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The Effects of Carbon Monoxide on the Heart: An In Vitro Study[1]

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McGRATH, J J *The effects of carbon monoxide on the heart An in vitro study*. PHARMACOL BIOCHEM BEHAV 21: Suppl 1, 99–102, 1984.—Experiments were conducted to assess the effects of increasing concentrations of carbon monoxide (CO) on the isolated spontaneously beating rat heart. Hearts removed from male Sprague Dawley rats were perfused via the aorta with Krebs-Henseleit solution Coronary flow was timed and collected in a calibrated vessel. Heart rate and pulse pressure were measured by a catheter inserted in the left ventricle and attached to a pressure transducer. After 30 min, the hearts were challenged for 10 min with perfusate containing increasing concentrations of CO and decreasing concentrations of 0_2. Coronary flow increased in response to CO concentrations below 50% After 8 min, coronary flow increased by 40% in response to 10% CO challenge Heart rate and pulse pressure were generally depressed by CO Heart rate was depressed at the end of 8 min by 5, 10, 38, and 64%, respectively, by solutions equilibrated against 10, 25, 50, and 95% CO. Pulse pressure decreased with concentrations of 50% CO and above These results indicate that coronary flow appears to be the most sensitive indicator of CO toxicity in the isolated heart.

Carbon monoxide Heart rate Pulse pressure Coronary flow

THE mechanism by which carbon monoxide (CO) exerts its toxic effects was described first by Haldane in [9] 1895 and has been verified since by numerous other workers [17,18] Briefly, CO reduces oxygen transport by binding with blood hemoglobin to form carboxyhemoglobin (COHb). The resulting decrease in the amount of hemoglobin available for oxygen transport and the leftward shift of the oxyhemoglobin dissociation curve produce a generalized tissue hypoxia. There are, also, several reports in the literature suggesting that CO may have other effects on isolated tissue [4, 6, 12] as well as intact animal preparations [8, 10, 23, 24].

The purpose of these experiments was to determine the effects of increasing doses of CO on the performance of the isolated rat heart perfused with hemoglobin-free Krebs-Henseleit solution.

METHOD

Experimental animals were male Sprague Dawley rats (King Animal Laboratories, Oregan, WI) weighing 225 to 250 g. Each rat was heparinized (2.5 mg IP) and guillotined; its chest was opened, and the heart excised rapidly and washed in ice cold saline. The isolated heart was perfused by a modification of the method described by Neely *et al.* [14]. The aorta was cannulated, and the heart was mounted on a modified Langendorf apparatus and perfused with oxygenated (95% 0_2–5% CO_2) Krebs-Henseleit solution. The perfusion medium contained (in mM): glucose, 5.2; $NaHCO_3$, 23.9; NaCl, 114, KCl, 4.6; $CaCl_2 \cdot H_20$, 2.9, $MgSO_4 \cdot 7H_20$, 1.1; kH_2PO_4, 1.2; and EDTA (disodium salt), 0.5. The fluid reservoirs were elevated to maintain a hydrostatic pressure of 80 mm Hg throughout the experiment. The perfusion media were gassed with 10, 25, or 95% CO–5% CO_2—balance 0_2.

A 17-cm, saline-filled catheter (PE-90) was implanted in the left ventricle and attached to a pressure transducer to record pressure changes [16]. Signals from the transducer were fed into a data logger (Buxco) and displayed on a pen recorder (Physiograph). The frequency response of the system was 22 cps. Changes in heart rate and left ventricular pressure were determined from this record. Perfusate flow was measured by collecting the effluent from the heart in a graduated cylinder.

After a 10-min washout period, the hearts were perfused with oxygenated perfusate for a 30-min control period. The hearts were switched to a parallel perfusion system and challenged with a CO test solution. After 10 min of CO challenge, the hearts were switched back to the oxygenated medium for another 10 min. The perfusion was stopped, and the hearts removed from the perfusion apparatus, weighed, and dried overnight at 100°C to determine dry weight.

Student's paired t-test was used to test the significance of differences between corresponding control and CO values [20]. A p value less than 0.05 was considered the minimal level of significance

RESULTS

Coronary flow increased in response to CO levels of less than 50% (Table 1). The response was abrupt and sustained throughout the CO challenge (Fig. 1) and was greatest with 5

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TABLE 1

CORONARY FLOW (ml min^{-1} g DRY TISSUE) BEFORE AND AFTER 8 MIN CARBON MONOXIDE-INDUCED HYPOXIA

Treatment	N	Control	8 Min	p
5% CO	(11)	49 3 ± 4 9	69 0 ± 6 7	<0 001
10% CO	(12)	44 8 ± 2 4	63 1 ± 3 0	<0 001
25% CO	(7)	49 4 ± 5 2	59 6 ± 2 9	<0 02
50% CO	(5)	49 5 ± 6 2	48 4 ± 8 4	NS
95% CO	(6)	50 4 ± 7 8	45 0 ± 10 2	NS

Values are Means ± SEM Student's paired t-test used to test significance

TABLE 2

HEART RATE (BEAT MIN^{-1}) BEFORE AND 8 MIN AFTER CARBON MONOXIDE-INDUCED HYPOXIA

Treatment	N	Control	8 Min	p
10% CO	(9)	297 7 ± 7 1	283 7 ± 8 2	<0 05
25% CO	(7)	293 9 ± 16 0	263 4 ± 14 0	<0 001
50% CO	(6)	295 3 ± 42 6	181 0 ± 26 2	<0 01
95% CO	(6)	286 2 ± 12 4	100 5 ± 14 6	<0 01

Values are Means ± SEM Student's paired t-test used to test significance

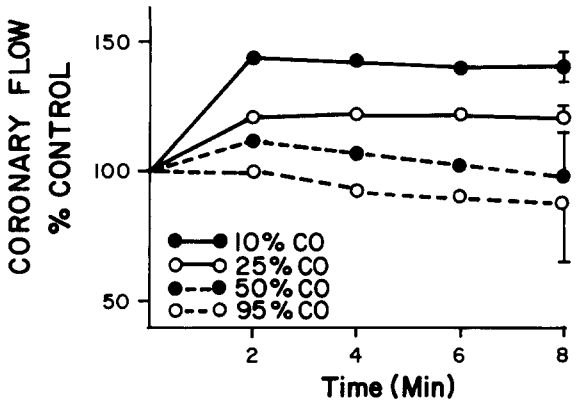

FIG 1 Changes in coronary flow in response to CO challenge ±SEM marked off at 8 min

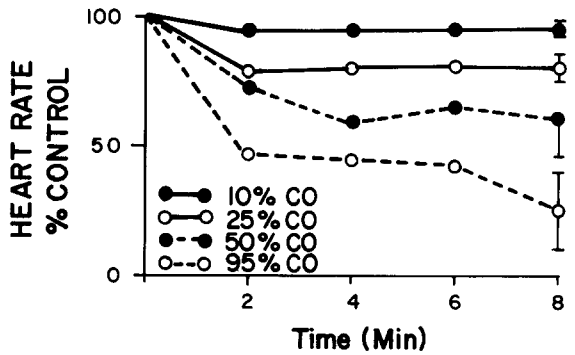

FIG 2 Changes in heart rate in response to CO challenge ±SEM marked off at 8 min

and 10% CO. At the end of 8 min, flow increased by 40 and 41%, respectively, in hearts challenged with 5 and 10% CO With 10% CO, flow increased at the end of 8 min by 40% (from 44 8 to 63.1 ml min^{-1} g dry tissue). The increase in flow with 25% CO was significantly greater than during the control period but somewhat less than that seen at the lower CO levels. It was also sudden and endured throughout the CO exposure. At 50% CO, the response was biphasic with a slight initial increase followed by a decrease to control values after 8 min

Heart rate decreased significantly in response to CO levels of 10% and above (Fig. 2, Table 2). With 10% CO, heart rate decreased at the end of 8 min by 5% (from 297.7 to 283.7 beats min^{-1}). The decrease in heart rate occurred in a dose-response manner, with heart rate decreasing by 38 and 64%, respectively, in hearts challenged with 50 and 95% CO In all cases, the decrease was sudden and sustained throughout the CO challenge

Pulse pressure decreased with levels of CO of more than 50% (Fig. 3, Table 3) The decrease occurred in a dose-response manner with pulse pressure decreasing by 24 and 52%, respectively, in hearts challenged with 50 and 95% CO In all cases, the decrease was more gradual than that occurring with heart rate, but it was sustained throughout the challenge period

DISCUSSION

These results indicate that the heart responds to CO challenge in vitro in a dose-response manner (Fig 4) Coronary flow increases at levels of CO below 50%, despite the decrease in heart rate and pulse pressure

The increased coronary flow rate is consistent with observations that coronary flow increases with CO exposure in vivo Thus, Adams *et al* [1] reported increased coronary flow and decreased myocardial oxygen consumption in anesthetized dogs breathing 1,500 ppm CO for 30 min Young and Stone [25] reported an increase in coronary flow with no change in myocardial oxygen consumption in awake dogs with COHb levels of 30% Einzig *et al* [7] 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 subendocardial layer of the left ventricle It was also apparent from our studies that at CO levels greater than 25%, there was a tendency for coronary flow to remain unchanged or decrease from control levels, suggesting the possibility of more sustained damage.

Berne and Rubio [2] have reviewed in detail the factors affecting myocardial blood flow and concluded that a reduced PO_2 releases adenosine, which vasodilates the coronary circulation. Thus, in our studies, the hypoxia caused by increasing concentrations of CO may have caused the release of adenosine or other vasodilating metabolites that dilated the coronary circulation and increased coronary flow

That hypoxia was not the only factor eliciting the increased flow response at CO concentrations of 10% or less is

TABLE 3

PULSE PRESSURE (mm Hg) BEFORE AND 8 MIN AFTER CARBON MONOXIDE-INDUCED HYPOXIA

Treatment	N	Control	8 Min	p
10% CO	(7)	63 9 ± 0 9	62 7 ± 0 9	NS
25% CO	(5)	70 4 ± 2 9	67 8 ± 2 0	NS
50% CO	(6)	66 2 ± 5 8	50 2 ± 5 0	NS
95% CO	(6)	64 8 ± 4 8	31 0 ± 6 3	<0.02

Values are Means ± SEM Student's paired *t*-test used to test significance

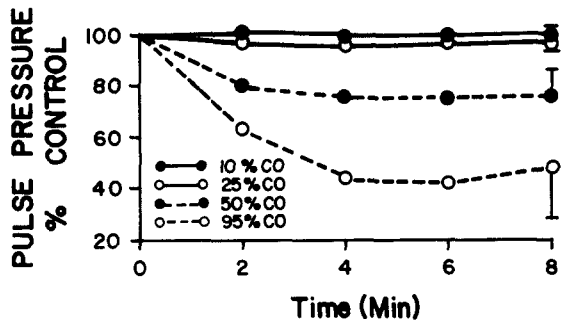

FIG 3. Changes in pulse pressure in response to CO challenge. ±SEM marked off at 8 min

suggested by our earlier observations that 10% CO elicited a greater flow response than 10% NO_2 [13].

These studies do not rule out the possibility that CO may release a vasodilator or may dilate vascular smooth muscle. The latter possibility is supported by the studies of Duke and Killick [6], who reported that pulmonary arterial pressure decreased in isolated lungs ventilated with a CO-air mixture but increased in isolated lungs ventilated with a nitrogen-air mixture. Because the response could not be blocked by nerve blocking agents the authors concluded that CO may act by dilating some part of the vascular bed directly. Further evidence comes from the work of Coburn [4], who demonstrated a decrease in isometric tension in the isolated aorta challenged with CO at a constant organ-bath PO_2

CO depressed heart rate at all levels tested. Depression was sudden, and the levels reached at 8 min were commensurate with those seen earlier [11]. It is possible that in addition to its hypoxic effects, CO may also have a direct effect on the excitation-conduction system. This possibility is consistent with the observations that 95% CO-5% CO_2 depressed heart rate more rapidly than 95% N_2-5% CO_2 in unstimulated but not in stimulated preparations [3,11] and is supported by the observations of Dergal *et al* [5] that cardiac pacing during CO poisoning increased the chances of survival in dogs.

Heart rate changes with CO inhalation in vivo are less clear cut. Thus, heart rate has been reported to remain unchanged in humans [19,21] and rats [22], however, Patajan *et al.* [15] reported a decrease in heart rate in rats after a transient increase, and Lass *et al* [10] described episodes of bradycardia due to heart block in awake rats exposed to CO.

Pulse pressures were relatively unaffected by CO challenges of 50% or less. At 95% CO, pulse pressure decreased significantly to levels commensurate with those seen in earlier studies [11]; however, the change was not so abrupt as the changes in coronary flow or heart rate. This suggests that the decrease in pulse pressure could be attributed to a global hypoxia rather than a direct effect of CO.

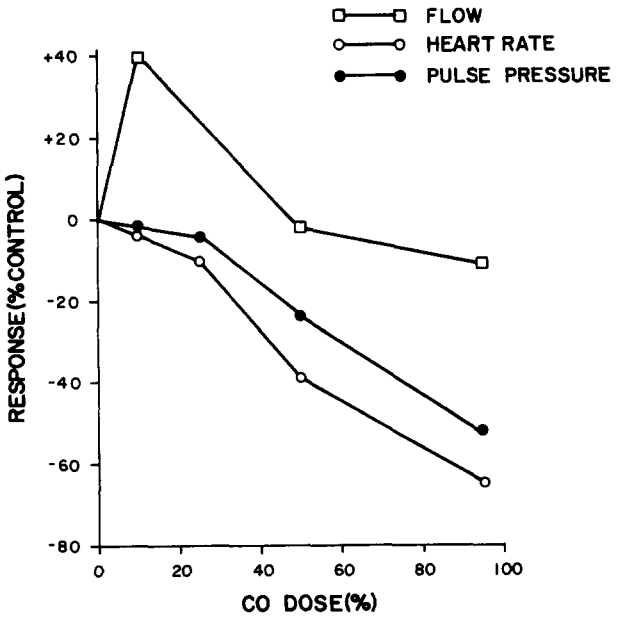

FIG 4 Changes in flow, heart rate, and pulse pressure at 8 min in response to varying CO doses

REFERENCES

1 Adams, J D , H H Erickson and H L Stone Myocardial metabolism during exposure to carbon monoxide in the conscious dog *J Appl Physiol* **34**: 238–242, 1973.

2 Berne, R M and R Rubio Coronary circulation In *Handbook of Physiology, Section 2 The Cardiovascular System,* edited by R M Berne, N Sperlakis and S. R Geiger Bethesda, MD American Physiological Society, 1979, pp 873–952

3 Chen, K C and J J McGrath Effects of nitrogen and carbon monoxide on the stimulated rat heart. *Fed Proc* **37**: 230, 1978

4 Coburn, R F Mechanisms of carbon monoxide toxicity *Prev Med* **8**: 3310–3322, 1979

5 Dergal, E., H Hoojati, L Goldbaum and K Absolon. Effects of cardiac pacing in acute carbon monoxide intoxication in dogs. *Chest* **70**: 424, 1976.

6 Duke, H. and E Killick. Pulmonary vasomotor responses of isolated, perfused cat lungs to anoxia *J Physiol* **117**: 303–316, 1952.

7 Einzig, S , D Nicoloff and R J Lucas, Jr Myocardial perfusion abnormalities in carbon monoxide poisoned dogs *Can J Pharmacol* **58:** 396–405, 1980
8 Goldbaum, L R , R. G. Ramirez and K Absalon Joint committee on aviation pathology XIII What is the mechanism of carbon monoxide toxicity? *Avat Space Environ Med* **46:** 1289–1291, 1975
9 Haldane, J S The action of carbonic oxide on man *J Physiol (Lond)* **45:** 430–462, 1895
10 Laas, R , J Igloffstein and S Meyerhoff Cerebral infarction due to carotid occlusion and cerebral carbon monoxide exposure I Pathophysiological and neuropathological observations *J Neurol Neurosurg Psychiatry* **46:** 756–767, 1983
11 McGrath, J J and K C Chen Comparative effects of nitrogen and carbon monoxide on the isolated rat heart *Toxicol Appl Pharmacol* **45:** 231, 1978
12 McGrath, J J and L G Martin Effect of carbon monoxide on isolated heart muscle *Proc Soc Exp Biol Med* **157:** 681–683, 1978
13 McGrath, J J and D Smith Comparative effect of carbon monoxide and nitrogen hypoxia on heart *Fed Proc* **41:** 1618, 1982
14. Neeley, J R , H Liebermeister, E J Battersby and H E Morgan Effect of pressure development on oxygen consumption by isolated rat heart. *Am J Physiol* **212:** 804–814, 1967
15 Patajan, J H , S C Packham, D B Frens and B. G Dinger Sequalae of carbon monoxide-induced hypoxia in the rat *Arch Neurol* **33:** 152–157, 1976
16 Penpargkul, S and J Scheuer The effect of physical training upon the mechanical and metabolic performance of the rat heart *J Clin Invest* **49:** 1859–1868, 1970
17 Root, W S Carbon monoxide In *Handbook of Physiology, Section 3 Respiration*, edited by W O Fern and H Rahn Washington, DC American Physiology Society, 1965, pp 1087–1098
18 Roughton, F J W The equilibrium of carbon monoxide with human hemoglobin in whole blood *Ann NY Acad Sci* **74:** 177–188, 1970
19 Shephard, R J The influence of small doses of carbon monoxide upon heart rate *Respiration* **29:** 516–521, 1972
20 Steel, R G and J H Torrie *Principles and Procedures of Statistics* New York McGraw Hill, 1960, pp 78–80
21 Stewart, R D , T N Fischer, M J Hosko, J E Peterson, E D Baretta and H G Dodd Experimental human exposure to methylene chloride *Arch Environ Health* **25:** 342–348, 1972
22 Weiss, H R and J A Cohen Effects of low levels of carbon monoxide on rat brain and muscle PCO *Environ Physiol Biochem* **4:** 31–39, 1974
23 Winston, J M , J M Creighton and R J Roberts Alterations of carbon monoxide and hypoxic hypoxia-induced lethality following phenobarbital, chlorpromazine, or alcohol pretreatment *Toxicol Appl Pharmacol* **30:** 458–465, 1974
24 Winston, J M and R J Roberts Effect of potassium cyanate on carbon monoxide and hypoxic hypoxia-induced lethality *J Toxicol Environ Health* **2:** 625–631, 1977
25 Young, S H and H L Stone Effect of a reduction in arterial oxygen content (carbon monoxide) on coronary flow *Aviat Space Environ Med* **47:** 142–146, 1976