carbon monoxide at concentrations leading to approximately 5, 10, and 15 percent COHb do not increase the incidence of ventricular arrhythmias in dogs with healed myocardial infarction when these animals are challenged with mild exercise and acute coronary artery occlusion. Susceptible and resistant dogs did not differ in their lack of arrhythmogenic response to carbon monoxide exposure. Systolic blood pressure in animals in both groups was also not affected by carbon monoxide exposure.

Heart rates, both at rest and during exercise, were found to be higher in animals exposed to carbon monoxide than in nonexposed animals, an effect that was statistically significant when the COHb levels reached 15 percent; this was not entirely unexpected (Ayres et al. 1970; Penney 1988). The heart rate response to acute myocardial ischemia and exercise was affected by 15 percent COHb levels in both animal groups, and this effect was opposite to what one might have predicted. The previously reported decrease in heart rate in the first minute of ischemia during exercise that is typical of the resistant dogs (Schwartz 1986) occurred much earlier after carbon monoxide exposure. Moreover, the heart rate increase that the authors usually observe in susceptible dogs during the first minute of coronary occlusion during exercise (Schwartz 1986) was significantly dampened in animals with 15 percent COHb levels, a phenomenon that was not observed after coronary occlusion in resting animals. Although this might be misinterpreted as indicating that carbon monoxide was protective, alternative explanations for the effects of carbon monoxide exposure on heart rate are possible. Carbon monoxide is known to cause increased coronary vasodilation and decreased metabolism when compared to an oxygen deficiency of the same level (hypoxic hypoxia—the amount of functional hemoglobin remains the same but the number of oxygen molecules is reduced). This mechanism may explain the fall in heart rates noted with carbon monoxide exposure after ischemic occlusion.

The reported findings of carbon monoxide–induced effects on heart rate, as well as the apparent lack of effect on arrhythmias, should be interpreted with caution because of the aforementioned experimental problems; these include the high mortality rates inherent in the model, the small

sample size and incomplete data set at each dose, the possible bias in the animal sample tested, the COHb variability, and the lack of adequate documentation of exposure conditions and degree of infarction.

IMPLICATIONS FOR FUTURE RESEARCH

The role of carbon monoxide–induced hypoxia in inducing ventricular arrhythmias and fibrillation still needs careful evaluation to determine whether or not arrhythmias induced by ischemia can be aggravated by exposure to carbon monoxide. Future studies in laboratory animals need to include strict controls for respiration and metabolism levels, exposure conditions, and surgical preparations. Such factors cannot be held constant in human clinical trials, but, when controlled for, are advantages of studies in animals.

CONCLUSIONS

The effect of acute exposure to carbon monoxide on heart rate and cardiac arrhythmias was examined in dogs with healed myocardial infarctions and acute myocardial ischemia. The results suggest, but do not prove, that short-term exposure to high levels of carbon monoxide has no effect on the incidence of ventricular fibrillation under these experimental conditions. Heart rate, however, was affected. The authors report that heart rates were elevated in animals exposed for short periods of time to high levels of carbon monoxide (resulting in 15 percent COHb), and that the typical heart rate responses observed during ischemia and exercise in this model were modified in an unexpected way. Given the small sample size in the different subgroups, the high mortality inherent in the experimental system, and other technical limitations, these results should not be directly extrapolated to human subjects with coronary artery disease. The findings are preliminary and require confirmation in a well characterized experimental preparation.

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Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research (Supplement)	January 1988

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