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Response of Rat Coronary Circulation to Carbon Monoxide and Nitrogen Hypoxia (41922)

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Abstract. The effects of nitrogen (N_2) or carbon monoxide (CO) hypoxia on coronary flow were assessed in the isolated nonworking rat heart perfused via the aorta with oxygenated (95% O_2–5% CO_2) Kreb's Henseleit solution. After 30 min, the hearts were challenged with solutions containing either CO (10% CO–85% O_2–5% CO_2) or N_2 (10% N_2–85% O_2–5% CO_2) for 2 min (Challenge I). After recovery in oxygenated solution, the hearts were challenged with the alternate test solution (Challenge II). There were no significant differences in heart rate or pulse pressure between the hearts challenged with CO or N_2. Coronary flow was significantly higher in the hearts challenged with CO regardless of the challenge sequence. Coronary flows ($ml \cdot min^{-1} \cdot g$ dry wt) in the CO- and N_2-treated hearts, respectively, were 61.5 ± 4.5 and 52.9 ± 1.3 after Challenge I, and 64.3 ± 2.6 and 56.4 ± 3.0 after Challenge II. Because PO_2 and oxygen content were the same in both test solutions, the results suggest that CO has a direct effect on coronary artery vascular smooth muscle.

The mechanism of carbon monoxide (CO) toxicity was described by Bernard in 1857 and has since been verified by numerous other workers (1). CO combines with hemoglobin to form carboxyhemoglobin (COHb), which decreases the oxygen carrying capacity of the blood and shifts the oxyhemoglobin dissociation curve to the left. Thus, the overall indirect effect is tissue hypoxia.

There are, however, several reports in the literature suggesting that CO may also have a direct effect at the tissue level (2–11). In an earlier study (12), we reported that coronary flow increased in isolated hearts perfused with hemoglobin-free solutions containing up to 50% CO and that coronary flow was the most sensitive of the responses observed. In that study, it was not possible to distinguish between effects caused by CO and those caused by the reduced PO_2. The studies reported here were conducted to compare the effects of 10% CO on coronary blood flow with those caused by 10% N_2 at a constant PO_2. A preliminary report of these observations has appeared (13).

Materials and Methods. Isolated, nonworking rat hearts were perfused by a modification of the method described by Neely and Rovetto (14). Experimental animals were male, Sprague–Dawley rats weighing 225 to 250 g. Each rat was heparinized (2.5 mg) and guillotined; its chest was opened and the heart excised rapidly and washed in ice-cold saline.

The aorta was cannulated and the heart was mounted on a modified, nonrecirculating Langendorf apparatus and perfused with 95% O_2–5% CO_2 (oxygenated), 85% O_2–10% N_2–5% CO_2 (N_2) or 85% O_2–10% CO–5% CO_2 (CO) Krebs–Henseleit solution (Fig. 1). The PO_2 of each perfusate was measured by an oxygen electrode (Transidyne General) and determined to be as follows: 95% O_2–5% CO_2, 598 Torr; 85% O_2–10% N_2–5% CO_2, 539 ± 11 Torr; 85% O_2–10% CO–5% CO_2, 539 ± 11 Torr. The perfusion medium contained the following (in m*M*): glucose, 5.2; $NaHCO_3$, 23.9; NaCl, 114; KCl, 4.6; $CaCl_2 \cdot 2H_2O$, 2.9; $MgSO_4 \cdot 7H_2O$, 1.1; KH_2PO_4, 1.2; and EDTA (disodium salt), 0.5. The pH and temperature of the perfusion medium were 7.4 and 38°C, respectively. These did not change with N_2 or CO gassing. The fluid reservoirs were elevated to maintain a hydrostatic pressure of 80 mm Hg throughout the experiment.

A 17-cm, saline-filled catheter (PE-90) was implanted through the wall of the left ventricle and attached to a pressure transducer to record pressure changes (15). Signals from the transducer were fed into a data logger (Buxco), averaged over 9 sec, and displayed

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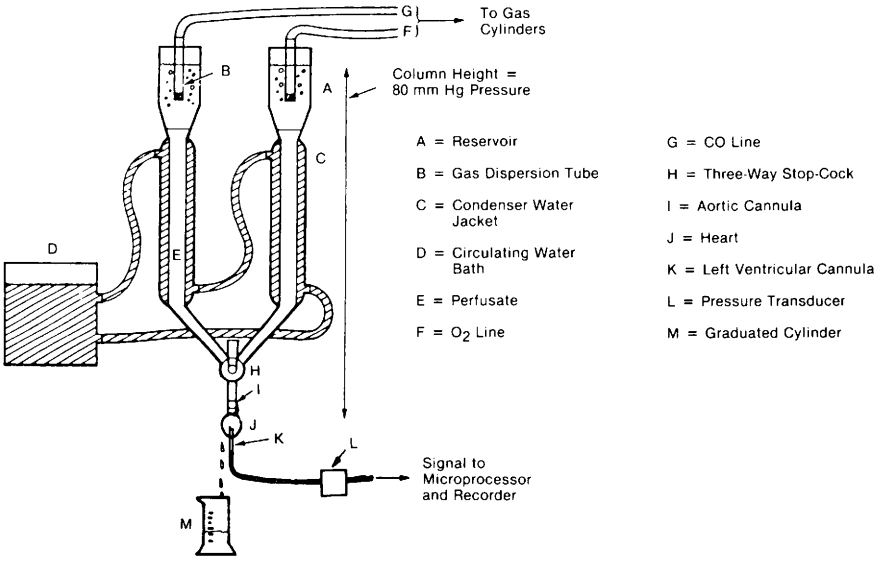

FIG. 1. Isolated heart perfusion apparatus.

in digital form every 12 sec. A continuous record of the wave form was also obtained via a chart recorder (Physiograph). Changes in heart rate and left ventricular pressure were determined from this record. The frequency response of the system was 22 cycles per second. Perfusate flow was measured by collecting the effluent from the heart in a graduated cylinder.

Experiment I. After a 10-min washout period, the hearts were perfused with oxygenated perfusate for a 30-min control period. At this time, the gas mixture bubbling through the perfusate was changed to 10% N_2–85% O_2–5% CO_2 (N_2) or 10% CO–85% O_2–5% CO_2 (CO). After 2 min of the test challenge, the gas bubbling through the perfusate reservoir was switched back to 95% O_2–5% CO_2, and the hearts allowed to recover for 10 min.

Experiment II. After recovery, the hearts were rechallenged with the alternate test gas for 2 min (i.e., hearts initially challenged with CO were subsequently challenged with N_2). The perfusion was stopped and the hearts removed from the perfusion apparatus, weighed, and dried overnight at 100°C to determine dry weight. Tissue water content was 80.8 ± 0.4% (SEM) in hearts treated initially with CO and 80.3 ± 0.4% in hearts treated initially with N_2. Because tissue water content did not change as a result of treatment with CO or N_2, coronary flow data are expressed in terms of dry weight.

Student's grouped *t* test was used to test the significance of differences in heart rate, pulse pressure, and coronary flow (16). A *P* value of less than 0.05 was considered the minimal level of significance.

Results. To test the stability of the preparation, eight control hearts were perfused with hemoglobin-free, Krebs–Henseleit solution equilibrated with 95% O_2–5% CO_2 for 50 min. There was no significant deterioration in heart rate, coronary flow, or pulse pressure in these control hearts for the duration of the experimental period.

Experiment I. Coronary flow increased 10% when the perfusate O_2 content was decreased to 24.6 μg/ml by N_2; with CO, coronary flow increased 27% (Fig. 2). At the end of 2 min, the mean increased in coronary flow was 4.8 ± 1.1 and 13.2 ± 3.1 $ml \cdot min^{-1} \cdot g$ dry tissue, respectively, in the N_2 and CO challenged hearts (Table I). The difference between these values is statistically significant ($P < 0.05$). The increase in flow was reversed completely by perfusion with oxygenated medium, and, at the end of 10 min, there was no longer any significant difference in coronary flow between the two groups.

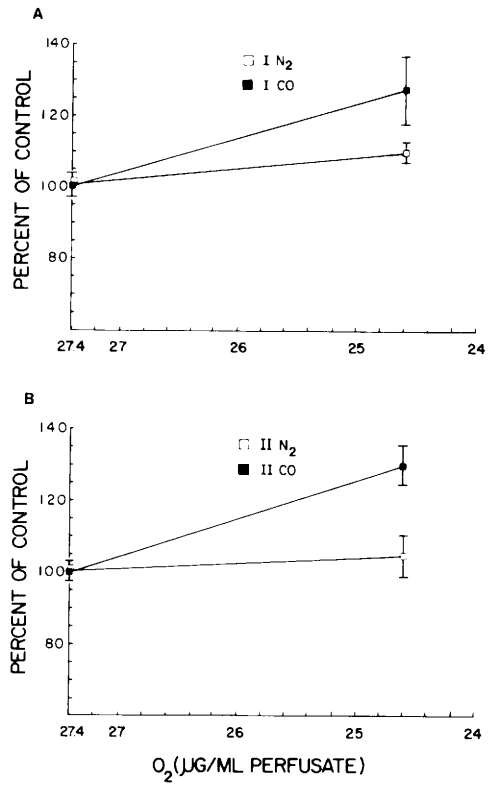

FIG. 2. Effects of a reduction in O_2 content by CO and N_2 on coronary flow in (A) Experiment I, and (B) Experiment II.

Experiment II. After recovery, coronary flow increased 5% in response to N_2 challenge and 30% in response to CO challenge (Fig. 2). At the end of 2 min, the mean increase in coronary flow was 2.5 ± 1.3 and 14.8 ± 2.3 ml·min^{-1}·g dry tissue, respectively, in the N_2- and CO-challenged hearts (Table I). The difference between these values is statistically significant ($P < 0.05$).

There were no significant changes in heart rate or pulse pressure in response to challenges with either N_2 or CO. After the first challenge, heart rate was 251.8 ± 4.6 beats·min^{-1} and 262.1 ± 5.7, respectively, in the N_2- and CO-challenged hearts. After the second challenge, heart rate was 260.8 ± 5.5 and 251.8 ± 5.1 beats·min^{-1}, respectively, in the N_2- and CO-challenged hearts. The differences are not statistically significant (Table I).

Pulse pressure after 2 min of challenge was 67.9 ± 1.1 and 67.2 ± 1.2 mm Hg, respectively, in the N_2- and CO-challenged hearts. This difference is not statistically significant (Table I). After the second challenge, pulse pressure was 66.8 ± 1.2 and 67.8 ± 1.1 mm Hg, respectively, in the N_2- and CO-challenged hearts. The differences are not statistically significant.

Discussion. These results indicate that both CO and N_2 hypoxia dilate the coronary vessels

TABLE I. CORONARY FLOW, HEART RATE, AND PULSE PRESSURE IN HEARTS CHALLENGED WITH N_2 (10% N_2–85% O_2–5% CO_2) OR CO (10% CO–85% O_2–5% CO_2)

	Experiment	Treatment	*N*	Control	Challenge	Difference
Flow (ml·min^{-1}·g dry tissue)	I	N_2	8	48.1 ± 1.6	52.9 ± 1.3	4.8 ± 1.1
		CO	8	48.3 ± 1.8	61.5 ± 4.5	13.2 ± 3.1*
	II	N_2	8	53.9 ± 2.7	56.4 ± 3.0	2.5 ± 1.3
		CO	8	49.5 ± 1.3	64.3 ± 2.6	14.8 ± 2.3*
Heart rate (beats·min^{-1})	I	N_2	8	252.0 ± 5.3	251.8 ± 4.6	−0.3 ± 2.0
		CO	8	260.4 ± 6.2	262.1 ± 5.7	1.8 ± 0.7[a]
	II	N_2	8	257.9 ± 5.3	260.8 ± 5.5	2.4 ± 1.3
		CO	8	249.4 ± 4.9	251.8 ± 5.1	2.9 ± 1.3[a]
Pulse pressure (mm Hg)	I	N_2	8	67.7 ± 1.1	67.9 ± 1.1	0.2 ± 0.3
		CO	8	65.8 ± 1.6	67.2 ± 1.2	1.3 ± 0.9[a]
	II	N_2	8	66.7 ± 1.2	66.8 ± 1.2	0.1 ± 0.5
		CO	8	67.3 ± 1.2	67.8 ± 1.1	0.5 ± 0.5[a]

Note. Values are means ± SEM. Student's *t* test used to test significance between differences.
[a] Not significant.
* Significant ($P < 0.05$).

but to a different degree. Berne and Rubio (17) have reviewed in detail the factors affecting myocardial blood flow and concluded that reduced PO_2 releases adenosine, which vasodilates the coronary circulation. Thus, in our studies, the hypoxia caused by 10% N_2 or 10% CO may have caused the release of adenosine or other vasodilating metabolites that dilated the coronary circulation and increased coronary flow. CO had the greater vasodilating effect, however, because the increase in flow was significantly greater after the CO challenge, even though PO_2 was constant. That the greater flow response to CO may result from augmented release of vasodilators or interaction with other cellular constituents is suggested also by the rapid reversal of the response when CO was withdrawn. This concentration of CO did not have a positive chronotropic or inotropic effect that could account for the flow response, because heart rate and pulse pressure remained constant throughout this experiment. In an earlier pilot study, we had observed that higher CO concentrations (up to 50% for 10 min) depressed heart rate and pulse pressure and increased coronary flow in the isolated heart (12).

The possibility that CO has a direct effect on vascular smooth muscle has been suggested by other studies of isolated organ preparations (3, 4). Duke and Killick (4) demonstrated that CO dilates directly the pulmonary vascular bed of isolated cat lungs perfused through the pulmonary artery and ventilated with CO or nitrogen. Ventilating the lungs with nitrogen increased pulmonary arterial pressure, whereas ventilating with CO or CO in nitrogen decreased pulmonary arterial pressure. Further evidence that CO may have a direct vasodilating effect on vascular smooth muscle was provided by Coburn (3), who demonstrated a decrease in isometric tension in the isolated aorta challenged with CO at a constant organ-bath PO_2.

Bassett and Fisher (2) demonstrated in isolated rat lungs that production of lactate and pyruvate increased and production of CO_2 decreased more with CO than with N_2 ventilation. The authors concluded that CO displaces O_2 from the ventilating gas and binds competitively with cytochrome oxidase. These investigators suggested also that CO may have other effects on lung tissue than those related to cytochrome oxidase activity.

Other studies have reported effects on the heart that cannot be attributed to the hypoxic effects mediated by the formation of carboxyhemoglobin (7). Scharf *et al.* (7) reported that coronary flow doubled with hypoxic hypoxia and tripled with CO hypoxia in the isolated, supported, dog-heart preparation. The authors concluded that CO and hypoxic hypoxia produced different adrenergic effects on the heart.

McGrath and Martin (18) compared the effects of N_2-induced anoxia to CO-induced anoxia in the isolated, rat, right-ventricle preparation stimulated to contract in hemoglobin-free Krebs–Ringer solution. Both gas stresses decreased developed tension; however, after 10 min of reoxygenation, recovery was significantly greater in the CO-stressed muscle. Recovery from either anoxia was further reduced by CO during the recovery phase. The results suggest also that the effects of CO on functioning tissue may be more complex than those caused solely by anoxia.

The increased coronary flow observed to occur with exposure to CO in these studies is consistent with *in vivo* observations: thus, Adams (19) reported increased coronary flow and decreased myocardial oxygen consumption in conscious dogs breathing 1500 ppm CO for 30 min. Young and Stone (11) reported an increase in coronary flow with no change in myocardial oxygen consumption in dogs with COHb levels of 30%.

The increased flow in response to CO reported herein and by others is not necessarily beneficial. Einzig *et al.* (20) reported that although myocardial blood flow increased in dogs inhaling CO, there was a decrease in subendocardial–subepicardial blood flow ratios, indicating a relative underperfusion of the left ventricle subendocardial layer. The Einzig study may also suggest a possible topical difference in vascular reactivity to CO.

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