Pharmacology Biochemistry & Behavior, Vol 21, Suppl , 1, 103–107, 1984 © Ankho International Inc Printed in the U S A 0091-3057/84 $3 00 + 00

Cardiorespiratory Responses to Intestinal Injection of Carbon Monoxide[1]

JAMES J. McGRATH AND CARL LEVISEUR

Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430

McGRATH, J J. AND C. LEVISEUR. *Cardiorespiratory responses to intestinal injection of carbon monoxide* PHARMACOL BIOCHEM BEHAV **21**: Suppl 1, 103–107, 1984 —Experiments were conducted to investigate whether direct, intestinal injection of carbon monoxide (CO) can be used as a reliable method of exposing rats to CO Laboratory rats were anesthetized with urethane A laparotomy was performed and the small intestine isolated. The pylorus and ileocaecal valve were ligated and air or 100% CO (75 ml/kg) was injected directly into the small intestine. Heart rate and respiratory rate were monitored by impedance pneumography. COHb and blood lactate concentrations were determined spectrophotometrically. CO was rapidly absorbed from the intestinal tract and COHb levels reached 70.9±1.3% one hr after treatment. After 45 min, heart rate decreased in the CO-injected animals to 86% of pre-injection value. Blood pressure decreased rapidly to levels significantly lower than controls 5 min after treatment. Respiratory rate decreased to levels significantly lower than controls 55 min after treatment. CO treatment resulted in a 4-fold increase in blood lactate concentration The results indicate that 100% CO injected directly into the small intestine is absorbed rapidly and the resulting elevated COHb levels cause a rapid decrease in blood pressure, a decrease in heart and respiratory rates, and an increase in the blood's lactic acid concentration.

Blood pressure Carbon monoxide Carboxyhemoglobin Heart Rate Hypoxia Lactic acid Respiration rate

THE physiological responses to inhaled carbon monoxide (CO) are numerous and well documented in mammals [1, 7, 15, 17]. Inhaled CO combines with hemoglobin in the lungs to form carboxyhemoglobin (COHb) which causes a functional anemia by decreasing the amount of hemoglobin available for oxygen transport and by causing the oxyhemoglobin dissociation curve to shift to the left. The physiological factors influencing the amount of hemoglobin formed in this manner in man have been quantified by Coburn *et al.* [3] and include endogenous production of CO alveolar ventilation, diffusion capacity of the lung, mean oxygen tension in the pulmonary capillaries, and the concentration of CO in inspired air.

Several workers [2, 4, 5, 11] have studied responses to elevated COHb levels produced by methods other than CO inhalation. The following study was conducted to determine whether direct, intestinal injection of CO is a reliable alternative to inhalation for producing elevated COHb levels and to observe the effects of CO injection on heart rate, blood pressure, and respiration rate.

METHOD

Animals used in this experiment were Sprague Dawley male rats weighing between 150 and 250 g. The animals were anesthetized with urethane (1.2 mg/kg) injected IP and remained anesthetized throughout the experiment. A laparotomy was performed and the intestine isolated according to the method of Poupa *et al.* [10]. The pylorus and ileocaecal valve were ligated. The carotid artery was cannulated for blood pressure measurements and sample withdrawal. Heart rates and respiratory rates were monitored continuously throughout the experiment by impedance pneumography using needle electrodes inserted subcutaneously on the thorax. Signals from the electrodes were displayed on the chart of a Narco physiograph recorder. Blood pressure was measured by a pressure transducer (Narco RP-1500) and displayed on the recorder. The test gas was obtained from a cylinder of 100% CO (Matheson Gas) with a 50-cc glass syringe according to the method described by Nelson [8]. (In this method, gas flows from the cylinder through a section of rubber tubing. The tubing is pierced, and the syringe is flushed three times with the gas before it is filled and withdrawn.) The gas was then injected directly into the intestine, distal to the pyloric ligature (Fig. 1). Experimental rats were injected with 75 ml CO/kg. Control rats were injected with air

Blood samples (0.25 ml) were taken at 15-min intervals for 1 hr. COHb levels were determined spectrophotometrically according to the method of Small *et al.* [14]. Blood lactate concentrations were determined enzymatically (Sigma Chemical Company) on 1-ml samples drawn at the end of one hr. Data were analyzed for statistical significance by Student's *t*-test.

RESULTS

Blood COHb levels increased rapidly with intestinal in-

[1]This study was supported in part by the American Heart Association, Texas Affiliate, and NIH Biomedical Research Support Grant number 2507RR05773-06

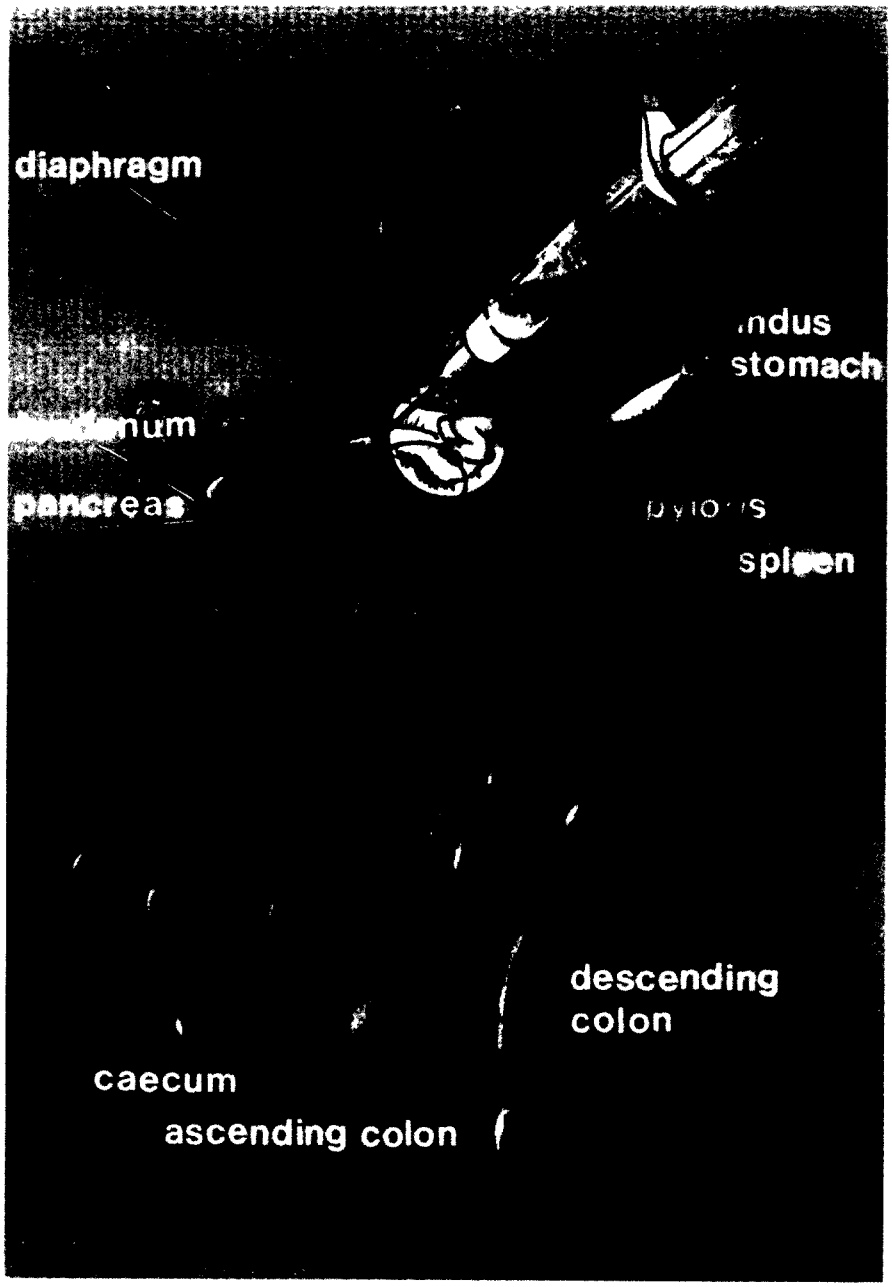

FIG 1 Schematic showing site of CO injection

jection of CO (Fig 2). At the end of 15 min, COHb levels in animals injected with 75 ml/kg CO had increased to 70 8%, after which it plateaued for the remainder of the one-hr experimental period.

Injected CO caused a slight (22 beats/min) increase in heart rate occurring 5 min after treatment (Fig. 3) Thereafter, heart rate returned to control levels At 45 min, heart rate had decreased to 78% of its pre-test value in the CO-injected rats and was significantly lower than in the air-injected controls ($p<0$ 01). Air injection had little effect on heart rate during the experimental period in the control animals

Injected CO depressed blood pressure to levels that were significantly lower than control values (Fig 4) within 5 min ($p<0.01$) Thereafter, blood pressure continued to decline After one hr, blood pressure had declined from a pre-injection value of 114 4±8.3 to 70.1±9.0 mm Hg Blood pressure decreased slightly from 117 3±7 1 to 105 3±11 1 mm Hg in the air-injected animals

CO caused a slight (88 3±3 5 vs 82.3±3 4 breaths/min) increase in respiration five min after injection (Fig 5) After 55 min, the respiration rate was significantly ($p<0.05$) decreased from that of controls, and, at the end of one hr, respiration rate had decreased to 68% of its pre-test value in

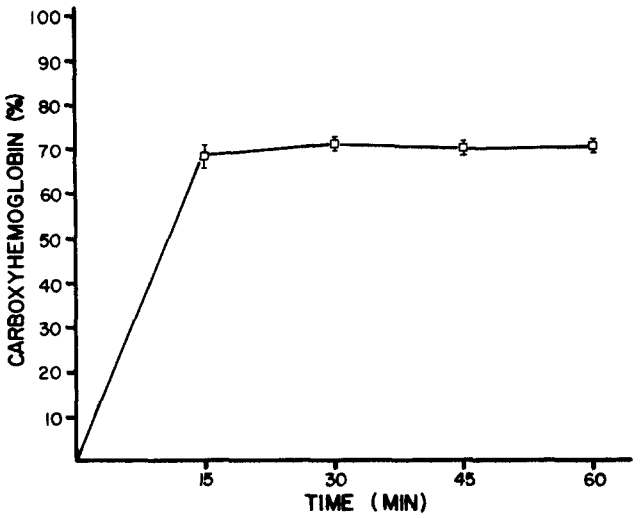

FIG 2 Changes in blood carboxyhemoglobin levels after injection with air or 75 ml CO/kg body weight at time 0. Each point represents the mean of 10 control or 16 experimental animals SEM marked off for each mean

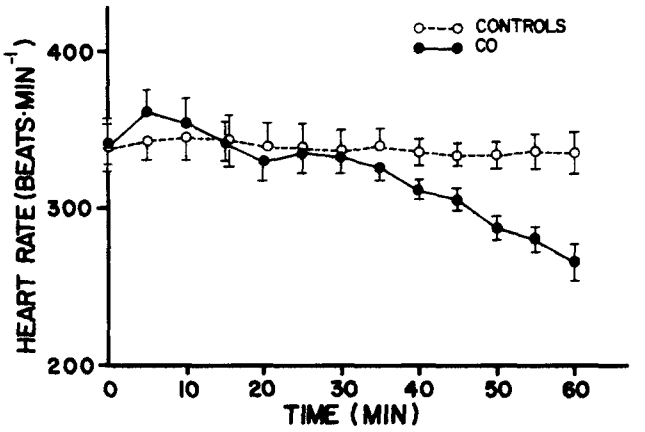

FIG 3 Changes in heart rate after injection with air or 75 ml CO/kg body weight at time 0. Each point represents the mean of 10 control or 16 experimental animals SEM marked off for each mean.

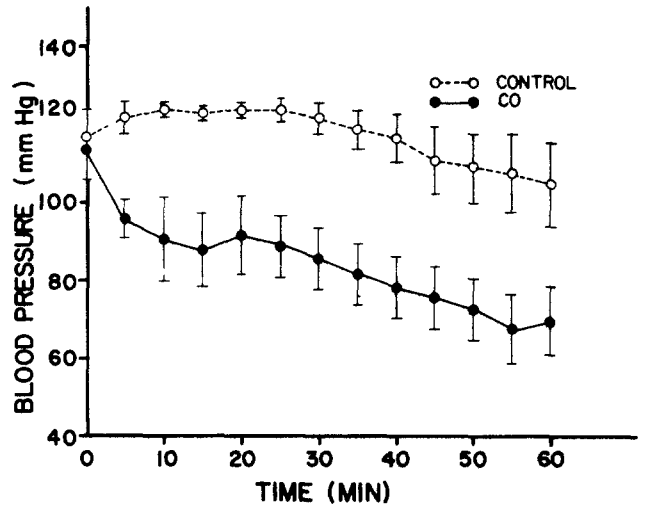

FIG 4 Changes in mean blood pressure after injection with air or 75 ml CO/KG body weight at time 0. Each point represents the mean of 10 control or 16 experimental animals SEM marked off for each mean

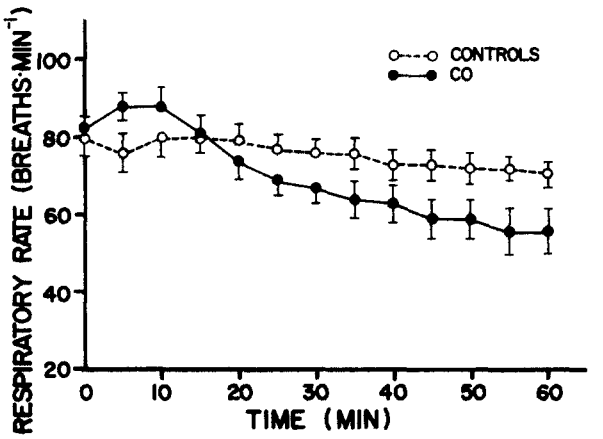

FIG. 5 Changes in respiration rate after injection with air or 75 ml CO/kg body weight at time 0 Each point represents the mean of 10 control or 16 experimental animals SEM marked off for each mean.

the CO-injected rats. Respiratory rates decreased slightly in the air-injected animals (from 79.9±4.6 to 71.1±2.9 breaths/min) during the experimental period.

CO treatment caused a 4-fold increase in blood lactate concentrations (Fig. 6). At the end of one hour, lactate levels had increased to 126.4 mg% in the CO-injected animals and were significantly higher than in controls ($p<0.001$). Lactate levels were 26.7±2.5 mg% in the air-injected animals at this time.

DISCUSSION

We have shown that relatively high levels of COHb may be produced by injecting 100% CO directly into the small intestine of anesthetized animals. The method is rapid and reliable and produced COHb values with small standard errors. The rapid rise in COHb levels produced by this method is associated with a rapid depression in blood pressure, a reduction in respiratory and heart rates, and a pronounced increase in blood lactate levels.

COHb has been shown to cause a tissue hypoxia by reducing the oxygen carrying capacity of the blood and shifting the oxyhemoglobin dissociation curve to the left so that the remaining oxygen is bound more tightly [12,13]. The intensity of the hypoxic exposure in these studies is evident in the

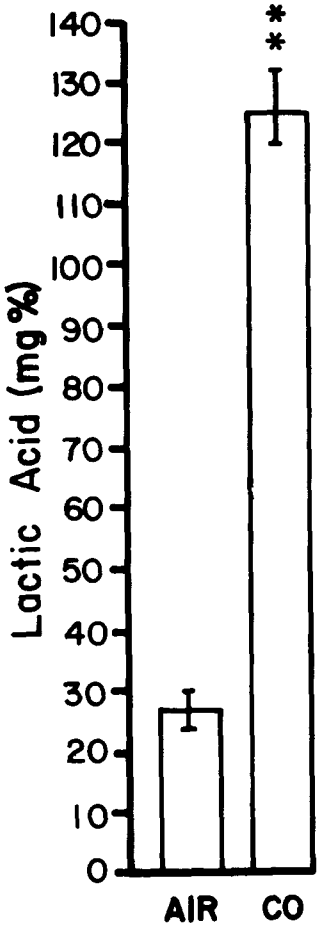

FIG 6 Blood lactic acid concentrations in rats 1 hr after injection with air or 75 ml CO/kg body weight Three control and 11 experimental animals used for each point SEM marked off for each mean The Student t-test used to test significance $^{**}p<0.01$

4-fold increase in the concentration of lactic acid in the CO-injected rats.

In general, injected CO depressed respiratory rate, however, a transitory increase in respiratory rate occurred approximately 5 min after CO injection An earlier pilot study revealed that COHb levels during this time were approximately 35% The increase in respiratory rate was concurrent with a depression in blood pressure and elevation in heart rate It is likely that respiration was stimulated initially but that the worsening hypoxemia and concomitant hypercapnia and acidemia depressed the respiratory center rapidly The respiratory response seen in these studies is similar to that reported by Petajan *et al* [9] in animals exposed to CO by inhalation

Injected CO depressed heart rate After one hour, heart rate was depressed by approximately 22% At this time, COHb levels had reached 71%, a level usually associated with cardiorespiratory failure and death in humans [15] Although CO depressed heart rate, there was a slight initial increase that, although not statistically significant, concurred with a significant decrease in blood pressure This suggests that the depression in blood pressure elicited a baroreflex that was blunted by the rapidly occurring hypoxemia The depressed heart rate was caused most likely by the sytemic hypoxia associated with the elevated COHb levels and was exacerbated by the subsequent hypercapnia and acidosis. These results are also similar to those reported for rats inhaling CO [9]

Injected CO depressed blood pressure Blood pressure was significantly depressed after 5 min of exposure and was depressed by 44% one hour after treatment These results are in agreement with those of Petajan *et al* [9], who produced comparable COHb levels in rats that inhaled CO These workers suggested that the degree of hypoxia is less of a factor than the lowering of blood pressure in the loss of both brain and nerve activity, and that the decline in nervous system function can be correlated directly to the decrease in blood pressure

Our results are essentially in agreement with those of Petajan *et al* [9], however, other workers reported not only decreased blood pressure but also variable changes in heart rate in animals inhaling CO [7,16] Korner [6] reported increased heart rate, decreased blood pressure, and no change in ventilation in animals breathing gas mixtures containing CO Sylvester *et al* [16] reported variable heart rate changes, increased cardiac output, and decreased arterial blood pressure in dogs anesthetized with pentobarbital and ventilated with CO The close agreement of our results with those of other workers indicates that direct intestinal injection of CO is a reliable, relatively simple method for studying the cardiorespiratory effects of CO, singly or in combination with other agents

REFERENCES

1 Ayres, S , S Gianelli and H Mueller Myocardial and systemic response to carboxyhemoglobin *Ann NY Acad Sci* **174:** 268–293, 1970

2 Chen, K C , J J McGrath and P S Lee Effects of intraperitoneal injection of carbon monoxide on circulating blood glucose and lactate *Fed Proc* **38:** 1313, 1979

3 Coburn, R F , R E Forster and P. B Kane. Consideration of the physiological variables that determine the blood carboxyhemoglobin concentration in man *J Clin Invest* **44:** 1899–1910, 1965

4 Goldbaum, L R , T Orellano and E Dergal Mechanisms of the toxic action of carbon monoxide *Annal Clin Lab Sci* **6:** 372–376, 1976

5 Goldbaum, L R , T Orellano and E Dergal Studies on the relation between carboxyhemoglobin concentration and toxicity *Aviat Space Environ Med* **48:** 969–970, 1977

6 Korner, P I Central neurons control of autonomic cardiovascular function In *Handbook of Physiology, The Cardiovascular System, vol 1, The Heart*, edited by R M Berne Bethesda, MD American Physiological Society, 1979, pp 691–740

7 McGrath, J J Physiological effects of carbon monoxide In *Air Pollution–Physiological Effects*, edited by J J McGrath and C D Barnes New York Academic Press, 1982, pp 148–182

8 Nelson, G O *Controlled Test Atmospheres* Ann Arbor, MI Ann Arbor Science Publishers, 1976, p 63

9 Petajan, 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
10 Poupa, O , M Kopecky and F Chytil. Effect of aeration of the small intestine on the oxyhaemoglobin content of portal blood *Nature* **179:** 1080–1081, 1957
11 Ramirez, R , A Solomon, J C Agostini, A P Basu, L R Goldbaum and K B Absolon Lack of toxicity of transfused carboxyhemoglobin red blood cells and carbon monoxide inhalation *Surg Forum* **25:** 165–168, 1974
12 Roughton, F J W and R C Darling The effect of carbon monoxide on the oxyhemoglobin dissociation curve *Am J Physiol* **141:** 17–31, 1944
13 Roughton, F. J W *Ann NY Acad Sci* **174:** 177–188, 1970
14 Small, K A , E P Radford, J. M Frazier, F L Rodkey and H A Collison Rapid method for simultaneous measurement of carboxy- and methemoglobin in blood *J Appl Physiol* **31:** 154–160, 1971
15 Stewart, R D The effect of carbon monoxide on humans. *Annu Rev Pharmacol* **15:** 409–423, 1975.
16 Sylvester, J T , S M Scharf, R D Gilbert, R S Fitzgerald and R J Traystman Hypoxic and CO hypoxia in dogs hemodynamics, carotid reflexes, and catecholamines *Am J Physiol* **236:** 1422–1428, 1979
17 Wright, G. R and R J Shephard Physiological effects of carbon monoxide In *Environmental Physiology*, vol 3, edited by D Robert Shaw Baltimore University park Press, 1979, pp 311–368